

### **To the editor**

Dear editor,

Following the reviewers' comments, I have carefully revised the manuscript. I now believe the manuscript is more transparent and friendly to the readers. In the following, I have attached my point-to-point responses to the reviewers' comments. Also included in the submission are (1) a clean revision with all changes incorporated and (2) a tracking change revision indicating where changes were made.

I sincerely appreciate your efforts in handling my manuscript. I am looking forward to hearing back from you soon.

Sincerely,

Jinyun

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### **Response to reviewer 1**

**Comment 1:** *The study speaks strongly via solid mathematical derivations for replacing the MM and RMM formulations by ECA kinetics. Evidence of testing the application of ECA in modeling plant-microbe interactions was discussed through recently published work by Zhu et al (2015) and the author himself. Other potential literature in microbial models may also need to be considered (i.e. Li J, G. Wang et al 2014; Manzoni S, G. Pineiro et al 2012). Except a few typos that need to be corrected, the current paper deserves a rapid publication so that it is expected to motivate more studies to emerge in order to advance the soil biogeochemical model development.*

**Response:** Thanks very much for your positive comments. I corrected the typos in the revised manuscript.

**Comment 2:** *Possible typos: Equation 21: denominator  $[E]_T$  should be  $[S]_T$  Figure 4 caption: enzyme should be changed to substrate.*

**Response:** Sorry for the typos that I missed in the proof reading, now they are corrected in the revised manuscript.

## Response to Dr. Thomas Wutzler

**Overall comments:** *J.Y. Tang in this paper show the relationship between the recently introduced Equilibrium Chemistry Approximation (ECA) kinetics with commonly used other formulation of substrate kinetics. This is an important topic and valuable to the modelling community, as this substrate kinetics is central to soil organic matter modelling.*

*While I strongly suggest publishing the paper, I give some constructively meant critiques that hopefully help to convey the message of the paper better to the reader.*

**Response:** I sincerely appreciate your positive comments. I addressed your comments point by point in the following.

**Comment 1:** *Note that GM journal also addresses readers that do not have a very strong mathematical background. Please, give some more aid, so that the readers can follow the derivations (Some suggestion are given in the specific section below)*

**Response:** I revised the manuscript by following your specific suggestions below.

**Comment 2:** *Both the abstract and the main part of the paper present a mathematical treatment without sufficient user-aid on how to interpret the results. Why are the parametric sensitivities important? What does it mean for modelling the processes?*

**Response:** Correct parametric sensitivity is important for model calibration and model interpretation. All calibration techniques (either explicitly or implicitly) rely on the parametric sensitivity to adjust parameter values and wrong parametric sensitivity would mislead empirical measurements to do incorrect measurements. I added this discussion in the revised manuscript.

**Comment 3:** *Beware of confounding the concepts of microbial uptake and enzyme kinetics (e.g. p. 7680 ll line 14). The ECA, as I understood it, deals with enzymatic breakdown of soil organic matter (SOM) into smaller compounds. The Monod-Description of microbial uptake of these components has a different more empirical background. While with the assumption of enzymatic breakdown to be the limiting step, models can apply ECA also for microbial growth, the two concepts should be kept clear.*

**Response:** Sorry I missed some nuance for this part in the paper. As described in Tang and Riley (2013), ECA is derived for generic purposes including, but are not limited to, enzymatic breakdown of SOM, microbial growth and predator-prey relationships, with appropriate setting up of the stage. The use of enzymatic reaction in the paper is just for a convenience of presentation. I clarified this nuance in the revised manuscript (P5. L25-29).

**Comment 4:** *ECA are based on total concentrations including the enzyme-substrate complex. Most SOM models are formulated on a more abstract level. How to deal with this practically? What are the consequences when total concentrations would be replaced by modelled pure concentrations or by pools in mass units? Under which conditions is this is viable?*

**Response:** I below explain briefly this technical nuance, but more details can be found through the examples in Tang and Riley (2013). In most applications, the total substrate concentration is equivalent to the free substrate concentration as used in the Monod

kinetics. However, as I explained in the paper, when free substrate concentration is very low, application of the Monod kinetics or the MM kinetics violates their condition of validity. When total concentrations are replaced by modeled pure concentrations or by pools in mass units, the ECA kinetics only requires all units of substrates, affinity parameters and enzymes (or microbes) are consistently defined. The major difference (between ECA and MM) occurs when one applies ECA for modeling microbial DOC uptake in presence of mineral surface adsorption. In ECA, the total DOC concentration means the total of adsorbed and free DOC, whereas in the MM kinetics, only free DOC is used. As shown in Tang and Riley (2013; Figure 6), this difference in treatment would lead the MM kinetics to predict very inaccurate decomposition dynamics.

**Comment 5:** *The introduction is written well, and the importance becomes clear. The main message of the paper to me is that ECA for one substrate-one enzyme is a mass-balanced approximation of the general QSS (quasi steady state) solution and that generalizes both MM and RMM. The derivation (from eq. 11 to 12), however, is too condensed to understand without more mathematical efforts. Did you generate the Taylor series at  $E=0$  and  $S=0$ ? Did you truncate second order terms of  $E$  and  $S$ ? What does it mean to truncate for  $\epsilon$ ?*

**Response:** The ECA is a mass-balanced approximation for arbitrary number of enzymes (or competitors in general) and substrates, and this paper focuses on the one-substrate-one-enzyme example to analytically tease apart the differences and connections between ECA, MM and RMM because such analytical analysis is not possible for the most general case involving many substrates and many enzymes. In the derivation, the Taylor expansion is performed with respect to  $\epsilon$ , and the first order approximation is defined with respect to  $\epsilon$ . I made it logic from Eq. (11) to Eq. (12) more straightforward in the revised manuscript (P7. L7-8)

**Comment 6:** *Can you, please, extend the explanation of the points at the end of section 2.1? To what and how is Eq. 12 applied? Is eq. 13 not just a re-statement of eq. 5? In what way does this form the tQSSA?*

**Response:** I did my best in the revised manuscript to clarify this (P8. L1-4). To put it simple, the QSSA means taking the temporal derivative of Eq. (4) to zero. The total substrate concentration means adding together the free substrate and enzyme-substrate complex. Therefore, tQSSA means adding Eq. (3) and Eq. (4) together. Mathematically, Eq.(13) is equivalent to a restatement of Eq. (5), yet, they mean different things. A more detailed analysis of such difference lies in the perturbation analysis of the tQSSA, however, that is very lengthy and involved, but if interested the paper by Borghans et al. (1996) and some references they cited explained it very well.

**Comments 7:** *Maybe also move the equations of the parametric sensitivity analysis to the appendix and focus in the main text on the figures and their interpretation for modelling. Why were the sensitivities normalized? Especially why multiplied by the rates? How are these normalized sensitivities interpreted?*

**Response:** I too have struggled in deciding where I should put those equations, but I finally decided to include them in the main text to satisfying the requirements from both readers enjoy mathematical rigorousness and readers that are less math-oriented. The

normalization follows from the tradition in analyzing chemical kinetics. Such normalization assures that all parametric sensitivities are not unit-dependent. Mathematically, the normalized parametric sensitivity indicates the relative change in dependent variable (reaction velocity here) in response to the relative change in the free parameter.

**Comment:** *Section 3.2. can be shortened by noting that the sensitivities are 1- the sensitivities of 3.1. I could not follow derivation from eq. B2 to B3. When I insert  $v_{ECA}$  and  $K_{ES}$  in the second term on the right of B3, I arrived at a result different from B2. (to editor: I did not check Taylor expansion of eq. 10 nor Appendix A)*

**Response:** As for the length of section 3.2 and section 3.1., I decide to put them as they are, so readers can understand both without referring to each other. For mathematical derivation, I double-checked the math, it is correct.

## 2 Specific comments

**Comment:** *P.7670 L.1: suggest aid: By inserting  $[E]$  solved from (7) and  $[S]$  from (8) into (6) one arrives at the following quadratic equation.*

**Response:** Per your suggestion, I added these manipulation details in the revised manuscript (P7. L1-2).

**Comment:** *P.7670 L.9, L12: Some more details are required.*

**Response:** I made it clear that the Taylor expansion is done with respect to  $\epsilon$  (P7. L7-8). I also added a reference to help readers understand the mathematics, although the details for more generic case can be found in Tang and Riley (2013).

**Comment:** *P. 7671: L15: What does the error in parametric sensitivities mean for modelling?*

**Response:** They could either mean the model will fail in calibration (see example of litter decomposition in Tang and Riley (2013)) or the model interpretation are incorrect.

**Comment:** *P.7675 L.8: term predictions refer to sensitivities or reaction rates?*

**Response:** They refer to sensitivity. I removed this ambiguity in the revision.

**Comment:** *P.7675 L.14: Color scale in Fig. 1 goes to -9% instead of 5% in the text. What is the difference?*

**Response:** Note -0.09 is the value normalized with respect to the sum of parametric sensitivity from both the ECA approximation and exact solution; therefore 5% is about (half of 9%) the actual relative difference.

**Comments:** *Figs (1-3d) are hard to understand. Why do you apply log in single variables in the derivatives instead of log(sensitivity). Also with so much overplotting the figure is obscured. Where does the spread come from?*

**Response:** I was comparing the parametric sensitivity calculated by the three approximations to the true parametric sensitivity as calculated from the exact solution. This comparison tells how well the MM, RMM and ECA kinetics approximate the exact solution. The spread comes from the poor performances of the MM and RMM kinetics. I

also have redrawn the plots to have a clearer visual.

**Comments:** *Two Typos after eq. B3 (Then, refer to eq. B3 instead of B2)*

**Response:** Typos corrected.

**Comments:** *P.7680 L.13. Important sentence, but very long. Can be broken up.*

**Response:** I broke it up.

## **References**

Tang, J. Y., and W. J. Riley (2013), A total quasi-steady-state formulation of substrate uptake kinetics in complex networks and an example application to microbial litter decomposition, *Biogeosciences*, 10(12), 8329-8351.

Borghans, J. A. M., R. J. DeBoer, and L. A. Segel (1996), Extending the quasi-steady state approximation by changing variables, *B Math Biol*, 58, 43-63, doi: 10.1007/Bf02458281.

## Response to Prof. J. Schimel

**Overall comments:** *In this paper, Tang has followed up on his earlier work assessing the nature of microbial kinetics to use in microbially explicit biogeochemical models. The earlier generations of microbially implicit models assumed first-order kinetics for substrate movement from a source pool to a sink pool ( $dC/dt = k \cdot C$ ). The models (e.g. CENTURY) are powerful and simple, but they have limitations that researchers have been trying to overcome with newer models that treat microbes as actual drivers of processes, drivers whose population and characteristics can change dynamically and so must be represented explicitly.*

*The challenge Tang notes is that authors have used different kinetic expressions in such models, depending on whether the model assumes that substrates are mobile and can saturate the enzyme active site (leading to Michaelis-Menten kinetics) or whether substrates are immobile and enzymes can saturate potential reaction sites (leading to reverse Michaelis-Menten kinetics). In an earlier paper, Tang and Riley had shown that these two formulations were really end members of a more general model which can shift between those states and doesn't require an assumption of either enzymes or substrates being a functionally immobile entity. That is the ECA model. In this paper, they further develop the analysis of these different approaches to modeling microbial kinetics. This is unquestionably a useful activity. I really appreciate developing a single integrated expression that isn't as constrained as any of the equations that place greater constraints in the assumptions.*

**Response:** Many thanks for your positive comments. We'll keep doing the good work.

**Comment 1:** *Despite that, I have some questions as to the utility of getting deeply mechanistic in the derivation of fundamental chemical kinetics for these expressions.*

**Response:** I addressed your comments point by point below, and some of those discussions are integrated into the revised manuscript where I found it appropriate.

**Comment 2:** *First, classical kinetics deals in activities, not concentrations, and assumes that the activity of any material that is not dissolved is equal to 1. Yet, many of the decomposition reactions involving exo-enzymes are likely mixed phase, in which the substrate is not in solution, the enzyme may be, and the products certainly are. So at least for applying to a real-world situation, does the shift between single phase (dissolved or vapor) and mixed phase (some in solution, some not) change how we should view the real mechanistic interpretation of these expressions? It should, I think, as it converts a true mechanistic model into an empirical approximation of one in which we can use concentration terms that are per gram soil, for example.*

**Response:** Thanks for raising this question and I will take this opportunity to clarify the goal of my manuscript. Yes, classical kinetics deals with activities, not concentration; however, the substrate uptake process as we are dealing with in soil biogeochemical modeling can be conceptualized as analogues to the predator-prey relationship, or, more generally, the resource competition problem. This generalization allows us to directly deal with the concentrations even though such generalization does make the solution to the problem slightly more empirical. Specifically, the prey-searching rate and prey-attacking rate together establish a dynamic equilibrium between prey and predator concentrations. This is analogous to the binding process between substrates and enzymes,

and could be achieved without referring to the phase of existence for either the enzymes or substrates. Therefore, mathematically, as we discussed in the ECA paper (Tang and Riley, 2013), the problem can be formulated into the equilibrium chemistry form. Such analogy is also supported by the derivation of MM kinetics even for a single molecule enzyme (where substrate is unlimited and the definition of phase for enzyme becomes ill-defined; English et al., 2006). Because of the equilibrium binding as implied behind the conceptual model, we can establish the relationships between MM, RMM and ECA kinetics as I attempted in this manuscript.

**Comment 3:** *Second, in a physically constrained, diffusion-limited system, are these simple concentration-defined rate expressions accurate or appropriate? I suspect that they all “work” to capture the overall dynamics of major organic matter components in soil and plant litter (using bulk concentration), but maybe not because they meet the assumptions of the actual chemical models.*

**Response:** As I explained in the response to comment 2, when diffusion is unlimited, the adoption of the equilibrium binding is eligible. When diffusion limitation comes into place, the control of diffusivity can be incorporated accordingly to derive a revised kinetics (e.g., Tang and Riley, 2013). Application of such revised kinetics in marine ecosystems has indicated very successful results (Bonachela et al., 2011).

**Comment 4:** *That latter issue underlies a slight misrepresentation of the Schimel and Weintraub model’s development of reverse M-M kinetics (page 7665, line 21). The author’s statement that Schimel and Weintraub explored straight M-M kinetics is inaccurate (though unimportant). S&W didn’t get that far! Rather they explored linear kinetics and noted that if the reaction rate expression was linear on enzyme concentration ( $dC/dt = k * [Enz] * [Substrate]$ ) the system was inherently unstable and would always either explode or crash.*

**Response:** Sorry for this misinterpretation, I corrected it in the revision.

**Comment 5:** *They proposed reverse-MM kinetics because it offered a mathematically simple equation to generate an asymptotic response; calling it reverse-MM kinetics gave a plausible rationale for using the equation, but the important thing was to get the needed general asymptotic shape. There was no fundamental chemical mechanism suggested in their use of the equation (even if one can be derived). With any mechanism to produce a system in which, as the enzyme pool increases, the activity per unit enzyme decreases, the system becomes potentially stable as it avoids the problem that if an enzyme returns more C over its lifetime than it cost to produce the enzyme then the enzyme pool would continue to grow and accelerate decomposition (and vice versa as if the enzyme never paid for itself, it would run down). There must be a variable marginal return on investment, but there can be multiple mechanisms that produce that pattern. It could be that as there are more enzymes, microbes become more likely to target them as a substrate, it may involve increased growth of “cheaters” as enzyme activities increase and the bioavailable substrate pool grows, it may even involve increasing diffusion path lengths and so slowing the link between enzyme production and substrate recovery. The model imperative of non-linear kinetics need not, in fact, ever involve the explicit reverse M-M assumption of enzymes competing for binding to potential reaction sites on*



*substrates (and so may not have a real  $K_{es}$  term in the sense implied by Tang's ECA model). In fact, multiple specific mechanisms may well be involved in creating the overall non-linearity that is required for model (and actual system) stability.*

**Response:** Thanks for this detailed explanation of how RMM was motivated.

Interestingly, the motivation that “*it [RMM] avoids the problem that if an enzyme returns more C over its lifetime than it cost to produce the enzyme then the enzyme pool would continue to grow and accelerate decomposition*” clearly points to the deficit of the MM kinetics resulting from its incomplete consideration of the substrate limitation as I discussed in the manuscript (which implies that if enzymes have a small turnover, the system will become unstable as enzyme concentration increases). Therefore, it seems that the mechanism, which works, for enzyme-substrate system could be (conceptually) scaled up to the overall system of carbons and microbes, indicating the scaling power of ECA as a first-principle based mathematical theory. This scaling property also seems to support the hypothesis that a single rate limiting “master reaction” controls the overall response of microbial activity (Johnson and Lewin, 1946), which is implied in the Monod kinetics. Therefore, considering the benefit from process scaling, I suggest approaches such as that used in deriving ECA should be preferred, even though the parameters in the derived equations are up-scaled versions of those measured in a tube.

**Comment 6:** *Such phenomena leave me uncertain just how useful a pure chemical kinetic derivation of these equations really is as they may describe the rough behavior of the system that is produced by several mechanisms working in parallel (or at odds with each other), such that the parameters that drive the equations are not clean chemical rate or equilibrium constants, but empirical terms to give the right rough shape to the overall responses. To some degree this is analogous to the difference between Michaelis-Menten enzyme kinetics and Monod microbial growth kinetics. The equations have identical structures but are fundamentally different: M-M kinetics is derived from 1<sup>st</sup> principles, while Monod growth has no such basis. The half-saturation constant in Monod growth is purely empirical. Would that be the case with the  $K_{es}$  term in Equation 12 in the ECA model if it were integrated into a soil C model? I think so. Might that make it a more difficult term to consider and apply? Maybe because to use the model in a biogeochemical model, it would have to be the empirically derived term rather than a real “affinity constant” that could be evaluated in a test-tube. But because it is an interaction term for the enzyme-substrate reaction it might be more sensitive to whether the non-linearity is being driven by substrate movement to the enzyme or to enzyme movement to the substrate. Please note, I'm not saying that would necessarily be the case (at least to within the bounds of experimental variation) but it remains a possibility. Such issues should be addressed more clearly by the authors, who I think somewhere should note the difference between a rate expression that is derived from fundamental chemical kinetics and one that may look the same but is only as an empirical approximation to force the model system to behave in reasonable, non-linear, patterns. I don't think that any such discussion need be long or involved, but I think it should be present.*

**Response:** I share your sympathy towards the complexity of the soil organic carbon decomposition problem that we are trying to model. However, I think adopting an approach as close as possible to the first principles is more valuable than a more

empirical approach, although they both require significant level of genius to work appropriately and sometimes may even appear similar (such as the MM kinetics and Monod kinetics). Compared to the empirical approach, the first-principle based approach would allow a more consistent and probably more mechanistic explanation to how the complexity of SOM decomposition could be scaled up and resolved by incrementally adding new identifiable processes one after another. For instance, the ECA approach would allow a consistent combination of microbe-substrate binding and substrate-mineral surface binding, such that it would naturally predict that k-strategist would be favored over the r-strategist with the increase of mineral adsorption, therefore both modelers and experimentalists could have a better clue to explain the measurements. Similarly, as we showed in Tang and Riley (2015), such combination enabled our model to explain many behaviors that are empirically observed, but otherwise require significant recalibration of the empirical approach for different experimental configurations or sometimes call for additional ad hoc parameterizations (e.g., the CENTURY-BGC module as we implemented in CLM4.5 requires a parameterization of decreasing decomposition rate with depth, which however will become ridiculous that by simply putting the same soil at different depth under same soil physical conditions will produce different respiration rates). Further, even it is arguable that the assumption underlines both the MM kinetics and Langmuir isotherm, or more generally, the law of mass action, are empirical, they all can be organized with a single statement, that there are two processes involved in the substrate uptake by consumers, i.e. find (or bind) the substrate and assimilate it. This simple assumption allows the consistent scaling of all mechanisms that are contributing to the SOM decomposition dynamics, therefore avoiding the necessity to propose a new empirical relationship when something new fails the model, such as replacing the MM kinetics with the RMM kinetics for enzyme degradation of SOM, because RMM is asymptotically more stable.

**Comment:** Minor points:

7665, 16: *This may be a linguistic battle I'll lose, but "uptake" is not a verb. Microbes take up a substrate.*

7677, 6: *"normalized" there's a typo*

7679, 15: *"very critical"? I'd delete "very."*

**Response:** Thanks for your careful examination. I corrected these issues.

## References

English, B. P., Min, W., van Oijen, A. M., Lee, K. T., Luo, G. B., Sun, H. Y., Cherayil, B. J., Kou, S. C., and Xie, X. S.: Ever-fluctuating single enzyme molecules: Michaelis-Menten equation revisited, *Nat Chem Biol*, 2, 87-94, 2006.

Johnson, F. H. and Lewin, I.: The Growth Rate of E-Coli in Relation to Temperature, Quinine and Coenzyme, *J Cell Compar Physl*, 28, 47-75, 1946.

Tang, J. Y. and Riley, W. J.: A total quasi-steady-state formulation of substrate uptake kinetics in complex networks and an example application to microbial litter decomposition, *Biogeosciences*, 10, 8329-8351, 2013.

Tang, J. Y. and Riley, W. J.: Weaker soil carbon-climate feedbacks resulting from microbial and abiotic interactions, *Nat Clim Change*, 5, 56-60, 2015.

1 **On the relationships between Michaelis-Menten kinetics, reverse**  
2 **Michaelis-Menten kinetics, Equilibrium Chemistry**  
3 **Approximation kinetics and quadratic kinetics**

4

5 **J. Y. Tang**

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8

9 **Abstract**

10 The Michaelis-Menten kinetics and the reverse Michaelis-Menten kinetics are two  
11 popular mathematical formulations used in many land biogeochemical models to describe  
12 how microbes and plants would respond to changes in substrate abundance. However, the  
13 criteria of when to use which of the two are often ambiguous. Here I show that these two  
14 kinetics are special approximations to the Equilibrium Chemistry Approximation kinetics,  
15 which is the first order approximation to the quadratic kinetics that solves the equation of  
16 enzyme-substrate complex exactly for a single enzyme single substrate biogeochemical  
17 reaction with the law of mass action and the assumption of quasi-steady-state for the enzyme-  
18 substrate complex and that the product genesis from enzyme-substrate complex is much  
19 slower than the equilibration between enzyme-substrate complexes, substrates and enzymes.  
20 In particular, I show that the derivation of the Michaelis-Menten kinetics does not consider  
21 the mass balance constraint of the substrate, and the reverse Michaelis-Menten kinetics does  
22 not consider the mass balance constraint of the enzyme, whereas both of these constraints are  
23 taken into account in deriving the Equilibrium Chemistry Approximation kinetics. By  
24 benchmarking against predictions from the quadratic kinetics for a wide range of substrate  
25 and enzyme concentrations, the Michaelis-Menten kinetics was found to persistently under-  
26 predict the normalized sensitivity  $\partial \ln v / \partial \ln k_2^+$  of the reaction velocity  $v$  with respect to the  
27 maximum product genesis rate  $k_2^+$ , persistently over-predict the normalized sensitivity  
28  $\partial \ln v / \partial \ln k_1^+$  of  $v$  with respect to the intrinsic substrate affinity  $k_1^+$ , persistently over-predict

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1 the normalized sensitivity  $\partial \ln v / \partial \ln [E]_T$  of  $v$  with respect to the total enzyme concentration  
2  $[E]_T$  and persistently under-predict the normalized sensitivity  $\partial \ln v / \partial \ln [S]_T$  of  $v$  with  
3 respect to the total substrate concentration  $[S]_T$ . Meanwhile, the reverse Michaelis-Menten  
4 kinetics persistently under-predicts  $\partial \ln v / \partial \ln k_2^+$  and  $\partial \ln v / \partial \ln [E]_T$ , and persistently over-  
5 predicts  $\partial \ln v / \partial \ln k_1^+$  and  $\partial \ln v / \partial \ln [S]_T$ . In contrast, the Equilibrium Chemistry  
6 Approximation kinetics always gives consistent predictions of  $\partial \ln v / \partial \ln k_2^+$ ,  $\partial \ln v / \partial \ln k_1^+$ ,  
7  $\partial \ln v / \partial \ln [E]_T$  and  $\partial \ln v / \partial \ln [S]_T$ , indicating that ECA-based models will be more  
8 calibratable if the modeled processes do obey the law of mass action. Since the Equilibrium  
9 Chemistry Approximation kinetics includes the advantages from both the Michaelis-Menten  
10 kinetics and the reverse Michaelis-Menten kinetics and it is applicable for almost the whole  
11 range of substrate and enzyme abundances, soil biogeochemical modelers therefore no longer  
12 need to choose when to use the Michaelis-Menten kinetics or the reverse Michaelis-Menten  
13 kinetics. I expect removing this choice ambiguity will make it easier to formulate more robust  
14 and consistent land biogeochemical models.

## 15 1 Introduction

16 The recent recognition that the typical turnover pool based soil carbon models cannot  
17 model the priming effect has revived the interest in developing microbe explicit soil  
18 biogeochemistry models. This has been manifested in a long list of microbial models that  
19 were published in the last few years (e.g., Schimel and Weintrub, 2003; Moorhead and  
20 Sinsabaugh, 2006; Allison et al., 2010; German et al., 2012; Wang et al., 2013; Wieder et al.,  
21 2013; Li et al., 2014; He et al., 2014; Riley et al., 2014; Xenakis and Williams, 2014; Tang  
22 and Riley, 2015; Sulman et al., 2015; Wieder et al., 2015). To build a microbial model, the  
23 substrate kinetics is fundamental as it describes the rate that microbes would take up a  
24 substrate and represents the first step towards describing how microbes would decompose the  
25 soil organic matter. Under the assumption that a single “master reaction” limits the growth of  
26 microbes (Johnson and Lewin, 1946), the substrate kinetics even completely determines the  
27 microbial dynamics as done in many models (e.g., the Monod model). Among the many  
28 mathematical formulations of substrate kinetics (see Tang and Riley (2013) for a review), the  
29 Michaelis-Menten (MM) kinetics is used mostly, because it succeeded in many applications

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1 ever since its birth in the early 20<sup>th</sup> century (Michaelis and Menten, 1913). However, Schimel  
2 and Weintraub (2003) proposed in their study that the decomposition rate should vary more  
3 like an asymptotic function of enzyme abundance such that the Reverse Michaelis-Menten  
4 (RMM) kinetics would better model the soil carbon decomposition dynamics. The proposal of  
5 RMM kinetics was motivated by the empirical observation that, as enzyme concentration  
6 increases, microbial growth cannot increase continuously without a limit, therefore some  
7 dynamic feedbacks between the different components must stabilize the system. In contrast,  
8 the MM kinetics predicts that substrate degradation is proportional to enzyme concentration,  
9 and therefore, like the linear kinetics as used in Schimel and Weintraub (2003), it will predict  
10 unstable decomposition dynamics. The success by Schimel and Weintraub has led to a  
11 number of studies to use the RMM kinetics as the backbone of their microbial models,  
12 including Moorhead and Sinsabaugh (2006)'s model of litter decomposition, Drake et al.  
13 (2013)'s model for root priming, Waring et al. (2013)'s model for change in microbial  
14 community structure in soil carbon and nitrogen cycling, and Averill (2014)'s model for  
15 change in microbial allocation in soil carbon decomposition.

16 Wang and Post (2013) pointed out that both the MM kinetics and the RMM kinetics  
17 (although the latter is empirical) are special approximations to the quadratic kinetics that  
18 exactly solves for the enzyme-substrate complex under the quasi-steady-state approximation  
19 (QSSA), which states that the enzyme-substrate complexes are in instantaneous equilibrium  
20 with enzyme and substrate concentrations (Borghans et al., 1996). They further concluded  
21 that the MM kinetics is applicable when the substrate concentration far exceeds the enzyme  
22 concentration, and the RMM kinetics is applicable when either the enzyme concentration far  
23 exceeds the substrate concentration or vice versa. The condition for the MM kinetics to be  
24 applicable as provided by Wang and Post (2013) was however much narrower than that was  
25 proposed in some earlier studies. For instance, Borghans et al. (1996) showed that the MM  
26 kinetics is a good approximation to the quadratic kinetics when the enzyme concentration is  
27 far smaller than the sum of the substrate concentration and the Michaelis-Menten constant  
28 (Palsson, 1987; Segel, 1988; Segel and Slemrod, 1989). To handle enzyme-substrate  
29 interactions under high enzyme concentrations, Borghans et al. (1996) proposed the total  
30 quasi-steady-state approximation (tQSSA) and obtained a substrate kinetics that was a special  
31 case of the later proposed Equilibrium Chemistry Approximation kinetics by Tang and Riley  
32 (2013). Tang and Riley (2013) applied the law of mass action with tQSSA and derived the

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1 ECA kinetics to describe the formation of enzyme-substrate complexes in a network of an  
2 arbitrary number of enzymes and substrates.

3 The consistent application of mathematical formulations to describe a dynamic system  
4 is critical for the model to successfully resolve the empirical measurements that observe the  
5 dynamic system. This consistency requirement has been raised in several studies using  
6 microbe explicit models. For instance, Maggi and Riley (2009) have found the MM kinetics  
7 has to be revised to resolve the evolution of  $\delta^{15}\text{N-N}_2\text{O}$  in their data of nitrification and  
8 denitrification. Druhan et al. (2012) later used Maggi and Riley (2009)'s revision to obtain an  
9 improved modeling of the  $\delta^{34}\text{S}$  data collected in the acetate-enabled uranium bioremediation  
10 at the US Department of Energy's Rifle Integrated Field Research Challenge site. Tang and  
11 Riley (2013) showed that the MM kinetics failed to resolve the evolution of lignocellulose  
12 index during a litter decomposition experiment. I was not able to find any example of using  
13 the RMM kinetics to model the kinetic isotope fractionation. However, because the RMM  
14 kinetics is a linear function of the substrate concentration, its application for modeling kinetic  
15 isotope fractionation will be doomed inevitably. Therefore, a substrate kinetics that merges  
16 the advantages from both the MM kinetics and the RMM kinetics would be a better choice for  
17 developing robust microbial models.

18 The call for a substrate kinetics that can consistently work across a wide range of  
19 substrate and enzyme (or more broadly competitor) concentrations becomes more imperative  
20 when the land biogeochemical models are required to resolve plant-microbe interactions. In  
21 plant-microbe interactions, both substrates and competitors evolve constantly and their  
22 concentration ratios could vary orders of magnitudes. For instance, when a soil is seriously  
23 nitrogen limited, the aqueous nitrogen concentration is much lower than the volumetric  
24 density of competitors and substrate uptake may follow more linearly with respect to the  
25 substrate concentration and be of an asymptotic function of competitors as described by the  
26 RMM kinetics. However when a large dose of fertilizer is added, the soil quickly becomes  
27 nitrogen saturated, such that the uptake dynamics would follow more linearly with respect to  
28 the variation of competitors (or enzymes) as represented in the MM kinetics. To consistently  
29 model the soil nitrogen dynamics that fluctuates between status of nitrogen limitation and  
30 nitrogen saturation, one therefore has to constantly choose between the MM kinetics and  
31 RMM kinetics, making a consistent mathematical formulation theoretically impossible.  
32 Therefore, an approach that includes the advantages from both the MM kinetics and RMM

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1 kinetics will significantly advance our capability in modeling soil biogeochemical processes.  
2 Fortunately, such kinetics (aka the ECA kinetics) was already derived in Tang and Riley  
3 (2013), but my coauthor and I did not give a theoretical analysis for the relationships between  
4 MM kinetics, RMM kinetics and the ECA kinetics, nor did we explain how the parametric  
5 sensitivity would vary depending on the choice of substrate kinetics and whether the ECA  
6 kinetics is superior across the whole range of feasible kinetic parameters. Because all model  
7 calibration methods either explicitly or implicitly rely on the parametric sensitivity to tune  
8 model predictions with respect to observations (e.g. Tang and Zhuang, 2009; Zhu and  
9 Zhuang, 2014), correct parametric sensitivity of the model formulation is a requisite for  
10 delivering a robust model. An analysis of the differences in their predicted parametric  
11 sensitivities will also help reveal the pitfalls that may exist in biogeochemical models that rely  
12 on MM kinetics (Allison et al., 2010) or RMM kinetics (e.g. Averill, 2014) or the  
13 combination of the two (e.g. Sihi et al., 2015), when the model is otherwise benchmarked  
14 against its equilibrium chemistry based formulation that solves the biogeochemical system  
15 exactly under the tQSSA (readers please refer to Tang and Riley (2013) for a thorough  
16 discussion on why the equilibrium chemistry formulation should be the benchmark for models  
17 based on MM kinetics, RMM kinetics and ECA kinetics).

18 In this study, I first review how the ECA kinetics could be derived from the quadratic  
19 kinetics and how the MM kinetics and the RMM kinetics could be derived from the ECA  
20 kinetics or directly from the equilibrium chemistry formulation of the enzyme-substrate  
21 interaction. Then I analyze how accurate the MM kinetics, the RMM kinetics and the ECA  
22 kinetics could approximate the parametric sensitivity, as one would derive from the quadratic  
23 kinetics that is exact for the one enzyme and one substrate biogeochemical reaction. Based on  
24 these analyses, I finally give recommendations on how to obtain more robust microbial  
25 models for soil biogeochemical modeling. Note, although the following analysis is for a  
26 single enzyme and single substrate system in an aqueous solution, the results are applicable to  
27 a wide range of problems, including predator-prey, microbial growth, Langmuir adsorption  
28 and any process that can be appropriately formulated as an equilibrium binding problem  
29 (Tang and Riley, 2013).

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## 1 2 The Mathematical relationship between different kinetics

2 Below I first review how one could obtain the quadratic kinetics under the QSSA for a  
3 biogeochemical reaction that involves one enzyme and one substrate. Then I show how one  
4 could derive the ECA kinetics, the MM kinetics and the RMM kinetics.

5 The biogeochemical reaction of the system is



6 where  $E$ ,  $S$ ,  $ES$  and  $P$  are, respectively, enzyme, substrate, enzyme-substrate complex and  
7 product. The three kinetic parameters are intrinsic substrate affinity  $k_1^+$  ( $\text{m}^3 \text{mol}^{-1} \text{s}^{-1}$ ),  
8 backward enzyme-substrate dissociation constant  $k_1^-$  ( $\text{s}^{-1}$ ) and product genesis rate  $k_2^+$  ( $\text{s}^{-1}$ ).

9 By the law of mass action, the governing equations for biogeochemical reaction (1) are

$$\frac{d[E]}{dt} = -k_1^+[S][E] + (k_1^- + k_2^+)[ES] \quad (2)$$

$$\frac{d[S]}{dt} = -k_1^+[S][E] + k_1^-[ES] \quad (3)$$

$$\frac{d[ES]}{dt} = k_1^+[S][E] - (k_1^- + k_2^+)[ES] \quad (4)$$

$$\frac{d[P]}{dt} = k_2^+[ES] \quad (5)$$

10 Here and below, I use  $[ ]$  to designate the concentration ( $\text{mol m}^{-3}$ ) of a given state variable.

11 Under the QSSA, Eq. (4) is approximated as

$$[S][E] = K_{ES}[ES] \quad (6)$$

12 where  $K_{ES} = (k_1^- + k_2^+)/k_1^+$  ( $\text{mol m}^{-3}$ ) is the Michaelis-Menten constant.

13 For a small temporal window when the amount of the product is negligible, it holds

14 that  $[P] \ll [ES] + [S] = [S]_T$ , then  $[ES]$  could be solved from Eq. (6) under the constraints

$$[ES] + [E] = [E]_T \quad (7)$$

$$[ES] + [S] = [S]_T \quad (8)$$

1 By solving  $[E]$  from Eq. (7),  $[S]$  from Eq. (8), and entering the results into Eq. (6),  
 2 one then obtains the quadratic equation

$$[ES]^2 - (K_{ES} + [E]_T + [S]_T)[ES] + [E]_T[S]_T = 0 \quad (9)$$

3 Therefore, if one applies the quadratic formula to Eq. (9) and takes the physically  
 4 meaningful solution,  $[ES]$  is then found as

$$[ES] = \frac{(K_{ES} + [E]_T + [S]_T)}{2} \left( 1 - \sqrt{1 - \frac{4[E]_T[S]_T}{(K_{ES} + [E]_T + [S]_T)^2}} \right) \quad (10)$$

## 5 2.1 The Equilibrium Chemistry Approximation kinetics

6 To obtain the ECA formulation of the enzyme-substrate complex, one assumes

$$\varepsilon = \frac{[E]_T[S]_T}{(K_{ES} + [E]_T + [S]_T)^2} \ll 1 \quad (11)$$

7 Then by substitution of the first order approximation  $\sqrt{1 - 4\varepsilon} \approx (1 - 2\varepsilon)$  into the square  
 8 root term of Eq. (10), the ECA formulation of  $[ES]$  is obtained

$$[ES] = \frac{[E]_T[S]_T}{K_{ES} + [E]_T + [S]_T} \quad (12)$$

9 The application of Eq. (12) implies

$$\frac{d[S]_T}{dt} = -k_2^+ [ES] \quad (13)$$

10 which together with the QSSA forms the tQSSA (Borghans et al., 1996).

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## 1 2.2 The Michaelis-Menten kinetics

2 The MM kinetics can be derived in two different approaches. In the first approach, by  
3 assuming  $K_{ES} + [S]_T \gg [E]_T$ , Eq. (12) gives the MM formulation of  $[ES]$

$$[ES] \approx \frac{[E]_T [S]_T}{K_{ES} + [S]_T} \quad (14)$$

4 In the second approach, one solves  $[ES]$  from Eq. (6) and (7) and obtains

$$[ES] = \frac{[E]_T [S]}{K_{ES} + [S]} \quad (15)$$

5 Note  $[S] = [S]_T - [ES] < [S]_T$ , and because  $[ES]$  is a monotonically increasing function of  
6  $[S]$ ,  $[ES]$  computed from Eq. (14) will be greater than that from Eq. (15). However, almost  
7 all existing applications do not differentiate between Eqs. (14) and (15). The strict application  
8 of Eq. (14) requires the substrate evolution to be computed by the tQSSA form Eq. (13),  
9 whereas under the QSSA the strict application of Eq. (15) requires

$$\frac{d[S]}{dt} = -k_2 [ES] \quad (16)$$

10 When  $[S]$  is low, or when enzyme concentration  $[E]_T$  is high, equating  $[S]$  to  $[S]_T$  and  
11 ignoring the contribution of  $[E]_T$  in calculating the enzyme-substrate complex  $[ES]$  will  
12 cause significant error in computing the parametric sensitivities as I will show in section 3.

13 The sufficient condition  $K_{ES} + [S]_T \gg [E]_T$  (which always leads to  $\varepsilon \ll 1$ , the  
14 sufficient condition to derive the ECA kinetics) for the MM kinetics to be applicable was well  
15 recognized in early studies; however, it was often misinterpreted as  $[S]_T \gg [E]_T$  (see a  
16 discussion in Borghans et al. (1996)). Yet, more importantly, I note that the derivation of the  
17 MM kinetics does not take into account the mass balance constraint for substrate (Eq. (8)). As  
18 I will show in section 3, the negligence of mass balance constraint for substrate will lead to

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1 poor predictions of parametric sensitivity by the MM kinetics when benchmarked with the  
2 quadratic kinetics.

### 3 2.3 The reverse Michaelis-Menten kinetics

4 There are also two approaches to derive the RMM kinetics. In the first approach, one  
5 assumes  $K_{ES} + [E]_T \gg [S]_T$ , then from Eq. (12), obtains the RMM formulation of  $[ES]$

$$[ES] \approx \frac{[E]_T [S]_T}{K_{ES} + [E]_T} \quad (17)$$

6 In the second approach, one solves  $[ES]$  from Eqs. (6) and (8)

$$[ES] = \frac{[E][S]_T}{K_{ES} + [E]} \quad (18)$$

7 Note  $[E] = [E]_T - [ES] < [E]_T$ , and because  $[ES]$  is a monotonically increasing function of  
8  $[E]$ ,  $[ES]$  calculated from Eq. (17) will be greater than that from Eq. (18). Like the MM  
9 kinetics, existing applications have treated Eq. (17) and (18) as equivalent.

10 Here the condition  $K_{ES} + [E]_T \gg [S]_T$  (which always leads to  $\varepsilon \ll 1$ , the sufficient  
11 condition to derive the ECA kinetics) for the RMM kinetics to hold is more general than the  
12 condition  $[E]_T \gg [S]_T$  proposed in Wang and Post (2013). I also note that the derivation of  
13 the RMM kinetics does not take into account the mass balance constraint for enzyme (Eq.  
14 (7)). This negligence of the mass balance constraint for enzyme will lead the RMM kinetics to  
15 predict poor parametric sensitivities when benchmarked with the quadratic kinetics.

### 16 3 Parametric sensitivity analyses

17 I below analyze the sensitivities of the reaction velocity with respect to the four  
18 parameters as predicted by the four kinetics. The four parameters are (1) maximum product  
19 genesis rate  $k_2^+$ ; (2) intrinsic substrate affinity  $k_1^+$ ; (3) the total enzyme concentration  $[E]_T$   
20 and (4) the total substrate concentration  $[S]_T$ . The reaction velocities predicted by the four  
21 different kinetics are, respectively, for the quadratic kinetics,

$$v_{QD} = \frac{k_2^+ (K_{ES} + [E]_T + [S]_T)}{2} \left( 1 - \sqrt{1 - \frac{4[E]_T[S]_T}{(K_{ES} + [E]_T + [S]_T)^2}} \right) \quad (19)$$

1 for the **ECA** kinetics,

$$v_{ECA} = \frac{k_2^+ [E]_T [S]_T}{K_{ES} + [E]_T + [S]_T} \quad (20)$$

2 for the **MM** kinetics,

$$v_{MM} = \frac{k_2^+ [E]_T [S]_T}{K_{ES} + [S]_T} \quad (21)$$

3 **and**, for the **RMM** kinetics

$$v_{RMM} = \frac{k_2^+ [E]_T [S]_T}{K_{ES} + [E]_T} \quad (22)$$

4 In evaluating the parametric sensitivity, I made the conventional assumption that  
 5  $k_1^- \ll k_2^+$  to obtain a better presentation of the results (although excluding this assumption will  
 6 not change the conclusion below). This assumption leads to  $K_{ES} = k_2^+ / k_1^-$ , which states that  
 7 the apparent substrate affinity  $1/K_{ES}$  is a linearly decreasing function of  $k_2^+$ , a relationship  
 8 that has been used to characterize the K-r tradeoff (e.g. Litchman et al., 2008). Because  $K_{ES}$  is  
 9 a function of  $k_2^+$ , the intrinsic affinity  $k_1^-$  better describes the substrate affinity for the  
 10 enzymes.

11 In addition, to simplify the presentation, I define  $y = K_{ES} + [E]_T + [S]_T$  and  
 12  $x = 4[E]_T[S]_T / y^2$ . Since the derivations for the MM and RMM kinetics related parametric  
 13 sensitivities could be derived from the ECA predictions straightforwardly, I only provide  
 14 details to derive the results for the quadratic and ECA related parametric sensitivities  
 15 (Appendix A and B). Nevertheless, to help the readers to visualize the differences in the  
 16 predicted parametric sensitivities by using different kinetics, I have summarized the

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1 comparison in four different figures: [Figure 1](#) for  $k_2^+$ , [Figure 2](#) for  $k_1^+$ , [Figure 3](#) for  $[E]_T$ , and  
2 [Figure 4](#) for  $[S]_T$ . All sensitivities are evaluated over the 2D normalized substrate-enzyme  
3 concentration domain  $[0.001,1000] \times [0.001,1000]$ , with both  $[E]_T$  and  $[S]_T$  normalized  
4 by  $K_{ES}$ . In addition, because the quadratic kinetics is exact under the QSSA, its predictions  
5 are used to benchmark the predictions made by the ECA kinetics, MM kinetics and RMM  
6 kinetics (see (d) panels in the figures). For comparison between predictions by the ECA  
7 kinetics and the quadratic kinetics, I plotted the normalized sensitivities as 2D functions of the  
8 normalized substrate  $[S]_T/K_{ES}$  and  $[E]_T/K_{ES}$  (see (a) and (b) panels in the figures), and  
9 evaluated their differences using the index  $(a_{QD} - a_{ECA}) / (a_{QD} + a_{ECA})$  (see (c) panels in the  
10 figures), where the subscripts *QD* and *ECA* indicate, respectively, sensitivities predicted by  
11 the quadratic kinetics and the ECA kinetics.

12 In all the analyses below, I represent the parametric sensitivity using the normalized  
13 form  $\partial \ln v / \partial \ln s$  to remove the unit dependency of the results. The normalized sensitivity  
14 represents the relative change of reaction velocity  $v$  in response to a relative change in  
15 parameter  $s$ , where  $s$  could be any of the four parameters being analyzed.

### 16 3.1 Reaction velocity vs. $k_2^+$

17 The normalized sensitivity of the reaction velocity vs.  $k_2^+$  are, respectively, for the  
18 quadratic kinetics.

$$\frac{k_2^+}{v_{QD}} \frac{\partial v_{QD}}{\partial k_2^+} = 1 + \frac{K_{ES}}{y} - \frac{K_{ES}}{y} (1 - \sqrt{1-x})^{-1} (1-x)^{-1/2} x \quad (23)$$

19 for the ECA kinetics,

$$\frac{k_2^+}{v_{ECA}} \frac{\partial v_{ECA}}{\partial k_2^+} = 1 - \frac{K_{ES}}{K_{ES} + [E]_T + [S]_T} \quad (24)$$

20 for the MM kinetics,

$$\frac{k_2^+}{v_{MM}} \frac{\partial v_{MM}}{\partial k_2^+} = 1 - \frac{K_{ES}}{K_{ES} + [S]_T} \quad (25)$$

1 and, for the RMM kinetics,

$$\frac{k_2^+}{v_{RMM}} \frac{\partial v_{RMM}}{\partial k_2^+} = 1 - \frac{K_{ES}}{K_{ES} + [E]_T} \quad (26)$$

2 From above, it is observed that both the MM kinetics and the RMM kinetics predict a  
 3 less variable and lower parametric sensitivity than does the ECA kinetics, because the ECA  
 4 kinetics predicts a more variable and larger denominator in the second term (in Eq.(24)) as  
 5 compared to that by the MM kinetics (Eq. (25)) and the RMM kinetics (Eq. (26)). Large  
 6 deviations between predicted sensitivities by the MM kinetics and the ECA kinetics are  
 7 expected at high enzyme concentrations, whereas large deviations between predictions by the  
 8 RMM kinetics and the ECA kinetics are expected at high substrate concentrations. Predicted  
 9 sensitivities by the MM kinetics and RMM kinetics are also smaller than that by the quadratic  
 10 kinetics (green and black dots in Figure 1d). In contrast, the ECA kinetics consistently  
 11 captures the variability of the normalized sensitivity, with some over-estimation (but the  
 12 relative difference is no greater than 5%) under moderate enzyme and substrate  
 13 concentrations (Figure 1c), where the normalized sensitivity is, however, small or moderate  
 14 (Figure 1a).

### 15 3.2 Reaction velocity vs. $k_1^+$

16 The normalized sensitivity of the reaction velocity vs.  $k_1^+$  are, respectively, for the  
 17 quadratic kinetics,

$$\frac{k_1^+}{v_{QD}} \frac{\partial v_{QD}}{\partial k_1^+} = -\frac{K_{ES}}{y} + \frac{K_{ES}}{y} (1-x)^{-1/2} (1-\sqrt{1-x})^{-1} x \quad (27)$$

18 for the ECA kinetics,

$$\frac{k_1^+}{v_{ECA}} \frac{\partial v_{ECA}}{\partial k_1^+} = \frac{K_{ES}}{K_{ES} + [E]_T + [S]_T} \quad (28)$$

19 for the MM kinetics,

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$$\frac{k_1^+}{v_{MM}} \frac{\partial v_{MM}}{\partial k_1^+} = \frac{K_{ES}}{K_{ES} + [S]_T} \quad (29)$$

1 and, for the RMM kinetics,

$$\frac{k_1^+}{v_{RMM}} \frac{\partial v_{RMM}}{\partial k_1^+} = \frac{K_{ES}}{K_{ES} + [E]_T} \quad (30)$$

2 From Eqs. (28)-(30), it is inferred that both the MM kinetics and the RMM kinetics  
 3 predict a less variable and higher normalized sensitivity with respect to  $k_1^+$  than does the ECA  
 4 kinetics. Large difference between predicted sensitivities by the ECA kinetics and the MM  
 5 kinetics are expected at high enzyme concentrations, whereas large difference between  
 6 predicted sensitivities by the ECA kinetics and the RMM kinetics are expected at high  
 7 substrate concentrations. The predicted sensitivities by the MM kinetics and the RMM  
 8 kinetics are also lower than that by the quadratic kinetics (Figure 2d), whereas the ECA  
 9 kinetics predicts consistent parametric sensitivity for the wide range of enzyme and substrate  
 10 concentrations (Figure 2). The under-predicted sensitivity by the ECA kinetics is significant  
 11 only at high substrate and high enzyme concentrations (Figure 2c), where the parametric  
 12 sensitivity is close to zero (Figure 2a and Figure 2b).

### 13 3.3 Reaction velocity vs. $[E]_T$

14 The normalized sensitivity of the reaction velocity vs.  $[E]_T$  are, respectively, for the  
 15 quadratic kinetics

$$\frac{[E]_T}{v_{QD}} \frac{\partial v_{QD}}{\partial [E]_T} = \frac{[E]_T}{y} + \frac{[E]_T}{y} (1 - \sqrt{1-x})^{-1} (1-x)^{-1/2} \times \left( \frac{2[S]_T}{y} - x \right) \quad (31)$$

16 for the ECA kinetics

$$\frac{[E]_T}{v_{ECA}} \frac{\partial v_{ECA}}{\partial [E]_T} = 1 - \frac{[E]_T}{K_{ES} + [E]_T + [S]_T} \quad (32)$$

17 for the MM kinetics

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$$\frac{[E]_T}{v_{MM}} \frac{\partial v_{MM}}{\partial [E]_T} = 1 \quad (33)$$

1 | and, for the RMM kinetics,

$$\frac{[E]_T}{v_{RMM}} \frac{\partial v_{RMM}}{\partial [E]_T} = 1 - \frac{[E]_T}{K_{ES} + [E]_T} \quad (34)$$

2 | From above, it is observed that the MM kinetics predicts a constant normalized  
 3 | sensitivity of the reaction velocity with respect to the total enzyme concentration  $[E]_T$ . The  
 4 | RMM kinetics predicts the normalized sensitivity as a monotonically decreasing function of  
 5 | the normalized enzyme concentration  $[E]_T/K_{ES}$ . The predicted sensitivity by the ECA  
 6 | kinetics is a function of both the normalized substrate concentration  $[S]_T/K_{ES}$  and the  
 7 | normalized enzyme concentration  $[E]_T/K_{ES}$ . Compared to predictions by the quadratic  
 8 | kinetics, the MM kinetics persistently over-estimates the parametric sensitivity (green dots in  
 9 | Figure 3d), whereas the RMM kinetics persistently under-estimates the parametric sensitivity  
 10 | (black dots in Figure 3d). The ECA predicted sensitivity is largely consistent with that by the  
 11 | quadratic kinetics (Figure 3), albeit with some significant deviation, in regions of very high  
 12 | substrate and enzyme concentrations (Figure 3c), where the parametric uncertainty is  
 13 | moderate or low (Figure 3a and Figure 3b).

### 14 | 3.4 Reaction velocity vs. $[S]_T$

15 | The normalized sensitivity of the reaction velocity vs.  $[S]_T$  are, respectively, for the  
 16 | quadratic kinetics

$$\frac{[S]_T}{v_{QD}} \frac{\partial v_{QD}}{\partial [S]_T} = \frac{[S]_T}{y} + \frac{[S]_T}{y} (1 - \sqrt{1-x})^{-1} (1-x)^{-1/2} \times \left( \frac{2[E]_T}{y} - x \right) \quad (35)$$

17 | for the ECA kinetics

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$$\frac{[S]_T}{v_{ECA}} \frac{\partial v_{ECA}}{\partial [S]_T} = 1 - \frac{[S]_T}{K_{ES} + [E]_T + [S]_T} \quad (36)$$

1 for the MM kinetics,

$$\frac{[S]_T}{v_{MM}} \frac{\partial v_{MM}}{\partial [S]_T} = 1 - \frac{[S]_T}{K_{ES} + [S]_T} \quad (37)$$

2 and, for the RMM kinetics,

$$\frac{[S]_T}{v_{RMM}} \frac{\partial v_{RMM}}{\partial [S]_T} = 1 \quad (38)$$

3 Because  $[S]_T$  and  $[E]_T$  are symmetric in the quadratic kinetics and the ECA kinetics,  
 4 the predicted normalized sensitivity of the reaction velocity with respect to the total substrate  
 5 concentration  $[S]_T$  mirrors that of  $[E]_T$  along the lower left to upper right diagonal ([Figure](#)  
 6 [3](#) vs. [Figure 4](#)). Such symmetric relationships also exist in predictions by the MM kinetics and  
 7 the RMM kinetics, however, the MM kinetics persistently under-predicts the normalized  
 8 sensitivity of the reaction velocity with respect to  $[S]_T$ , and the RMM kinetics predicts a  
 9 constant sensitivity (Eq. (38)). The ECA kinetics once again predicts consistent parametric  
 10 sensitivity when compared with the quadratic kinetics.

#### 11 4 Discussions and conclusions

12 From the above analyses, I showed that the ECA kinetics is a better approximation to  
 13 the quadratic kinetics, which, obtained from the law of mass action and the quasi-stead-state  
 14 approximation, is the exact solution to the governing equation of substrate-enzyme  
 15 interaction. In contrast, the Michaelis-Menten kinetics and the reverse Michaelis-Menten  
 16 kinetics are inferior in approximating the quadratic kinetics over the wide range of enzyme  
 17 and substrate concentrations. The worse performances of the MM kinetics than the ECA  
 18 kinetics in approximating the quadratic kinetics stems from the negligence of mass balance  
 19 constraint of the substrate during the derivation of the MM kinetics; while the worse  
 20 performance of the RMM kinetics in approximating the quadratic kinetics is caused by the  
 21 negligence of mass balance constraint of the enzyme during the derivation of the RMM  
 22 kinetics. The failure to consider the mass balance constraints for both enzyme and substrate

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1 | during their derivations caused the MM kinetics and the RMM kinetics to predict significantly  
2 | biased normalized sensitivity of the reaction velocity with respect to the two kinetic  
3 | parameters  $k_1^+$  and  $k_2^+$ , the total enzyme concentration  $[E]_T$  and the total substrate  
4 | concentration  $[S]_T$ . Although being a first order approximation to the quadratic kinetics  
5 | under the assumption that  $[E]_T[S]_T \ll (K_{ES} + [E]_T + [S]_T)^2$ , because it considers the mass  
6 | balance for both substrate and enzyme, the ECA kinetics predicts consistent parametric  
7 | sensitivity with that by the quadratic kinetics over the wide range of normalized substrate and  
8 | enzyme concentrations.

9 | In modeling complex soil biogeochemical dynamics, the consistency between used  
10 | kinetics and equilibrium chemistry formulation of the relationships between enzymes,  
11 | substrates and enzyme-substrate complexes might be critical (Tang and Riley, 2013), but it  
12 | has been unfortunately under-appreciated in many previous studies. In Tang and Riley (2013),  
13 | it was shown that for a system involving three microbes competitively decompose three  
14 | carbon substrates, the MM kinetics failed wildly even with industrious calibration (see their  
15 | Figure 12). In an earlier study, Moorhead and Sinsabaugh (2006) have to prescribe the  
16 | relative decomposition between lignin and cellulose in order to resolve the lignocellulose  
17 | index dynamics. The ECA kinetics was able to consistently resolve the lignin-cellulose  
18 | dynamics during the litter decomposition by that it explicitly considers the mass balance  
19 | constraints for each of the substrates and enzymes (or, effectively, abundance of competitors;  
20 | Tang and Riley, 2013). The success of ECA kinetics and the failure of MM kinetics in studies  
21 | referred above can both be traced back to their capability in approximating the actual  
22 | parametric sensitivities of the specific dynamic system. Because all model calibration  
23 | techniques rely on model's parametric sensitivity to obtain improved agreement between  
24 | model predictions and measurements, wrong parametric sensitivity as formulated in the  
25 | adopted substrate kinetics would result in a non-calibratable or poorly calibratable model,  
26 | which could be manifested as systematic model biases or completely unreasonable model  
27 | predictions. This explained well why the MM kinetics based model in Tang and Riley (2013)  
28 | failed wildly even with intensive Bayesian model calibration.

29 | Therefore if the ecological dynamics involved in substrates processing by microbes,  
30 | does approximately obey the law of mass action and the total-quasi-steady-state  
31 | approximation (as it is already implied in any microbe explicit model that uses the MM

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1 kinetics or the RMM kinetics), then the analytically tractable ECA kinetics is a much more  
 2 powerful and mathematically more consistent tool than the popular MM kinetics and RMM  
 3 kinetics that are currently used in many microbial models. Indeed, a recent application (Zhu  
 4 and Riley, 2015) indicated that by representing plant-microbe competition of soil mineral  
 5 nitrogen using the ECA kinetics, the predicted global nitrogen dynamics became much more  
 6 consistent with that inferred from the  $\delta^{15}\text{N}$  isotopic data (Houlton et al., 2015). The ECA  
 7 kinetics was also found to satisfyingly model the plant-microbe competitions for phosphorus  
 8 and mineral nitrogen at several fertilized sites (Zhu et al., 2015) and predicted consistent  
 9 vertical nitrogen uptake profile measured at an alpine meadow ecosystem (Zhu et al. in prep).  
 10 Theoretically, because either the MM kinetics or the RMM kinetics works only in a small  
 11 subdomain of the parameters that are used in the original quadratic kinetics, models based on  
 12 MM kinetics or RMM kinetics may likely have much lower predictive capability than that is  
 13 implied in the mechanisms that the models are trying to represent (e.g. the law of mass action,  
 14 which is the foundation to all substrate kinetics). I therefore recommend modelers to use the  
 15 ECA kinetics to describe the substrate uptake processes in modeling microbe regulated  
 16 biogeochemical processes. As I showed above, with the same number of parameters as one  
 17 would use with either the MM kinetics or the RMM kinetics, the ECA kinetics achieved better  
 18 accuracy in approximating the exact quadratic kinetics for a biogeochemical reaction that  
 19 involves a single enzyme and a single substrate. The superior performance of ECA is also true  
 20 for systems that involve many substrates and many enzymes (Tang and Riley, 2013), which  
 21 are much more common in the natural environment that we are trying to model. Last and  
 22 more importantly, the ECA kinetics could save the modelers from the pain of when to use the  
 23 MM kinetics or the RMM kinetics to represent a soil that fluctuates between status of nutrient  
 24 limitation and nutrient saturation, for which neither the MM kinetics nor the RMM kinetics is  
 25 (but ECA is) theoretically consistent with the law of mass action, the best theory we have for  
 26 modeling biogeochemical reactions.

27

28 **Appendix A: Derivation of parametric sensitivities (Eqs. (23), (27), (31) and (35)) for**  
 29 **the quadratic kinetics**

30 Using the definition of  $y = K_{ES} + [E]_T + [S]_T$  and  $x = 4[E]_T[S]_T/y^2$ , one has the  
 31 following results

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$$1 \quad v_{QD} = \frac{k_2^+ y}{2} (1 - \sqrt{1-x}) \quad (\text{A-1})$$

$$2 \quad \frac{\partial x}{\partial k_1^+} = \frac{8[E]_T [S]_T}{(K_{ES} + [E]_T + [S]_T)^3} \frac{K_{ES}}{k_1^+} = \frac{8[E]_T [S]_T}{y^3} \frac{K_{ES}}{k_1^+} \quad (\text{A-2})$$

$$3 \quad \frac{\partial x}{\partial k_2^+} = -\frac{8[E]_T [S]_T}{(K_{ES} + [E]_T + [S]_T)^3} \frac{1}{k_1^+} = -\frac{8[E]_T [S]_T}{y^3} \frac{1}{k_1^+} \quad (\text{A-3})$$

$$4 \quad \frac{\partial x}{\partial [E]_T} = \frac{4[S]_T}{(K_{ES} + [S]_T + [E]_T)^2} - \frac{8[E]_T [S]_T}{(K_{ES} + [S]_T + [E]_T)^3} = \frac{4[S]_T}{y^2} - \frac{2x}{y} \quad (\text{A-4})$$

$$5 \quad \frac{\partial x}{\partial [S]_T} = \frac{4[E]_T}{(K_{ES} + [S]_T + [E]_T)^2} - \frac{8[E]_T [S]_T}{(K_{ES} + [S]_T + [E]_T)^3} = \frac{4[E]_T}{y^2} - \frac{2x}{y} \quad (\text{A-5})$$

$$6 \quad \frac{\partial \sqrt{1-x}}{\partial k_1^+} = -\frac{1}{2} (1-x)^{-1/2} \frac{\partial x}{\partial k_1^+} \quad (\text{A-6})$$

$$7 \quad \frac{\partial \sqrt{1-x}}{\partial k_2^+} = -\frac{1}{2} (1-x)^{-1/2} \frac{\partial x}{\partial k_2^+} \quad (\text{A-7})$$

$$8 \quad \frac{\partial \sqrt{1-x}}{\partial [E]_T} = -\frac{1}{2} (1-x)^{-1/2} \frac{\partial x}{\partial [E]_T} \quad (\text{A-8})$$

$$9 \quad \frac{\partial \sqrt{1-x}}{\partial [S]_T} = -\frac{1}{2} (1-x)^{-1/2} \frac{\partial x}{\partial [S]_T} \quad (\text{A-9})$$

$$10 \quad \frac{\partial y}{\partial k_1^+} = \frac{\partial K_{ES}}{\partial k_1^+} = -\frac{K_{ES}}{k_1^+} \quad (\text{A-10})$$

$$1 \quad \frac{\partial y}{\partial k_2^+} = \frac{\partial K_{ES}}{\partial k_2^+} = \frac{1}{k_1^+} \quad (\text{A-11})$$

$$2 \quad \frac{\partial y}{\partial [E]_r} = \frac{\partial y}{\partial [S]_r} = 1 \quad (\text{A-12})$$

3 Then from Eq. (A-1), one has

$$4 \quad \frac{\partial v_{QD}}{\partial k_2^+} = \frac{y}{2} (1 - \sqrt{1-x}) + \frac{k_2^+}{2} (1 - \sqrt{1-x}) \frac{\partial y}{\partial k_2^+} - \frac{k_2^+}{2} y \frac{\partial \sqrt{1-x}}{\partial k_2^+} \quad (\text{A-13})$$

5 By substitution of Eqs. (A-3), (A-7) and (A-11) into (A-13), and use the definition of  $v_{QD}$

6 from Eq. (A-1), one obtains

$$7 \quad \begin{aligned} \frac{\partial v_{QD}}{\partial k_2^+} &= \frac{y}{2} (1 - \sqrt{1-x}) + \frac{K_{ES}}{2} (1 - \sqrt{1-x}) - \frac{K_{ES}}{2} (1-x)^{-1/2} x \\ &= \frac{v_{QD}}{k_2^+} \left\{ 1 + \frac{K_{ES}}{y} - \frac{K_{ES}}{y} (1 - \sqrt{1-x})^{-1} (1-x)^{-1/2} x \right\} \end{aligned} \quad (\text{A-14})$$

8 which, after some rearrangements, gives Eq. (23) in the main text.

9 Similarly, from Eq. (A-1), one has

$$10 \quad \frac{\partial v_{QD}}{\partial k_1^+} = \frac{k_2^+}{2} (1 - \sqrt{1-x}) \frac{\partial y}{\partial k_1^+} - \frac{k_2^+ y}{2} \frac{\partial \sqrt{1-x}}{\partial k_1^+} \quad (\text{A-15})$$

11 which, after using Eqs. (A-2), (A-6) and (A-10), leads to

$$12 \quad \begin{aligned} \frac{\partial v_{QD}}{\partial k_1^+} &= -\frac{1}{2} K_{ES}^2 (1 - \sqrt{1-x}) + \frac{1}{2} K_{ES}^2 (1-x)^{-1/2} x \\ &= \frac{v_{QD}}{k_1^+} \left\{ -\frac{K_{ES}}{y} + \frac{K_{ES}}{y} (1-x)^{-1/2} (1 - \sqrt{1-x})^{-1} x \right\} \end{aligned} \quad (\text{A-16})$$

13 By multiplying  $k_1^+/v_{QD}$  to both side of Eq. (A-16), one easily obtains Eq. (27).

14 Take the partial derivative with respect to  $[E]_r$  in Eq. (A-1), one obtains

$$\frac{\partial v_{QD}}{\partial [E]_T} = \frac{k_2^+}{2} (1 - \sqrt{1-x}) \frac{\partial y}{\partial [E]_T} - \frac{k_2^+ y}{2} \frac{\partial \sqrt{1-x}}{\partial [E]_T} \quad (\text{A-17})$$

which, when combined with Eqs. (A-4), (A-8), and (A-12), becomes

$$\begin{aligned} \frac{\partial v_{QD}}{\partial [E]_T} &= \frac{k_2^+}{2} (1 - \sqrt{1-x}) + \frac{k_2^+}{2} (1-x)^{-1/2} \left( \frac{2[S]_T}{y} - x \right) \\ &= \frac{v_{QD}}{[E]_T} \left\{ \frac{[E]_T}{y} + \frac{[E]_T}{y} (1 - \sqrt{1-x})^{-1} (1-x)^{-1/2} \times \left( \frac{2[S]_T}{y} - x \right) \right\} \end{aligned} \quad (\text{A-18})$$

from which, after some rearrangement, one finds Eq. (31).

Note, because switching the order of  $[E]_T$  and  $[S]_T$  in Eq. (A-1) does not change the definition of  $v_{QD}$ , Eq. (35) could be derived from Eq. (31) by simply swapping  $[E]_T$  and  $[S]_T$ .

## Appendix B: Derivation of parametric sensitivities (Eqs. (24), (28), (32) and (36)) for the Equilibrium Chemistry Approximation kinetics

Using the definitions of  $x$  and  $y$ ,  $v_{ECA}$  is

$$v_{ECA} = \frac{k_2^+ [E]_T [S]_T}{y} \quad (\text{B-1})$$

From Eq. (B-1), one has

$$\frac{\partial v_{ECA}}{\partial k_2^+} = \frac{[E]_T [S]_T}{y} - \frac{k_2^+ [E]_T [S]_T}{y^2} \frac{\partial y}{\partial k_2^+} \quad (\text{B-2})$$

which, when combined with Eq. (A-11), becomes

$$\frac{\partial v_{ECA}}{\partial k_2^+} = \frac{v_{ECA}}{k_2^+} - \frac{v_{ECA}}{k_2^+} \frac{K_{ES}}{y} \quad (\text{B-3})$$

The by dividing both sides of Eq. (B-3) with  $v_{ECA}/k_2^+$ , one obtains Eq. (24).

Similarly, from Eq. (B-1), one has

$$\frac{\partial v_{ECA}}{\partial k_1^+} = -\frac{k_2^+ [E]_T [S]_T}{y^2} \frac{\partial y}{\partial k_1^+} \quad (\text{B-4})$$

Then by aid of Eq. (A-10), one finds

$$\frac{\partial v_{ECA}}{\partial k_1^+} = \frac{v_{ECA}}{k_1^+} \frac{K_{ES}}{y} \quad (\text{B-5})$$

which gives Eq. (28) by multiplying  $k_1^+/v_{ECA}$  to both sides.

For  $[E]_T$ , one can derive from Eq. (B-1)

$$\frac{\partial v_{ECA}}{\partial [E]_T} = \frac{k_2^+ [S]_T}{y} - \frac{k_2^+ [E]_T [S]_T}{y^2} \frac{\partial y}{\partial [E]_T} \quad (\text{B-6})$$

which, when combined with Eq. (A-12), leads to

$$\frac{\partial v_{ECA}}{\partial [E]_T} = \frac{v_{ECA}}{[E]_T} - \frac{v_{ECA}}{y} \quad (\text{B-7})$$

One then, by dividing both sides of Eq. (B-7) with  $v_{ECA}/[E]_T$ , obtains Eq. (32).

By using the symmetry between  $[E]_T$  and  $[S]_T$  in the definition of  $v_{ECA}$ , Eq. (36) could be obtained by swapping  $[E]_T$  and  $[S]_T$  in Eq. (32).

### Author contributions

JYT developed the theory, conducted the analyses, and wrote the paper.

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1 **References**

- 2 Allison, S. D., Wallenstein, M. D., and Bradford, M. A.: Soil-carbon response to warming  
3 dependent on microbial physiology, *Nat Geosci*, 3, 336-340, 2010.
- 4 Averill, C.: Divergence in plant and microbial allocation strategies explains continental  
5 patterns in microbial allocation and biogeochemical fluxes, *Ecol Lett*, 17, 1202-1210, 2014.
- 6 Borghans, J. A. M., DeBoer, R. J., and Segel, L. A.: Extending the quasi-steady state  
7 approximation by changing variables, *B Math Biol*, 58, 43-63, 1996.
- 8 Drake, J. E., Darby, B. A., Giasson, M. A., Kramer, M. A., Phillips, R. P., and Finzi, A. C.:  
9 Stoichiometry constrains microbial response to root exudation-insights from a model and a  
10 field experiment in a temperate forest, *Biogeosciences*, 10, 821-838, 2013.
- 11 Druhan, J. L., Steefel, C. I., Molins, S., Williams, K. H., Conrad, M. E., and DePaolo, D. J.:  
12 Timing the onset of sulfate reduction over multiple subsurface acetate amendments by  
13 measurement and modeling of sulfur isotope fractionation, *Environ Sci Technol*, 46, 8895-  
14 8902, doi: 10.1021/Es302016p, 2012.
- 15 German, D. P., Marcelo, K. R. B., Stone, M. M., and Allison, S. D.: The Michaelis-Menten  
16 kinetics of soil extracellular enzymes in response to temperature: a cross-latitudinal study,  
17 *Global Change Biol*, 18, 1468-1479, 2012.
- 18 He, Y., Zhuang, Q., Harden, J. W., McGuire, A. D., Fan, Z., Liu, Y., and Wickland, K. P.:  
19 The implications of microbial and substrate limitation for the fates of carbon in different  
20 organic soil horizon types of boreal forest ecosystems: a mechanistically based model  
21 analysis, *Biogeosciences*, 11, 4477-4491, 2014.
- 22 Houlton, B. Z., Marklein, A. R., and Bai, E.: Representation of nitrogen in climate change  
23 forecasts, *Nat Clim Change*, 5, 398-401, 2015.
- 24 [Johnson, F. H. and Lewin, I.: The Growth Rate of E-Coli in Relation to Temperature, Quinine  
25 and Coenzyme, \*J Cell Compar Physl\*, 28, 47-75, 1946.](#)
- 26 [Li, J. W., Wang, G. S., Allison, S. D., Mayes, M. A., and Luo, Y. Q.: Soil carbon sensitivity  
27 to temperature and carbon use efficiency compared across microbial-ecosystem models of  
28 varying complexity, \*Biogeochemistry\*, 119, 67-84, 2014.](#)
- 29 Litchman, E. and Klausmeier, C. A.: Trait-Based Community Ecology of Phytoplankton,

1 Annu Rev Ecol Evol S, 39, 615-639, 2008.

2 Maggi, F., and Riley, W. J.: Transient competitive complexation in biological kinetic isotope  
3 fractionation explains nonsteady isotopic effects: Theory and application to denitrification in  
4 soils, *J Geophys Res-Bioge*, 114, Artn G04012, doi: 10.1029/2008jg000878, 2009.

5 Michaelis, L., and Menten, M. L.: The kinetics of the inversion effect, *Biochem Z*, 49, 333-  
6 369, 1913.

7 Moorhead, D. L. and Sinsabaugh, R. L.: A theoretical model of litter decay and microbial  
8 interaction, *Ecol Monogr*, 76, 151-174, 2006.

9 Palsson, B. O.: On the Dynamics of the Irreversible Michaelis Menten Reaction-Mechanism,  
10 *Chem Eng Sci*, 42, 447-458, 1987.

11 Riley, W. J., Maggi, F., Kleber, M., Torn, M. S., Tang, J. Y., Dwivedi, D., and Guerry, N.:  
12 Long residence times of rapidly decomposable soil organic matter: application of a multi-  
13 phase, multi-component, and vertically resolved model (BAMS1) to soil carbon dynamics,  
14 *Geosci Model Dev*, 7, 1335-1355, 2014.

15 Schimel, J. P. and Weintraub, M. N.: The implications of exoenzyme activity on microbial  
16 carbon and nitrogen limitation in soil: a theoretical model, *Soil Biol Biochem*, 35, 549-563,  
17 2003.

18 Segel, L. A.: On the Validity of the Steady-State Assumption of Enzyme-Kinetics, *B Math*  
19 *Biol*, 50, 579-593, 1988.

20 Segel, L. A. and Slemrod, M.: The Quasi-Steady-State Assumption - a Case-Study in  
21 *Perturbation*, *Siam Rev*, 31, 446-477, 1989.

22 Sihi, D., Gerber, S., Inglett, P. W., and Inglett, K. S.: Comparing models of microbial-  
23 substrate interactions and their response to warming, *Biogeosciences Discuss.*, 12, 10857-  
24 10897, doi:10.5194/bgd-12-10857-2015, 2015.

25 Sulman, B. N., Phillips, R. P., Oishi, A. C., Shevliakova, E., and Pacala, S. W.: Microbe-  
26 driven turnover offsets mineral-mediated storage of soil carbon under elevated CO<sub>2</sub>, *Nat Clim*  
27 *Change*, 4, 1099-1102, 2014.

28 [Tang, J. Y. and Zhuang, Q. L.: A global sensitivity analysis and Bayesian inference](#)  
29 [framework for improving the parameter estimation and prediction of a process-based](#)  
30 [Terrestrial Ecosystem Model, \*J Geophys Res-Atmos\*, 114, 2009.](#)

1 Tang, J. Y. and Riley, W. J.: A total quasi-steady-state formulation of substrate uptake  
2 kinetics in complex networks and an example application to microbial litter decomposition,  
3 *Biogeosciences*, 10, 8329-8351, 2013.

4 Tang, J. Y. and Riley, W. J.: Weaker soil carbon-climate feedbacks resulting from microbial  
5 and abiotic interactions, *Nat Clim Change*, 5, 56-60, 2015.

6 Wang, G. S., Post, W. M., and Mayes, M. A.: Development of microbial-enzyme-mediated  
7 decomposition model parameters through steady-state and dynamic analyses, *Ecol Appl*, 23,  
8 255-272, 2013.

9 Wang, G. S. and Post, W. M.: A note on the reverse Michaelis-Menten kinetics, *Soil Biol*  
10 *Biochem*, 57, 946-949, 2013.

11 Waring, B. G., Averill, C., and Hawkes, C. V.: Differences in fungal and bacterial physiology  
12 alter soil carbon and nitrogen cycling: insights from meta-analysis and theoretical models,  
13 *Ecol Lett*, 16, 887-894, 2013.

14 Wieder, W. R., Bonan, G. B., and Allison, S. D.: Global soil carbon projections are improved  
15 by modelling microbial processes, *Nat Clim Change*, 3, 909-912, 2013.

16 Wieder, W. R., Grandy, A. S., Kallenbach, C. M., Taylor, P. G., and Bonan, G. B.:  
17 Representing life in the Earth system with soil microbial functional traits in the MIMICS  
18 model, *Geosci Model Dev*, 8, 1789-1808, 2015.

19 Xenakis, G. and Williams, M.: Comparing microbial and chemical kinetics for modelling soil  
20 organic carbon decomposition using the DecoChem v1.0 and DecoBio v1.0 models, *Geosci*  
21 *Model Dev*, 7, 1519-1533, 2014.

22 [Zhu, Q. and Zhuang, Q. L.: Parameterization and sensitivity analysis of a process-based](#)  
23 [terrestrial ecosystem model using adjoint method, \*J Adv Model Earth Sy\*, 6, 315-331, 2014.](#)

24 Zhu, Q. and Riley, W.J.: Improved modeling of soil nitrogen losses, *Nat Clim Change*, 5,  
25 705-706, 2015.

26 Zhu, Q., Riley, W. J., Tang, J. Y., and Koven, C. D.: Multiple soil nutrient competition  
27 between plants, microbes, and mineral surfaces: model development, parameterization, and  
28 example applications in several tropical forests, *Biogeosciences Discuss.*, 12, 4057-4106,  
29 doi:10.5194/bgd-12-4057-2015, 2015.

30

1 List of Figures

2 Figure 1. (a) ECA kinetics predicted normalized sensitivity of the reaction velocity with  
3 respect to the maximum product genesis rate  $k_2^+$ ; (b) predictions by the quadratic kinetics; (c)  
4 the normalized difference  $(a_{QD} - a_{ECA}) / (a_{QD} + a_{ECA})$  between the quadratic kinetics predictions  
5  $a_{QD}$  and the ECA kinetics predictions  $a_{ECA}$ ; (d) comparison of normalized sensitivity predicted  
6 by different kinetics.

7

8 | Figure 2. Similar as [Figure 1](#), but the sensitivity is evaluated against the intrinsic substrate  
9 affinity  $k_1^+$ .

10 | Figure 3. Similar as [Figure 1](#), but the sensitivity is evaluated against the total enzyme  
11 concentration  $[E]_T$ .

12 | Figure 4. Similar as [Figure 1](#), but the sensitivity is evaluated against the total [substrate](#)  
13 concentration  $[S]_T$ .

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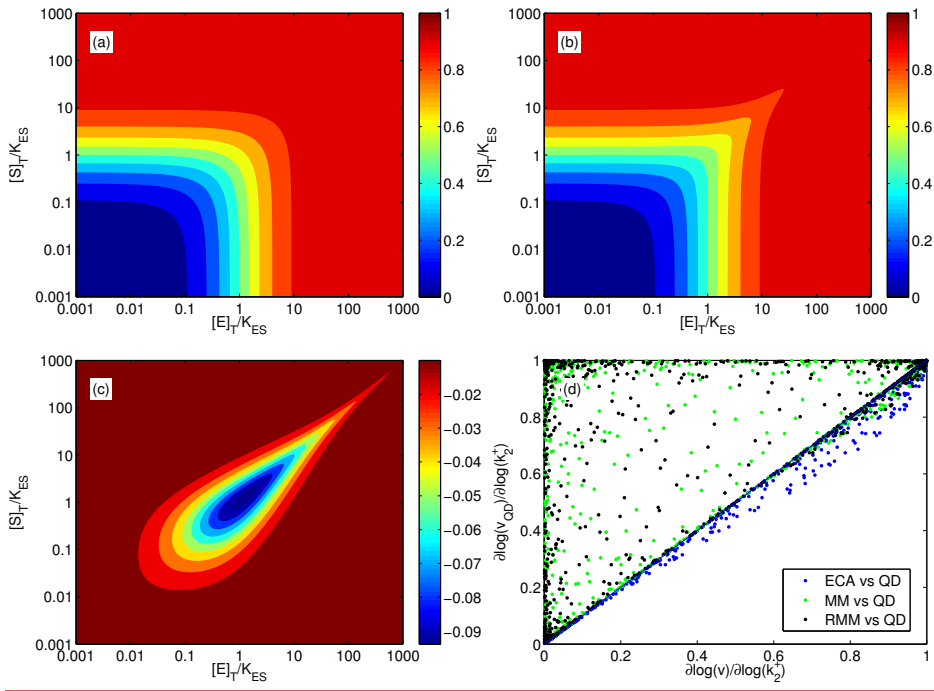
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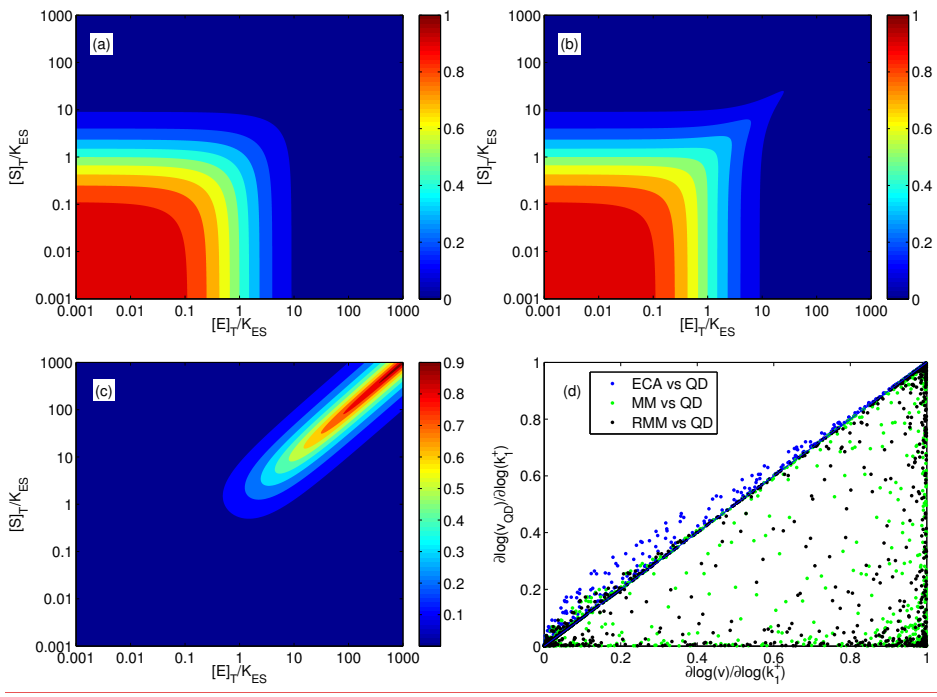
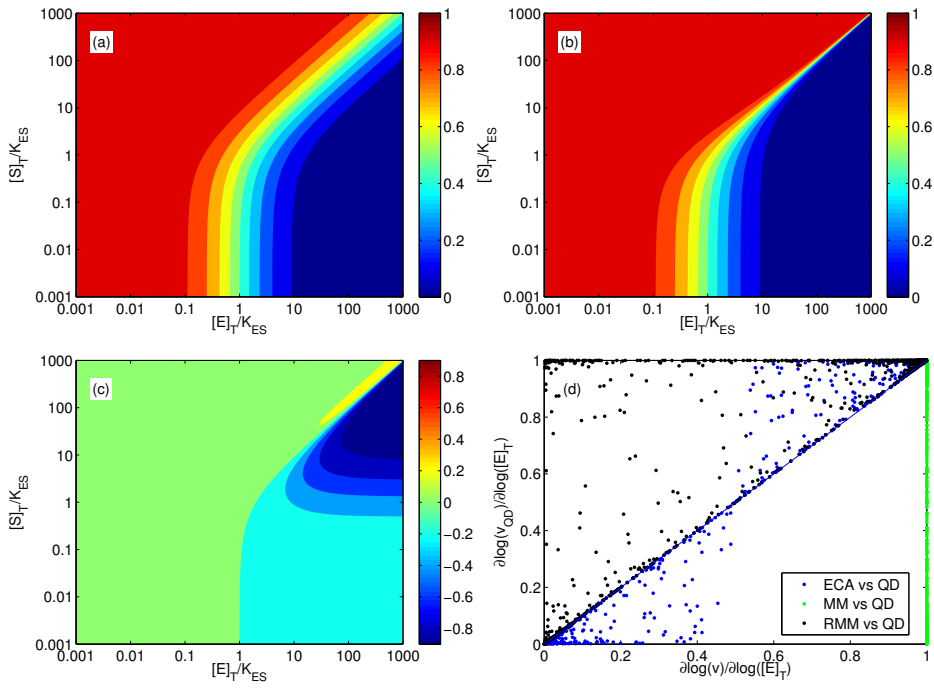


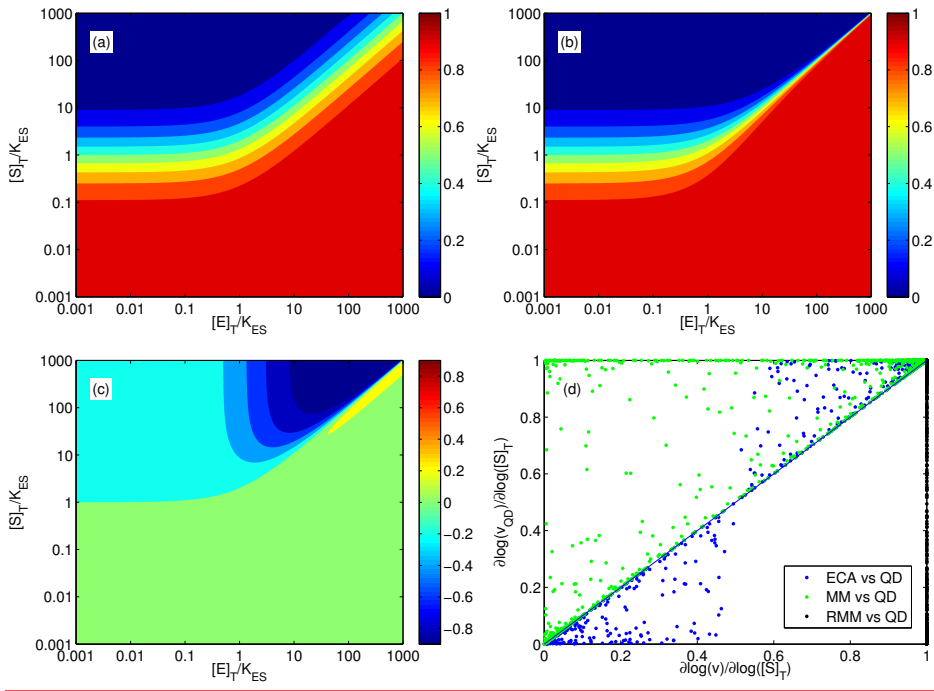
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