Cover Letter

Dear Editor,

Thank you for giving us the opportunity to submit the revised version of the manuscript, "Comparing models of microbial-substrate interactions and their response to warming" by D. Sihi, S. Gerber, P. W. Inglett, and K. S. Inglett for consideration of publication in *Biogeosciences*.

The comments of both the reviewers tremendously helped to improve the manuscript. In our current version, we followed Will Wieder's comment and separated different models families such that we now have first the base models (forward vs. reverse M-M models), then move on to better explain the effect of the short-term equilibrium assumption of microbial biomass, and finally modified formulations of respiration (splitting respiration in maintenance and growth term) in the base models. Adding subsequent layers in this way helped us to demonstrate clearly how different assumptions influence the dynamics of soil organic carbon, microbial biomass, carbon use efficiency, and respiration vary in short vs. long time-scale. Further, it allowed us to address both of the reviewers' concerns of short-term result (at microbial time-scale) and demonstrate the bridge between microbial decomposition models with the first-order decomposition models. The second major modification from the discussion paper is that we now let the long-term response deviate by parameterizing such the models have the same initial short term (instead of long-term)sensitivity to temperature.

Below, you find a point by point response to all the reviewers' comments, as well as a marked up version that highlights the differences between the discussion paper and this submission.

We believe that these improvements makes our revised manuscript an excellent addition to *Biogeosciences,*particularly also, because we show the mechanisms and consequences of different formulations of microbial carbon consumption resolved in current microbial models The quantification and prediction of soil organic matter decomposition, and its response to global change factor is critically important for global carbon cycle feedbacks, and therefore the questions raised in our manuscript and our insights in decomposition models are important to the readers of this Journal.

We look forward to hearing from you soon.

Sincerely,
Debjani Sihi (corresponding author)

Response to Reviewer 1:

General comments

As the authors mention, the behavior of models 1 and 2 is very similar [P10872, L23]. How does the additional complexity mathematically manifest itself in the emergent dynamics? Can the similarity be explained by the structure of the models? In the case of models 1 and 2, it seems that they share the same mathematical structure, in that model 2 can be reduced to model 1 as follows:

dS/dt = I – lambda_d*M – D where D = V_max*S*M/(K_E + S) [same in models 1 & 2]

dM/dt = (D - lambda_r *M)*(1-g) – lambda_d *M [form of model 2]
= (D – D*g - lambda_r *M + lambda_r *g*M) – lambda_d *M
= (D(1-g) – M(lambda_r - lambda_r *g + lambda_d)
= alpha*D – beta*M [same form as model 1]

Where alpha = (1-g) and beta = (lambda_r - lambda_r *g + lambda_d) for model 2.
How do these relate to epsilon and lambda_d in model 1, respectively? The authors briefly discuss concerns associated with adding parameters [P10873]. Is introducing uncertainty through extra parameters warranted here? The temperature sensitivity of the partitioned respiration model is indeed different, but the model structure is the same. Additional discussion on how the models relate when reduced mathematically would be a great addition to this manuscript. For example, why does model 4 with mu=0 behave so similarly to model 5?

Thank you for your detailed comments, which help us greatly to improve our Manuscript. In response to the suggestions from Reviewer 2, in our revised manuscript, we change the presentation of the models. We first introduce forward (FWD) vs. reverse (REV and OPT) Michaelis-Menten models, where the main difference is a decreasing marginal return in the REV model and the subsequent optimisation of enzyme productivity in the OPT model, for which we present a new figure 2.

We now put the question of separating maintenance vs. growth respiration into a much broader context of time scale partitioning. Across the model development, we assumed quasi-steady states at different timescales. For example the Michaelis-Menten equation assumes quasi-steady states of enzyme concentrations. Further the direct relationship between microbial biomass and depolymerisation assumes a quasi-steady state of dissolved organic carbon. As we move up the time scale we assumed for the REV and OPT models, that microbial biomass is at quasi-steady state with substrate supply (See Fig 1b). We motivate this by the approximation, that the timescale of the microbial turnover is much shorter than the time scale of soil organic matter turnover. That is, microbial biomass adjusts much faster to changes in environmental conditions than soil organic matter itself. Thus, over the timescale of microbes, soil organic matter can be approximated by a constant (it does not change that much). We can then substitute the expression for microbial biomass as obtained from dM/dt = 0 into the function of depolymerisation and, microbial death, and respiration, which is the microbial quasi-steady state. In the revised manuscript, we add a figure (see Fig 3 in the revised manuscript) that shows how the assumption of microbial equilibrium compares well against the fully dynamic models, with respect to the dynamics of decomposition and CO_2 flux. Further, this analytical trick helps to build the bridge to traditional first order models, because the formulations of decomposition are now independent of the microbial biomass. For example depolymerisation (D) in Model 3 (now REV model) now becomes

D = V_max*S – K_M*lambda_d/ epsilon

Where V_max is a maximum depolymerisation rate, S soil organic matter carbon, k_M the half saturation constant for microbes, and epsilon is carbon use efficiency.
The expression of depolymerisation above becomes independent of microbial biomass. This expression becomes a first order model, if k_M * lambda_d << V_max *(1-g). Similarly, in model 4 (now, OPT model) under mu = 0, and dM/dt = 0 (quasi-steady state) depolymerisation becomes

D = V_max*S
Microbial death = V_max*S* epsilon
And thus
dS/dt = I – V_max*S*(1- epsilon)

where I is the input.

Both reviewers mention that they have trouble seeing the value of the short-term equilibrium in Table 3. We explain this better in our revised manuscript.

Next, we compare each of the models (FWD, REV, and OPT) to a variant where we first introduce a separation between growth and maintenance respiration (3rd layer in the model families). As for the reviewer's analysis above, the reduction requires a temperature sensitivity of the term beta (instead of alpha, as used in model 1 in our discussion paper), and it does modify the respiration on the short microbial time scale. We are convinced that our reorganisation of first considering a microbial model without maintenance respiration, then assuming microbial steady state, and ultimately adding maintenance respiration helps to explain how nuances of microbial models impact the temperature response, and how they compare analytically to traditional first-order (FOD) models.
Overall, we believe with this new organization, we provide much more context, how one can move across the models (FWD, REV, OPT), and across the layers (base, quasi-steady state microbes, separation of respiration terms).

Overall, this manuscript is well-written and presents an interesting modeling analysis. It could be improved by providing a perspective on future models and giving specific recommendations that the reader could take away for model development.

Thank you for your positive review! This comment has also been raised by reviewer 2.
We add to the discussion, and more importantly in the conclusion how the evaluation of simple models can serve larger scale models. Our work clearly shows dynamical differences whether substrate-enzyme reactions are considered a rate limiting step, resulting in forward (Models 1 and 2 in the discussion paper) vs. reverse models (Model 3 and 4 in the discussion paper). We show that there are potential mechanisms (i.e. limitation of reaction sites, microbial enzyme consumption) that support a reverse model. Further, our OPT model ask the question, whether it is justified to link microbial enzyme production to microbial biomass. If we untether this relationship, but focus an optimizing returns on microbial investment, we obtain a first order model. Overall, we sought the make the link from previous thought-through microbial models and their formulations to first order models, which will help in analyzing and juxtaposing the different models.
On a minor side note, we discuss that even in simple models, the response to temperature is a composite of parameters that are hard to come by, including half saturation constants, sensitivity of microbial respiration to temperature, the amount of enzyme produced by microbes, as well as enzyme activity.

Specific comments

P10858, L8: It may be more appropriate to say that you "analyse five microbial decomposition models", as this is not a general analysis of all existing models, nor of models with multiple pools.

This sentence is not in the revised manuscript since we change the model setup from the previous submission.

P10858, L10-15: How does your proposed model compare to models that explicitly represent enzyme dynamics with finite potential binding sites, such as the MEND and DEB models?

To our knowledge the MEND model does not have finite potential binding sites, based on our reading of Wang et al. (2013). Their steady-state solution is fairly similar to our model 1 and 2, although the MEND model considers additional pools. However, in the same years Wang and Post, (2013), discuss the formulations of forward and reverse Michaelis-Menten Model, and also compare it with the Langmuir isotherm theory, and we include their derivation as an additional mechanism towards the diminishing return that occurs in the REV model. We highlight in the discussion section, that our suite of models does not include sorption onto mineral surfaces of microbes, enzymes and substrate, as it is considered in the DEB model. Perhaps of critical importance to the difference between forward and reverse models, which – based on your comments elsewhere, and based on Reviewer 2, we are hashing out much more.

P10858, L15: "fast responses" in relation what?

This sentence has been replaced in the revised manuscript.

P10858, L16: Why "short-term adjustment in microbial growth"? From the figures it appears that microbial biomass, as with carbon storage, reaches a new (long-term) steady-state.

The response to this question should come out of one of the major changes of the manuscript. We try to motivate a short (microbial) timescale and a longer (soil organic matter) timescale. On the short timescale, microbial biomass adjusts quickly to new environmental conditions (temperature), and on the long timescale, microbial biomass only adjusts to the slow decrease of soil organic matter (it is in quasi-equilibrium with soil organic matter). See also our response to the main concern above.

P10859, L17: Can add citation for Wieder et al. 2015 here.

We add this reference in our new submission.

P10859, L20: The citation for Li et al. 2014 would be appropriate here regarding CUE response to warming across models.

We add this reference in our new submission.

P10860, L15: Although additional parameters were added to separate microbial respiration sources, the form of model 2 can be reduced back to model 1, as shown in the main comments above. Does the parameterization drive the difference in decomposition dynamics, since the model structure is the same?

It is actually the model structure that drives the difference, since now different terms are temperature sensitive. That is, in the mathematical derivation under "main comments" parameter beta, instead of parameter alpha becomes temperature sensitive, when moving from model 1 to model 2. We further now show how the separation of maintenance and growth respiration affects all models, but discuss, why this separation is only important on the short (i.e. microbial) timescale.

P10861, L8: For clarity, it would be good to note that enzyme concentrations and microbial biomass go together and that you do not represent them as separate pools in the simulated differential equations; rather, you focus on the response of 2-pool, substrate-microbe models to warming. Can you confidently capture microbe and enzyme allocation/reaction/production dynamics without an explicit enzyme pool?

We now specifically discuss the assumption of quasi-steady states that feeds across the scales. I.e. the assumption of a quasi-steady state in the enzyme pool substitutes enzyme concentrations with a function of the microbial biomass. That is, the enzyme pool does now change in tandem with the microbial biomass changes. Given, the simple mechanisms that describe enzyme production and turnover, our equilibrium assumption is a valid simplification.

P10861, L19: It would be good to clarify that the "tendency" is the "derivative" when you first use it, as I feel that the latter is more commonly used among BG readers.

We have added in parenthesis "derivative with respect to time".

P10863, L19: To be consistent with the literature, it may be good to mention here that the final form you use for model 3 is a reverse Michaelis-Menten formulation, as in Schimel and Weintraub 2003.

We add the reference in the method, and now make explicit distinction between forward and reverse Michaelis-Menten models.

P10865, L3: Is there a negative sign missing in Eq. 14? Otherwise, dS/dt = constant*S with a constant > 0 would increase exponentially. Also, please check your mass balance: if a (1-epsilon) fraction leaves to respiration, then should a net – (1-epsilon) be leaving S, since –k*S +epsilon*ks in the mass balance?

Thank you for catching this! This equation should correctly say:

$$\frac{dS}{dt} = I - S * k * (1 - \varepsilon)$$

where I is input of fresh litter. Also please note that we have a separate section for temperature sensitivity terms now in the method section.

P10865, L10: Which are the traditional models (cite a few) and how do they represent the temperature sensitivity of CUE? Often CUE decreases linearly with temperature in simple models and often 'traditional', Century-type models include more than one pool of carbon.

We changed the description of how we set up our FOD model (previously model 5)which is the first order model in that we explicitly mention how this setup differs from traditional models such as CENTURY and Roth C. The two major differences are that our model only considers a single pool, while traditional models consider a series of different quality pools feeding into each other. We also mention that with a temperature dependent carbon use efficiency, a temperature increase changes the fraction of carbon processed becoming CO_2. This is not typical to traditional models, as the fraction respired is not a function of temperature.

P10865, L21: What do you mean by tuning factors for V_max1 and K_E and what are they tuned to for model 1 in addition to the German et al. parameters?

We have changed the formulation such that it becomes clear that we did not tune this part of the model, but instead worked the tuning factors directly into the parameters. It now reads: „ We use parameters as reported in German et al. (2012), with a few modification. Here, we report $V_{max,FWD}$ and K_E by considering 15°C as our reference temperature and by working their tuning factors directly into these two parameters. In other words, $V_{max,FWD}$ and K_E are the product of the reference values in German et al. (2012), their respective tuning parameters and their adjustment to our reference temperature, 15°C. “

P10866, L7: I think “maintenance estimation” should be “maintenance respiration”. Also, why one-third of the death rate? Please provide a reference or more reasoning.

Yes, a typo. We re-addressed the partitioning of maintenance vs. growth respiration. We had a hard time finding specific values in the literature, but motivated the partitioning based on vegetation models. LPJ (Sitch et al., 2003) and ED (Moorcroft et al., 2001) have a growth respiration factor of one-third. We then constrain the overall respiration by the carbon use efficiency in German et al., 2012, and obtain a maintenance respiration rate that is close to the microbial death rate (lambda_r,0 =1.25 * lambda_d).

P10866, L16: Here you say that you match the equilibrium values for CUE, M, S and decomposition. Matching equilibrium decomposition rates had not been mentioned before?

In the discussion paper, matching decomposition is actually not necessary, as it results from matching CUE, M, and S. You will notice, though, in our revised manuscript, it is our goal to match depolymerisation immediately after the temperature increase, and let the long-term responses deviate.

P10868, L5-15: This confuses me a little, as the two differential equations are coupled and respond together by necessity. The magnitude of change within each pool differs, as the pool sizes are significantly different. Please provide a bit more explanation and rationalization for this part of your analysis. In calculating the true equilibrium, dM/dt = 0 and dS/dt=0.

This confused both reviewers and we take great care in our revised manuscript to show the use of short-term (quasi) vs long-term (true) equilibrium. The turnover of soil organic matter is much slower than that of microbe. Therefore, over the timescale of microbial adjustment, there is little change in S. It therefore allows microbe to almost equilibrate with S. In other words, microbes are at quasi-steady state. As soil organic matter changes, the short-term equilibrium of microbial biomass (or the quasi-steady state) is changing along. In our revised manuscript, we show a plot (Fig 3), with the equilibrium microbial biomass as a function of time. We show that, over mid- to long-term the quasi-steady state of microbes is a good approximation of the actual microbial biomass. This paragraph is entirely rewritten in the revised manuscript. We further explain in the text, that this quasi-steady state assumption is also true for enzyme kinetics and for dissolved organic carbon.

P10870, L22: Can you show mathematically how model 4 reduces to the linear model when mu=0?

Equation 7 has the depolymerisation as D = Vmax * S – sqrt(K_P*c*V_max*S).

Where the second term on right hand side is the reduction of depolymerisation if there is a cost associated with decomposition (i.e. mu>0). If mu is 0, D becomes V_max*S, which is the form of the first order model. We reference the equation and how it changes under zero cost, in order to clarify and support our assertion.

P10875, L18: Considering putting (mu=0) for the negligible costs scenario, just to be clear.

We write mu->0 as marginal cost scenario in our improved manuscript.

P10876, L1: How realistic are the equilibrium values you fit to and how much do these vary in reality? If the parameters are fit to different values, how much might the dynamics and conclusions change? For example, the enzyme-substrate model in Allison et al. 2010 may or may not oscillate depending on the parameters.

The equilibrium values can vary a great deal across the globe, depending on climatic conditions and soil quality. Perhaps equally important is the question, by how much the parameters are constrained which determine the equilibrium values. We find that these values are fairly uncertain. As we compare models with each other, we felt that we should not pick arbitrary parameters, but choose them that the models are comparable in some way. In our discussion paper, based our parameters based on published values, or we use other justification. Our base parameter set starts off with German et al., 2012. In our discussion paper, we chose to force the model through equilibrium values at base and elevated temperature. Reviewer 2 pointed out that some of the results were too derived (in particular the apparent Q_10, Fig 4 in our discussion paper). We agree with reviewer 2, and we changed the parameterisation such that the equilibrium at reference temperature are the same, and that the initial response to a temperature perturbation is equal across the model.

As for the oscillation, Wang et al. (2014) showed the parameter space with respect to the oscillatory behavior. Large V_max compared to K_m dampens oscillation quickly. On the other hand, in the Allison et al. (2010) model, a large fraction of the input and microbial necromass was assumed to become DOC, which does not require enzymes for microbial consumption. This assumption also reduces the positive feedback between microbial growth and decomposition, because microbial growth can occur independent of enzyme production via consumption of readily available DOC, we added this possibility in the method and the discussion section

P10878, L2: Is there a +kr[ES] term missing from the expression given for d[E]/dt? If the reversibility of enzyme binding removes –kr[ES] from d[ES]/dt, then where does it go? Also, reversibility is not shown in the diagram of Fig. 1.

Yes, this is missing, but the mistake is editorial and does not affect the subsequent math. We show the reversibility in our new Fig 1.

P10878, L6: Please explain a little more in the text what P is and that it changes; i.e., that it is a rate proportional to microbial biomass.

Our revised manuscript say that P is the production of enzymes, and that in most microbial decomposition model, this is assumed to scale to microbial biomass. However, our **model 4 (OPT model)** relax this assumption and P is optimised.

P10878, L13: Why are you most interested in E_t? The Michaelis-Menten derivation using the quasi-steady state approximation for short-lived intermediates (i.e., d[ES]/dt = 0) is very standard in textbooks, but could be better explained here.

Starting from P26, L 2 we rearrange to the following:

„The Michaelis–Menten approximation for depolymerisation assumes that the system is in quasi-steady state in which the tendency $\frac{d[ES]}{dt}$ and $\frac{d[E]}{dt}$ are zero. This implies also that tendency of the total enzyme concentration $\frac{d[Et]}{dt}$ (with [Et] = [ES] + [E]) becomes zero.“

P10878, L15-16: This sentence seems to cut off prematurely, in which E_t … is?

This is now taken care of with the new formulation, where we explain the quasi-steady state in the Michaelis-Menten kinetics. (see our response to the previous comment).

P10878, L17-19: Consider using S_t for total sites instead of [S] which is also used as the transient free sites and is certainly not constant, otherwise d[S]/dt=0 would defeat the purpose. I think that the condition on S or St is not necessary for the derivation; Eq. (A2) = 0 by the quasi-steady state assumption of fast-reacting intermediates. Also note the missing period.

The S stands for the total substrate, and we would like to keep that in the main text. We changed the text, such that S refers to the concentration of the substrate. Importantly, we have S_f as the free available sites for enzyme-substrate reaction when these sites become limiting Appendix B, limited available substrate. You are correct, the condition on S_tot is not necessary. And thus we delete this part. What we meant, with Sthough, S_tot changes only marginally (quasi-steady state of E and ES) so that the relative concentrations of ES and S do not lag the substrate.

P10879, L10: Similarly to what? I would suggest moving P10879 L18 – P10880 L5 to above P10879 L10. It might be better to introduce the previous method and then what you do, instead of switching back and forth.

We agree. In our revised manuscript we now first deal with the simple enzyme turnover model, as used in Allison et al., 2010, introduce the concept of steady state in the enzyme pool, and then move forward to discuss the parameterization of differential turnovers of the enzyme substrate complex and the free enzymes. .

P10879, L13: Is Eq. (A7) missing a term? From [Et] = [E]+[ES], taking the derivative and substituting Eq. (A1) and (A2), you would get d[Et]/dt = P –lambda_E1*[E] – kcat + lambda_E2)*[ES]. This would then add a term to the denominators of (A8) and (A9) and carry through the expressions presented to (A25), etc. It would also be good to be consistent with your Et and [Et] notation, as they are used interchangeably in the appendix.

We actually missed kcat*[ES] in equation A1. However, some enzymes may be destroyed when the product is formed.. **lambda_E2** is thus a parameter that includes both, the destruction of enzymes when products form, as well as denaturizing of enzymes while they are complexed with substrate. We will also make sure to maintain consistency on [E_t] notation.

P10882, L4: It would be nice to keep consistent notation for [S]; for example, St = theta (S+ES), where S represents free, available sites.

We would like to keep S for the total amount of substrate, to be consistent with the main text. But we make sure, here and in the discussion of the Michaelis-Menten equiation, to inform the reader how S (all forms of S) relates to the available sites, also when much of the substrate becomes inaccessible.

P10882, L8-9: Can you explain a bit more why you take a Taylor series expansion (linearize) around the total sites S=0 versus linearizing around the equilibrium S? Also, you alternate between kE and KE.

We expand there. We assume that enzyme concentrations are much bigger than the potential reaction sites. That is E_t + K_E >> S/theta. Thus the term S/theta is in the vicinity of zero if compared to E_t + K_E. This allows us to expand around zero. We explain this now in the text. We also add that we obtain the same result, if S_f << K_E in Equation B7, (small amount of free sites) and thus

Equation B7

$S_f = S/\theta - S_f*E_t/(K_E + S_f) \sim= S/\theta - S_f*E_t/K_E$

Therefore

$S_f = S/\theta * K_E/(E_t + K_E)$
We also make sure to maintain consistency on K_E notation.

P10882, L12: Could you explain why the S/theta term is much smaller than E_t and K_E (as on P10883, L1) and dropped from the denominator of Eq. (A24)?

We add that this particular solution is for a small amount of binding sites, and enzymes compete for free sites. Thus E_t >> S/theta, and it can be dropped within the denominator.

P10884, L5: Is the final expression missing an M in the numerator?

Correct and nice catch! We add the microbial biomass as a factor.

P10885, L1-3: If P is a function of M as before, then M can also be written as a function of P. When taking the derivative of G in Eq. (A32) with respect to P, does the lambda_r*M term come into play? Similarly with substituting a function of M for P in the denominator of D (A35) when determining if an optimum exists.

In this solution, where the microbial community optimises enzyme production, P is independent of the microbial biomass, therefore the derivation of lambda_r*M is zero.

P10892, Table 2: For model 4, the value of KP is not given, does this mean that it carries over from the fitting of the other models? For clarity, please addμ to the table where Pc/D is given for model 4.

We realize, the ratio Pc/D in the table is confusing. We will use mu, as suggested, and motivate its usage better in the method section (10867 L8-10 in the discussion paper).
Values for both parameter K_P and c are hard to come by. But in our solution they always occur together in a product (K_P*c). Moreover, the fraction of enzyme expenditures in relation to depolymerisation can easily be expressed as a function of maximum depolymerisation (V_max*S) and the product K_P*c (Equation A8). Mu is then the fraction of carbon that is used for enzyme production compared to the potential depolymerisation rate, as it would occur without cost, evaluated at steady state. We think this makes enzyme expenditures a bit more tangible because we relate these costs to processing rates. Once we defined mu, we can derive K_P*c, for any Vmax and S. We note that the potential depolymerisation rate at steady state is also the input of fresh litter (I). We added the values of K_P*c to the table 3.

P10893, Table 3: Should the short/fast time scale and long time scale have the same conditions (namely, S =eq. S) in the caption? Please clarify the methodology in the caption.

We improve Table 3, also in response to the improved modeling setup. In the short-term equilibrium (quasi-steady state), we let microbial biomass equilibrate with S (any potential value of S). This is motivated by the fact that microbial biomass turns over much faster than soil organic matter. We added a new column, that calculates depolymerisation if M is at equilibrium (with any given S). We also have carried out additional simulations that show the dynamics of soil organic carbon, respiration, and the diagnostic equilibrium microbial mass. We can show that the assumption of a microbial steady-state leads to similar results in the medium to long-term (but not in the short-term, see our new Fig 3).
We will explain the assumption of the short-term equilibrium (quasi-steady state) in the new method section (see also our response to an earlier comment to P10868, L5-15).

P10895-10897, Figures 2-4: Could you include a short descriptive model name for the four models in the legend or in the captions and briefly discuss why you chose a logarithmic x-axis? The log axis makes it harder to think through the dynamics and build intuition for shorter time scales; consider changing to a regular axis. 1,000 years is very long! Also, it looks like models 4 and 5 have the same orange color in the legend. Please make sure the five colors used are clearly distinguishable.
We changed the model names such that they are more descriptive (now FWD, REV, OPT, and FOR) for the forward, and reverse Michalis Menten, model, for optimising enzyme production, and for the traditional first order Model. 'Logarithmic axes are chosen' to better highlight differences in short-term responses' to the figure caption. If we switch to regular axis, differences with respect to the equilibrium assumption, and with respect to the implementation of respiration (explicit growth and maintenance respiration) simply disappear. We truncated our time axis to 200 years when the system finally reaches equilibrium. We have changed the color scheme to better highlight the differences between models.

Technical corrections

P10858, L5: "sufficient" should be "sufficiently"
Done

P10859, L11: Remove the word "a" to read "to more complex dynamics"
Done

P10860, L20: Sentence fragment – consider revising to: "models, each of which carries a single soil organic matter pool…"
This sentence has been replaced in the revised manuscript.

P10862, L1: "represented" might be a better word choice instead of "parameterized" when referring to the mathematical form (structure) of a process.
Done

P10862, L21: Consider changing "They dynamics…" to "The microbial pool is characterized by…" or if keeping the current sentence, change "is" to "are"
Now it reads as "Partitioning of microbial respiration into growth and maintenance respiration characterise the microbial pool as follows:"

P10864, L11: Consider changing "parameterized" to "represented" again.
Done

P10867, L8: Remove "the" from before model 4 and consider replacing "…and expressed them as…" by "expressed as"
We keep "the" as we added a name to the model. Now reads as "Here, we analyse the OPT model based on different levels of enzyme expenditures and expressed as enzyme costs per unit carbon depolymerised (mu =Pc/D), where μ is 0, 10, and 50 percent of the depolymerisation rate at reference temperature and at steady state".

P10868, L14: "response" to "respond"
This sentence has been replaced in the revised manuscript.

P10868, L23 – P10869, L1: Check sentence punctuation and rephrase; e.g., "response to warming: all catalytic…"
This sentence has been replaced in the revised manuscript.

P10869, L13: Consider changing to "… biomass also converges…"
This sentence has been replaced in the revised manuscript.

P10870, L19: Consider changing to "warming-induced increase"
This sentence has been replaced in the revised manuscript.

P10874, L8: Missing the word "of" in "a surplus of free enzymes"
The word "of" is added now

P10874, L23: Remove the word "they" from "that relate to"
Done

P10874, L27: Add the word "with" in "occur with infinite enzyme…"
Done

P10875, L7: Add the word "the" in "found in the…"
This sentence has been replaced in the revised manuscript.

P10875, L21: "… by introducing a variable…"
This sentence has been replaced in the revised manuscript.

P10878, L1: Dynamics is plural, so "dynamics… are"
Done

P10878, L6: [S] "is" instead of "are"
Done

P10879, L6: Note the missing space.
Done

P10879, L6: Remove the comma in "we assumed that DOC…"

Done

P10881, L3-4: Are lambda_E1,0 and lambda_E1 the same? It looks like there may be a notation typo.
No, they are not the same. lambdaE is a general decay rate, lambda_E1 is the decay of free enzymes, but in the REV model with microbial enzyme consumption we devided decay of the free enzymes further into lambda_E1,0 + lambda_E1,M*M

P10882, L14: No need to capitalize "Equations"
Done

P10883, L4: Consider changing the wording of "evaluate end member" to, for example, "evaluate the following scenarios"
Done

P10883, L15: The word enzymes should be plural.
Done

P10884, L11: Change wording to "where P is the…" and also Vmax "is…" versus "may be…"
Done

Thank you for reading the MS carefully and making note of grammatical and spelling mistakes. All these technical corrections (where applicable) have been addressed in the revised manuscript.

References cited in the response to reviewer comment:

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Response to reviewer 2

General comments

Sihi and co-authors present a nice study examining soil C dynamics projected by a series of simple models that make different assumptions about heterotrophic respiration and enzyme production. At a high level their findings could be interpreted as: 1) Forward Michaelis-Menten (M-M) models are crazy 2) Reverse Michaelis-Menten models look more reasonable and 3) Reverse models approximate first-order models so why bother with these silly microbial models that are a pain to parameterize and run? I this this paper has more to offer, however, and my suggestions are intended to give the paper broader insight and appeal.

Thank you for these comments. This is perhaps a little bit too simplified. However, our work was intended to contribute to the discussion how microbial decomposition models and microbial enzyme models are similar (or dissimilar). The subsequent question the reviewer raises, however, are very much to the point, and help us tremendously to sharpen our manuscript.

The discussion around Model 4 (P 10885) may be the most interesting nuance of the paper, but I wonder if one has to invoke a optimized enzyme production model to get this same result? Could an empirical function between temperature and turnover accomplish the same goal? What if a larger (or temperature sensitive) Km value was chosen (implying a lower affinity for substrates with increased temperatures)? More importantly, how do we quantify the "real" μ value that should be used for Model 4, if that's the important value to differentiate between first order and microbial explicit models?
What determines the cost of enzyme expenditures, and how may it be different in different soils.

Perhaps the central motivation to put forward the model with optimised enzyme production is that earlier models link enzyme production directly with microbial biomass. What determines the level of enzyme production? The optimisation of enzyme production may be viewed as an alternative to the "proportional" model, allowing microbes to adjust to the soil environment. In the REV model, it is always assumed that enzyme production scales to microbial biomass. An increase in enzyme production in the REV model is congruent with a reduction of K_M, which increases the overall affinity of microbes for substrates. Our OPT model relaxes the fixed relationship between M and enzyme production, but introduces instead a cost of enzyme production that allows microbes to optimise their growth. This replaces the production rate with another unknown parameter. At this point, we do not have a recipe to estimate the cost of enzyme expenditures rather we assumed it is a fraction of total carbon depolymerised at reference temperature.

We interpret enzyme expenditures in 2 ways:

1) The cost per unit enzyme produced, which may be related to the enzyme specifically to solubilise a particular polymer. This may be the easier term to determine experimentally or even theoretically, but may also be a function of temperature.

2) The cost of enzyme production relative to the amount of carbon depolymerised (roughly mu). Clearly, this depends on many parameters, including quality of the substrate, its accessibility, and the affinity of the enzyme for the substrate, none of which is easy to determine.

Given that all these parameter are unknown, the reviewer is right; there could be an empirical function that can get the same result. In fact, the first order solution is very close to the microbial model solution, particularly for marginal costs. Nevertheless, the microbial models and their analysis serve to lay theoretical fundament to understand microbial dynamics.

There are really two underlying modeling frameworks being used, the forward and reverse M-M kinetics (currently models 1 & 3 respectively). Overlying these basic structures the authors increase model complexity by adding maintenance respiration (Model 2), and enzyme production optimization (Model 4), but the order of these additions makes it unclear how maintenance respiration effects the reverse M-M model or how optimizing enzyme production may modify results from the forward model? I wonder if it makes more sense to restructure the results so we're able to: A) Compare forward vs. reverse configuration (these could be models 1a and 2a); then B) Layer on maintenance respiration costs (models 1b & 2b); and finally, C) Add Enzyme production optimization (models 1c & 2c).

The Reviewer is right. Rearranging the discussion and the figures generates a much clearer picture. The biggest model alterations are forward versus reverse M-M models. We keep the enzyme optimisation as part of layer 1 because this is makes a distinct change in the long term, and it fits thematically also with the discussion on how formulations of depolymerisation affect the models' dynamics. In revised manuscript, we add a 2nd layer, in which we analyse the model behavior under the conditions that microbial biomass adjusts fast to new temperature and new carbon availability. We note that this layer was not applicable to the forward M-M model, because of unstable microbial equilibrium in the short time scale. We can show that decomposition in the reverse model can be more simplified, without much loss of information. The exception is the initial response to a temperature increase. In the early phase of the temperature response, the microbial decomposition model lags the sudden increase in depolymerisation higher v_max vs the model where microbes are assumed to equilibrate quickly with the supply (See Fig 3). The fast adjustment models create a bridge between traditional and microbial model in an analytical fashion. This new set of analysis also highlights the use of the fast scale equilibrium for microbes in Table 3 in our revised manuscript, an issue raised by both reviewers. Then, we add another layer ofmaintenance respiration costs.

Based on the reviewer's suggestion we propose new figures to replace previous Figs 2 to 4 (See new Figs 2 to 4 in the revised manuscript).

The model simulations nicely compare results of the models evaluated here, but given the choice to modify parameters to achieve the same initial and final values of CUE, M, and S (P 10869, L 21) it's unclear how much the results in Fig 2 emerge because of the parameter values chosen vs. differences in model structure. Is there some apriori reason to expect these predefined responses of CUE, and substrate pools to warming?

I realize that Fig. 4 and section 4.2 tries to address this concern, but it's too derived to make much intuitive sense (beyond forward M-M models seem really wacky)- but that's a point already made in Fig. 2 and elsewhere (Wang et al. 2014).

Our challenge has been to parameterise each of these different models, such that they are comparable to each other. We chose in our first submission that to parameterise in order to create the same long-term response. We realise that this may be 'too derived' in order for the reader to be able to critically compare the models based on the figures themselves. Here we present an alternative: In layer 1, we adjust model parameter that

a) microbial biomass,CUE, and soil organic carbon are equivalent at reference temperature as in our first submission, and
b) that the initial response of respiration is the same across models.

This second parameterisation may be motivated, that short-term respiration responses are often measured in laboratory settings. This second requirement can be met by simply keeping the temperature sensitivity of maximum depolymerisation and of carbon use efficiency the same across models.
As a result, the long term changes in soil organic matter differs across the models (but not microbial biomass, (see Table 3 and Figure 2) in the final manuscript.
When we add the additional layers of microbes in quasi-steady state and maintenance respiration we do not change the parameters to fulfill requirement b)
nor do we change requirement a) when we add enzyme production cost in the enzyme optimisation model, keeping the format of our previous submission.

Because both short-term and long-term responses can now be inferred directly from the new figures (Figure 2 &3), the previous figures (in discussion the paper) with the apparent Q_10 become obsolete.

Would it be more illustrative to explore the parameter space that allows each model to hit the same initial conditions, but then potentially diverge in their responses to warming? This would provide more of a sensitivity analysis for the respective models, and illustrate potential issues with equifinality in the more complicated model (#4). Such considerations seem important, because I would assume that different parameterizations may project either an increase or decrease in microbial biomass, but currently only one set of parameters are used for each model (e.g. Model 3, discussed in the middle of page 10870).

Based on the previous comment (see above) we did change the models to hit the same initial conditions, and they now diverge in the long-term. We kept the setup for model 4 (OPT model), where the initial conditions are different, based on the carbon cost for enzyme production. I think this is usefule in such that at higher cost i) fewer microbes are able to live off a given supply of carbon, and ii) the rate of decomposition is lower, which then translates into overall higher soil organic carbon.
To address equifinality of the different cost models, we compare the relative change in soil organic matter and microbial biomass, which are smaller the higher the cost is. Similar values indeed suggest similar model behavior as in the no-cost model. We also found interesting

dynamics with respect to CUE: CUE sharply decreases, as in previous models. Yet in the model associated with cost, CUE further declines, as the substrate depletes. Lower SOM increases the fraction of carbon used towards enzyme production.

Specific comments

Introduction: There are so many clauses in the text that they become distracting to the main message being communicated. I understand this is highly editorial, but I'd recommend using more direct, precise language throughout the manuscript to directly convey the authors' intent.

We have re-addressed our introduction, and will use shorter sentences to more clearly convey our message. We hope that it helps to pose the questions that we address in this manuscript which are:
Microbial models suffer from oscillation, because there is a positive feedback between depolymerisation and microbial biomass. How do alternative formulations of depolymerisation affect this feedback?
Simple microbial decomposition models consider 1 respiration term. Does the separation of temperature dependent maintenance respiration and temperature-independent growth respiration plus other respiration trade-offs such as enzyme expenditures affect response to warming?
How do different microbial decomposition models compare against the traditional first order models?

Paragraph starting on P 10859, L 19-30: I'm not sure these features are unique to microbial models alone. (see Frey et al 2013 cited here, which uses CENTURY). Moreover, much of the partitioning of respiration fluxes could be done in first-order and microbial models. Separately, it's somewhat misleading to cite Hagerty et al. 2014, which is an observation based paper that doesn't really deal with models (the topic of the sentence here). Finally, is seems odd to cite Schimel 2013, which is a non-peer reviewed opinion / summary of Wieder et al. (2013).

The reviewer is right. The sensitivity to carbon use efficiency is not restricted to microbial models. In our revised manuscript we change that to:

Moreover, the response of soil organic matter to warming is very sensitive to microbial carbon use efficiency (CUE), because this parameter and its climate sensitivity defines the fraction of carbon remaining in the soil as processed organic matter vs. carbon removed via respiratory CO_2 (Allison et al., 2010; Frey et al., 2013; Kivlin et al., 2013; Tucker et al., 2013; Wang et al., 2013; Li et al., 2014).
We remove the Schimel (2013) reference and add wieder et al. (2013). We also remove the Hagerty et al. (2014) reference as it also does not deal with carbon use efficiency, but evaluates the effect of microbial turnover.

The paragraphs at end of the Introduction and beginning of the Materials & Methods section are nearly identical and summarize the modifications to the basic "German model". I appreciate the clear organization, but wonder if some redundancy can be removed.

We have reorganised both, the end of the introduction and the beginning of the method. The end of the introduction now lays out a road map of the paper where we say what kind of models we create, and how we analyse them. The beginning of the method we jump right into the specifics of the model families. The end of introduction now reads as "In the next section, we introduce 3 models that differ in their representation of depolymerisation. Each model will be further modified for different representation of microbial dynamics and respiration. To analyse model behavior we will evaluate the response of respiration, microbial biomass, CUE, and soil organic matter to a step increase in temperature. We will then discuss the models' behavior by comparing against traditional first order model."

Methods: I really appreciate Figure 1, which nicely summarizes the model modifications being investigated here. Is it worth adding Fig. 1b that shows the first-order model (#5) used too? Alternatively, this could be described more completely in the text (is it just a two pool model with SOM and microbial biomass (that doesn't do anything?)

We have changed Fig 1 now to explicitly show the different model families: Different formulations of depolymerisation (Fig 1a), equilibrium microbial model (where the microbial uptake at each time step is equal the microbial carbon loss via death or respiration, Fig 1b), which in the special case of Model 4 is the first order decomposition model, and and partitioning between maintenance and growth respiration (Fig 1c).

Model 3 is a reverse Michaelis-Menten models, which has been proposed and used in other microbial explicit models (e.g. Schimel & Wientraub 2003), as opposed to the forward configuration used by Allison et al 2010, on which the German model is built. References to models and the theory behind forward vs. reverse Michaelis-Menten models are likely relevant here.

We will add the Schimel and Weintraub (2003) reference to the reverse Michaelis-Menten model in the beginning of the model description section in the revised manuscript. We also provide extensive theory in the Appendix, and refer to Wang and Post,2013 for additional theoretical cases how it can emerge.

Results: The 'knife edge' results are mentioned in both results and discussion, but I'm not really clear what this refers to? Is it obvious is any of the display items? If not, could it be- it's such a strongly visual phrase it seems like it should be obvious in a figure?

In both instances, we refer to Schimel and Weintraub (2003), who used this term, and also showed the instability of the system (describe again Table 3, 2nd column? In order to get stable M, there has to be a perfect balance of parameters, i.e. referred to the knife-edge equilibrium). We feel it is not necessary to add a graph, particularly we do not want to create the impression that this finding is new (which it is not).

In Table 3 and results I'm not clear of the utility of the short times scale steady-state solution for M? Is this just to show that the forward models (#1 & 2) aren't stable & oscillate over short times scales (as evident in Fig 2b)? I'm also curious what causes the shift in the steady state equation for M in model 3 over longer times scales? It's also not

clear what part of Table 3 if being reference in the results (P 10868, L 10-12), specifically what's independent of 'M', steady state S pools? This is generally true of other microbial explicit models (see Wang et al. 2014). I'd suggest dropping the shorter times scale M response to focus on the longer time scale dynamics, or spend time discussing both.

Both reviewers mention that they have trouble seeing the value of the short-term equilibrium in Table 3 of the discussion paper. We reorganized the tables and it is table 2 now.The timescale of the microbial turnover is much shorter than the time scale of soil organic matter turnover. That is microbial biomass adjusts much faster to changes in environmental conditions than soil organic matter itself. Thus, over the timescale of microbes, soil organic matter can be approximated by a constant (it does not change that much). This allows microbe to equilibrate with the current level of soil organic matter (quasi-steady state, see also Menge et al., 2009). We can then substitute the quasi-steady state expression for microbes into the function of depolymerisation and, microbial death, and respiration. In the revised manuscript, we added a figure (Fig 3) that shows how the assumption of microbial equilibrium compares against the fully dynamic models with respect to the dynamics of decomposition and CO2 flux. Further, this analytical trick helps to build the bridge to traditional first order models, because the formulations of decomposition are now independent of the microbial biomass. For example depolymerisation in Model 3 now becomes:

D = V_max*S*epsilon– K_M*lambda_d

Similar, the decomposition in the OPT model is analytically the same as the first order decomposition model.

The authors never refer to Fig. 3 in the results, but I assume the first paragraph on P 10871 refers to these results?

Yes, that is correct, this paragraph described the Fig 3 results. All figures are referenced in our revised manuscript.

I wonder if the lack of apparent changes of Q_10 in the first order model (#5) are an artifact of the analysis done here, or the very simplified model structure being considered (see Koven et al. 2015).

Q_10 in the first order model is higher than 1, so there is a (albeit small) temperature response also in model 5. The much lower Q_10 stems from our initial modeling setup to force the results to the same beginning and end values for CUE, soil organic carbon and microbial biomass. This required us to set Q_10 for Vmax to be 1, while only respiration was temperature sensitive. Based on the reviewer's suggestion, we now do not force the model to the same end-points, but through the same initial response to temperature. The apparent Q_10 figure (Fig 4 in the discussion paper) was intended to compare short-term vs. long-term responses. The new modeling setup allows us now to compare short-term vs. long-term responses in a more direct fashion. Thus the 'too derived' Fig 4 in our initial manuscript becomes obsolete.

Discussion: The beginning of the discussion reads too much like the introduction. In my mind, the discussion should highlight key finding of the work presented here, not a literature review on microbial models.

In our revised manuscript, we shorten the first paragraph of the discussion. It was our intention to acknowledge earlier work. We tip our hats to these researchers now in appropriate places throughout the section, and more directly in conjunction with the discussion of our results.

I wonder if you really need the nuances of maintenance respiration and CUE to get a reverse Michaelis-Menten model to approximate a first order model? Just looking at equation 9, if Km is small (relative to M [P 10866, L 23]) then D = V_max * S (basically eq. 14).

This is correct, there is no need of nuanced respiration and CUE to get a first order model. We can demonstrate that now even better, with the suggested layering of the model. In previous model 4 (the OPT model now, and in absence of enzyme production cost), the decomposition equation is exactly a first order model. However, what needs to be considered in some way is a temperature dependent CUE. That is how much carbon is being rerouted back into soil organic carbon pool.

Material in the Appendix is frequently referred to in the discussion; however, it's not really clear what part of the Appendix readers should direct their attention. Moreover, it's not really clear if or how the mathematical derivations in the Appendix are (or are not) used in the main display items and results of the paper. If the material in the Appendixes are being used for simulations presented they should be clearly referenced in the main text. In my mind the Appendixes should NOT be used as a large parenthetical to house fancy mathematical derivations that don't inform the larger manuscript.

Our intention of the appendix was to not clutter the method section with detailed mathematical derivations, but provide the readers with the necessary tools to recreate the differential equations for depolymerization, and the quasi- steady state of enzymes and DOC pools. However, in retrospect we can relate to the reviewer (and readers) not seeing the link between the method and appropriate parts and equations in the appendices. In our revised manuscript, we have the appendix clearer referenced and clearer structured with sections and section titles.

I appreciate the need to use simple models like this to understand the mathematical dynamics of microbial explicit models, but how much do we lose by using such a simple model that it doesn't really represent soil C dynamics at large spatial, or long temporal scales? There's some of this at the end of the discussion, but greater introspection into how this study may inform ecosystem scale models (or larger) that are used for soil C projections would be helpful.

We will add to the discussion, and more importantly in the conclusion how the evaluation of simple models can serve larger scale models. In the discussion, we explain, that our framework provide ecosystem modelers with a mechanistic handle, when decomposition dynamics is expanded to include multiple substrate with different response to microbial processing. In the

conclusion, we highlighted how specific mechanisms lead to transformations from a forward to a backward, and what it means to relax the proportionality of microbial biomass and allow microbes to "choose" enzyme investment. Our manuscript also lays out what the specific parameters are composed off under these mechanisms. This provides ecosystem modelers insights when expanding to more complex representations, such as multiple quality pools.. Further, we show that even in simple models, the response to temperature is a composite of parameters that are hard to come by, including half saturation constants, sensitivity of microbial respiration to temperature, the amount of enzyme produced by microbes, as well as enzyme activity.
Finally, our work shows mathematical linkages between first order decomposition model and microbial models, which help to understand and potentially improve first order models, as more nuanced microbial models are being developed.

P 10858, L 5-6 This sentence is somewhat awkward and doesn't seem grammatically correct.

Changed to "Under sufficiently large substrate, this new feedback allows an unconstrained growth of microbial biomass."

P 10858, L 6 I'd recommending modifying the beginning of this sentence by adding 'often' or some other qualifier. For example: "A second phenomenon 'often' incorporated in microbial decomposition models"

We changed the abstract and talk now about different respiration at the end of the abstract.

P 10859, L 29 Wieder et al. 2014a doesn't deal with microbial models (as implied by the text in the sentence. A better references may be Wieder et al. 2015, Geoscientifc Model Development.

We substitute the reference as suggested.

L 10860, L 6 What are "dynamical consequences"?

We change that to "This differentiation can impact the dynamics of the microbial biomass".

I appreciate thorough documentation supplied in the Appendix, but to aid in reader understanding can specific parts of the Appendix be referred to in the main body of the text where appropriate (e.g., sections 2.1.3 & 2.1.4)? Were are A1, A2:: etc. referred to in the text? (see also P 10873 L 23 and P 10874 L 10)

We have separated appendix in Appendix A (short-term dynamics of the enzymes and DOC), B (derivation of the REV model), and C (derivation of the OPT model). Now, our revised manuscript have a clearer link to the specific parts in the appendix in the method section and throughout the text.

P 10869, L1-2 this statement is not obviously supported by results presented in this paper.

This part of the result section is different in the improved manuscript, due to the altered modeling setup.

Figure 3: It's not immediately obvious to what model this figure refers? The green color chosen is painful to look at.

We change the caption to make clear that the simulations refer to OPT model. We also have changed the color scheme.

P 10873 L 23: It's nice that the authors derived a reverse M-M model (from the forward configuration), but it seems like a lot of work to replace a term in the denominator of an established model seem like a lot of work. I'm not sure how much the derivation is warranted in the Appendix.

Respectfully, we would like to keep this part in the appendix, since we explicitly point to two specific mechanisms that can change a forward M-M model into a reverse model. Showing the full derivation helps the reader to understand that transition. We make a point to give specific examples how one arrives at a reverse Michaelis-Menten formulation, since they provide a juxtaposition to enzyme limited models.

Paragraph beginning on P 10875, L 10 should reference Fig 3.

This paragraph has changed. The warming response in model 4 is now not confined to the temperature sensitivity of microbial respiration, but also to the depolymerisation rate. We make clea reference to our new Fig 4 in the result and discussion section of the revised manuscript.

P 10877 L 17-19 This sentence is completely unsubstantiated and should be qualified & reference or removed.

The reviewer is right. Through the modifications of the modeling setup, this sentence is not needed.

P 10877 L 20-21 This seems like completely throw away sentence that should be removed since no discussion of experiments and observations are used or discussed earlier in the paper.

Both comments are taken care of by rewriting the conclusion.).

References cited in the response to reviewer comment:

Allison, S. D., Wallenstein, M. D., and Bradford, M. A.: Soil-carbon response to warming dependent on microbial physiology, Nature Geosci., 3, 336–340, doi:10.1038/ngeo846, 2010.

German, D. P., Marcelo, K. R. B., Stone, M. M. and Allison, S. D.: The Michaelis-Menton kinetics of soil extracellular enzyme in response to temperature: a cross-latitudinal study, Glob. Change Biol., 18, 1468–1479, doi:10.1111/j.1365-2486.2011.02615.x, 2012.

Hagerty, S. B., van Groenigen, K. J., Allison, S. D., Hungate, B. A., Schwartz, E., Koch, G. W., Kolka, R. K., and Dijkstra, P.: Accelerated microbial turnover but constant growth efficiency with warming in soil, Nature Clim. Change, 4, 903-906, doi:10.1038/nclimate2361, 2014.

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Menge, D. N. L., S. W. Pacala, and L. O. Hedin. 2009. Emergence and maintenance of nutrient Limitation over multiple timescales in terrestrial ecosystems. The American Naturalist, 173:164–175.

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Schimel, J. P. and Weintraub, M. N.: The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model, Soil Biol. Biochem., 35, 549-563, doi:10.1016/S0038-0717(03)00015-4, 2003.

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

Comparing models of microbial-substrate interactions and their response to warming

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Abstract

Recent developments in modelling soil organic carbon decomposition include the explicit incorporation of enzyme and microbial dynamics. A characteristic of these models is a positive feedback between substrate and consumers~~,~~ which is absent in traditional first order decay models. Under ~~sufficiently~~sufficient large substrate, this new feedback allows an unconstrained growth of microbial biomass. ~~We~~A second phenomenon incorporated in the microbial decomposition models is decreased carbon use efficiency (CUE) with increasing temperature. Here, first we analyse microbial decomposition models by parameterising changes in CUE based on the differentiation between growth and maintenance respiration. We then explore mechanisms that curb unrestricted microbial growth by including finite potential sites where enzymes can bind and by allowing microbial scavenging for enzymes. ~~We further developed~~Finally, we propose a model where enzyme synthesis is ~~not scaled to microbial biomass, but~~ associated with a respiratory cost and microbial population adjusts enzyme production in order to optimise their growth. ~~We then tested short and long term responses of these models to a step increase in temperature, and find that these models differ in the long term, when short term responses are harmonized. Oscillations that arise from a positive feedback between microbial biomass and depolymerisation are eliminated if limitations other than through enzyme-substrate interactions are considered. The model, where enzyme production is optimised to yield maximum microbial growth shows the strongest reduction of soil organic carbon in response to warming, and the trajectory of soil carbon largely follows that of a first order decomposition model. Modifications to separate growth and maintenance respiration generally yield short term differences, but results converge over time, because~~

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~~microbial biomass approaches a quasi-equilibrium with the new conditions of carbon supply and temperature.~~

When applying a step increase in temperature, we find fast responses that reflect adjustments to enzyme dynamics and maintenance respiration, a short-term adjustment in microbial growth, and the long-term change in carbon storage. We find that mechanisms that prevent unrestricted microbial growth lead to a similar response to warming as traditional first order decomposition models.

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1 Introduction

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Traditional soil organic matter decomposition models are based on first order kinetics, where decomposition scales to the pool size~~. The~~ and the scaling factor represents recalcitrance of a specific pool, ~~and is~~ modified by soil temperature, moisture, and other ~~soil properties~~factors (e.g. van Veen et al., 1984; Parton et al., 1987; Molina et al., 1990; Li, 1996; Chertov and Komarov, 1997). Recent modelling efforts have specifically included catalysis of polymeric soil organic carbon to dissolved organic carbon ~~(DOC)~~ by extracellular enzymes~~. This depolymerisation step~~ produced by microorganisms in soil, which is thought to be ~~a~~the rate-limiting step in organic matter decomposition process (Schimel and Weintraub, 2003; Fontaine and Barot, 2005). Further, these microbial models explicitly consider carbon use efficiency (CUE) as a function of soil temperature. The resulting prediction of soil carbon dynamics suggests that an increasing temperature attenuates the loss of soil organic matter compared to traditional models (Allison et al., 2010).

In traditional models, microbes are only considered as a simple donor-controlled pool (i.e, microbial biomass has no impact on decomposition), or in an implicit manner (Gerber et al., 2010). In contrast, in the microbial models, decomposition rates become a function of enzyme activity that is linked to microbial biomass. This leads to a more complex dynamics because decomposers feed back into soil organic matter degradation via microbial enzyme production affecting depolymerisation~~.~~, the first step of organic matter decomposition. This positive feedback between microbial biomass and depolymerisation causes soil organic carbon stocks and microbial biomass to oscillate after a perturbation (Li et al., 2014; Wang et al., 2014). ~~Nevertheless, microbial~~Microbial decomposition models have been shown to improve the prediction of soil carbon and perform well when compared against decomposition experiments (Lawrence et al., 2009; Wieder et al., 2013; Wieder et al., 2014a; Wieder et al.,

2014b; Wieder et al., 2015b). A comparison to traditional first order model show further that microbial model display an attenuated loss of soil organic matter to warming (Allison et al., 2010; Wieder et al., 2013).

MoreoverFurther, the response of soil organic matterthe microbial decomposition models to warming is very sensitive to microbial carbon use efficiency (CUE), because this parameter and its climate sensitivity defines the fraction of carbon remaining in the soil as processed organic matter vs. carbon removed via respiratory CO_2 (Allison et al., 2010; Frey et al., 2013; Kivlin et al., 2013; Schimel, 2013; Tucker et al., 2013; Wang et al., 2013; Li), and turnover (Hagerty et al., 2014). Temperature dependence of CUE is typically not considered in traditional decomposition models, rather the ratios between respired CO_2 and the transfer to a different quality pool are mostly constant parameters, or vary based on soil texture, and soil quality, and organic or inorganic nutrient (Parton et al., 1987; Gerber et al., 2010; but see Frey et al., 2013). Microbial respiration can be partitioned into a series of carbon expenditures that do not contribute to growth. These expenditures , which include growth respiration, maintenance respiration, respiratory cost expenditures for enzyme production, and overflow respiration (Manzoni et al., 2012; Moorhead et al., 2012). Each type of respiratory carbon expenditures may differdiffers in itstheir response to temperature. In addition, respiration may be parameterised based on different microbial properties: Maintenance respiration is assumed to scale with microbial biomass (Chapman and Gray, 1986; Fontaine and Barot, 2005) while growth respiration may scale to the amount of new tissues built. On the other hand, overflow respiration (Russell and Cook, 1995; Franklin et al., 2011) occurs during stoichiometric adjustment (Russell and Cook, 1995; Schimel and Weintraub, 2003; Frost et al., 2005; Franklin et al., 2011) whereas costs related to enzyme production may be governed by microbial demand and substrate availability and quality, resource diffusion, and microbial diversity (Allison, 2005). This differentiation can impact the dynamics of the microbial biomasshave dynamical consequences: For example, maintenance respiration costs would incur even in the absence of carbon uptake, which can lead to a reduction in microbial biomass. In contrast, growth respiration is only due when substrate for growth is available. However, inclusionInclusion of these microbial models in Earth System Modelsto the coupled climate models by following the framework of Todd-Brown et al. (2012, 2013) may have the potential to ultimately reduce uncertainty of climate-carbon feedback in the face of climate change, because of the explicit link between microbial activity and soil organic matter degradation (Todd-Brown et al. 2012, 2013; Wieder et al., 2015a).

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~~As microbial models are considered critical towards improvement of Earth System model, it is key to analyse and understand their structure and their dynamics. Here, we compare a series of microbial decomposition models with each other. Specifically, we analyse feedbacks between depolymerisation and microbial growth, consider constraints on depolymerisation and enzyme substrate interactions, the parameterisation of microbial enzyme productivity, and investigate the representation of microbial respiration and CUE.~~

Here, we apply a series of simple microbial decomposition models and investigate how different formulations of carbon use efficiency and depolymerisation of soil organic matter affect decomposition.

Our main questions are:

~~a~~a) How does separating microbial respiration into growth, maintenance, and enzyme production terms affect decomposition dynamics?

b) How do different model implementations of depolymerisation affect the feedback between microbial biomass and soil organic matter, if subjected to warming?

~~b) How does the consideration of functional respiration terms (growth, maintenance, and carbon acquisition expenditures) affect decomposition dynamics?~~

We organise the paper in the following way~~. In the next section~~: First, we introduce ~~3 simple models that differ in their representation of depolymerisation. Each model will be further modified for different representation~~a series of microbial ~~dynamics and respiration. To analyse model behaviour we will evaluate the response of respiration, microbial biomass, CUE, and~~ decomposition models. Each of which carries single soil organic matter ~~to a step increase in temperature.~~and a single microbial pool. In sequential model modifications, we include differentiation between growth and maintenance respiration, introduction of mechanisms where depolymerisation may be curbed by limited sites of enzyme-substrate reaction or by microbial scavenging for enzymes, and by respiratory costs associated with enzyme production. We ~~will~~ then ~~discuss the models' behavior by comparing~~present analytical equilibrium solutions to infer long-term values of carbon use efficiency, soil organic matter, and microbial biomass. For each model, we test its response to a 5°C warming. Finally, we compare the results against a traditional ~~first order~~decomposition model.

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2 Materials and methods

2.1 Model descriptions

~~We first introduce three model families that differ in the way depolymerisation is handled.~~

~~In all models the setup consists of a single soil organic matter pool and a single microbial pool (Fig. 1).~~We apply five different microbial decomposition models (Fig. 1, Table 1). We start off with a simple microbial-enzyme decomposition model as proposed by Allison et al. (2010) and modified by German et al. (2012). We sequentially alter the model as we make distinction between growth and maintenance respiration (model 2), then different implementations of depolymerisation: we develop a case for diminishing return where increasing enzyme concentrations or microbial biomass result in decreasing marginal depolymerisation (model 3), and provide a model, where the microbial population adjusts enzyme production to optimise growth (model 4). All models describe the dynamics of a single soil organic matter pool and a single microbial pool. However, all models also implicitly take into account interaction between enzymes and substrate, depolymerisation of substrate into a ~~DOC pool on which microbes can feed. Enzyme substrate reactions are based on Michaelis Menten kinetics (see Appendix A, Michaelis Menten kinetics with enzyme denaturation). We do not consider a specific enzyme pool, nor a specific DOC pool, but assume that the enzyme and DOC pool are in a quasi-steady state (Appendix A, DOC and enzyme dynamics). Thus, the amount of enzyme produced equals the amount of enzyme decay at every time step. Similarly, the amount of DOC produced is the same as the amount of DOC consumed by microbes. In contrast to Allison et al. (2010), but congruent with German et al. (2012), there is no "free" DOC, both fresh litter, and microbial necromass need to be depolymerised before it can be ingested by microbes.~~dissolved organic carbon pool on which microbes can feed. Further, both depolymerisation and microbial respiration are temperature dependent, causing increased depolymerisation and reduced microbial ~~CUE with warming.~~carbon use efficiency with warming. We then will evaluate these models under a step increase in temperature.

~~*2.1.1. Base Models*~~

Model 1: German Model

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The tendency ~~(derivative with respect to time)~~ for soil organic carbon and microbes in ~~all of~~ the ~~models are~~German et al. (2012) model is described with~~:~~

$$\frac{dS}{dt} = I + \lambda_d * M - D \quad (1)$$

$$\frac{dM}{dt} = D * \varepsilon - \lambda_d * M \quad (2)$$

where S and M are the soil organic matter and the microbial pool, respectively, I the input of fresh litter, λ_d the death rate of microbes, D the rate of depolymerisation, and ε the microbial ~~CUE.~~

~~Forward M-M Model (FWD)~~

~~In the forward model (FWD), depolymerisation~~growth efficiency. Depolymerisation is ~~represented~~parameterised as a Michaelis-Menten process~~, and stems from the simple microbial enzyme decomposition model as proposed by Allison et al. (2010) and modified by German et al. (2012) (Fig 1a).~~

with

$$D = \frac{\frac{V_{max,FWD} * S * M}{K_E + S}}{} \frac{V_{max1} * S * M}{K_E + S} \quad (3)$$

Where ~~D is the rate of depolymerization, $V_{max,FWD}$~~$Vmax_1$ is the maximum depolymerisation rate and K_E the half saturation constant for enzymes. Both, $Vmax_1$ and K_E are temperature dependent, where

$$V_{max1} = V_{max1,0} * Q_{10}^{(\frac{\Delta T}{10})} \quad (4)$$

$$K_E = K_{E,0} * Q_{10}^{(\frac{\Delta T}{10})} \quad (5)$$

where $V_{max1,0}$ and $K_{E,0}$ are the maximum rate of depolymerisation and the half saturation constant at reference temperature, respectively, and ΔT is the temperature difference compared to reference temperature.

ε depends linearly on temperature:

$$\varepsilon(\Delta T) = \varepsilon_0 + \Delta T * \varepsilon_{slope} \quad (6)$$

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where ε_0 is the carbon use efficiency at reference temperature, and ε_{slope} the change in carbon use efficiency per °C temperature (ΔT) change. Implicit in this model is that microbial enzyme productivity scales to microbial biomass (see also Appendix ~~A shows the derivation~~), and that depolymerised carbon is at steady state with rates of ~~this function, based on enzyme substrate~~depolymerisation and microbial uptake (German et al., 2012).

Model 2: Modified German Model (include maintenance respiration rate)

While the dynamics~~.~~ of the soil organic matter pool remains the same as in model 1, we partition microbial respiration into growth and maintenance respiration. The dynamics of the microbial pool is then characterised with

$$\frac{dM}{dt} = (D - \lambda_r * M)(1 - g) - \lambda_d * M \quad (7)$$

Where g is the growth respiration fraction and λ_r the maintenance respiration rate. The separation of microbial respiration in growth and maintenance terms is motivated by similar formulation in other microbial (Beefting et al., 1990; Van Bodegom, 2007), vegetation growth (Foley et al., 1996; Cannell and Thornley, 2000; Arora, 2002; Thornley, 2011; Pretzsch et al., 2014), and ecosystem-scale (Sistla et al., 2014) models. Growth respiration is applied after requirements for maintenance respirations are met. Maintenance respiration (respiration related to non-growth components) is typically proportional to microbial biomass (Van Bodegom, 2007). Growth respiration is typically much less sensitive to warming than maintenance respiration (Frantz et al., 2004). Hence, we apply a constant growth respiration and parameterise the temperature sensitivity of maintenance respiration with a Q_{10} function:

$$\lambda_r = \lambda_{r,0} * Q_{10}^{\left(\frac{\Delta T}{10}\right)} \quad (8)$$

Where $\lambda_{r,0}$ is the maintenance respiration rate at reference temperature.

Model 3: Diminishing Return ~~(REV)~~ Model

In the Appendix ~~B~~, we derive two ~~depolymerisation~~ models which show a diminishing increase of

depolymerisation as microbial mass increases. These models include a) a case where microbes are scavenging for free enzymes, and b) where potential sites of enzyme-substrate reactions are finite. ~~The implementations of~~ We simplified depolymerisation in these ~~factors lead~~

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~~to~~diminishing return models such that it becomes again a ~~reverse~~ Michaelis-Menten type ~~model (REV) as in Schimel and Weintraub (2003):~~function:

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$$~~D = \frac{V_{max,REV} * S * M}{K_M + M}~~ D = \frac{V_{max3} * S * M}{K_M + M} \quad (~~4~~9)$$

Where K_M is a half saturation constant that determines the diminishing return function. In the cases developed in the Appendix, K_M incorporates factors indicating the finite sites for enzyme substrate interactions ~~(Appendix B, model with limited available substrate),~~ or the efficiency with which microbes scavenge for free extracellular enzymes ~~(Appendix B, microbial consumption of enzymes). A version of the reverse Michaelis-Menten model also has been derived if only a fraction of the binding sites where a particular enzyme can adsorb to (Wang and Post, 2013).~~. A major difference to ~~the FWD model~~models 1 and 2 is that now the microbial biomass, instead of the amount of soil organic matter appears in the denominator. Therefore, the depolymerisation per unit biomass decreases as biomass increases (diminishing return).

~~Model 4:~~ Optimised Enzyme Production ~~(OPT)~~ Model

~~In this model, we relax the condition that microbial enzyme production scales to microbial biomass, an assumption that is present in many microbial models and which is also assumed in the FWD and the REV model above. Instead we~~We further probe a model where microbial enzyme production is optimised for growth. We motivate this by microbial competition (Allison, 2005), which will allow microbes to succeed if microbial enzyme production allows the highest possible return. Optimisation only has meaningful results for the case of limited substrate availability (i.e. a diminishing return, possibly through constraints in potential sites for enzyme-substrate reaction) and if there is a cost associated with microbial enzyme production.

Depolymerisation as a function of enzyme production can be ~~represented~~parameterised by

$$D(P) = ~~\frac{P * V_{max,OPT} * S}{K_P + P}~~ \frac{P * V_{max4} * S}{K_P + P} \quad (~~5~~10)$$

~~$V_{max,OPT}$~~Where P is the ~~maximum rate of depolymerisation~~microbial enzyme production and K_P ~~carries information on the affinity of the enzyme for the substrate and longevity of the enzyme~~a half saturation constant (see the Appendix ~~C,~~ for full derivation ~~of depolymerisation in the OPT model).~~and interpretations of V_{max4} and K_P) .

Microbial growth (G) is as in previous models but accounts for carbon expenditure of enzyme production:

$$G = \varepsilon * (= (1 - g)\,(D(P) - Pc) \quad (6 - \lambda_r * M) \qquad (11)$$

Where c is the respiratory cost per unit enzyme produced (Schimel and Weintraub, 2003).

Optimising growth by setting $\frac{dG}{dP} = 0$ yields:

$$D = V_{max,OPT} V_{max4} * S - \sqrt{K_P * c * V_{max,OPT} * S}\sqrt{K_P * c * V_{max4} * S} \qquad (712)$$

And A nd the cost per unit carbon depolymerised is then

$$\frac{Pc}{D} = \frac{\sqrt{\frac{K_P c}{S V_{max,OPT}}}}{} \qquad (8)$$

2.1.2. Equilibrium microbial models

While the previous models are fairly simple, we further reduce the complexity by removing microbial biomass as a state variable, but instead consider M at a quasi-steady state. In the equilibrium microbial models, the microbial uptake at each time step is thus equal to the microbial carbon loss via death or respiration (Fig 1b). This is similar to our treatment of DOC and enzymes, where production and removal of these substances are always balanced. This simplification is motivated by the fact that microbial biomass turns over much faster than soil organic matter, and therefore microbial biomass adjusts much faster to changes in environmental conditions than soil organic matter itself. The fast turnover of M compared to S allows microbial biomass to (quasi) equilibrate with the current level of soil organic matter (see also Menge et al., 2009).

In our equilibrium microbial models, we solve $\frac{dM}{dt} = 0$, in order to obtain a quasi-steady state microbial biomass, $\bar{M}$. $\bar{M}$ substitutes state variable M in the functions for depolymerisation and microbial death. We note that this is only possible for the REV and the OPT model. The FWD model yields no solution for M in $\frac{dM}{dt} = 0$, and the first order model does not consider a

microbial biomass in the first place. The equilibrium models, effectively becomes a one-pool model, where depolymerisation is not a direct function of microbial biomass, but an expression of S and a series of parameters. Table 2 (see formulations for Short/Fast timescale) shows the quasi-steady state for M, and the resulting depolymerisation function for the equilibrium models.

2.1.3. Partitioning between maintenance and growth respiration

While the dynamics of the soil organic matter pool remains the same as in base model setup, we alter the forward and the reverse Michaelis-Menten models as we make distinction between growth and maintenance respiration (Fig 1c). Partitioning of microbial respiration into growth and maintenance respiration characterise the microbial pool as follows:

$$\frac{dM}{dt} = (D - \lambda_r * M)(1 - g) - \lambda_d * M \quad (9)$$

Where g is the growth respiration fraction and λ_* the maintenance respiration rate. The separation of microbial respiration in growth and maintenance terms is motivated by similar formulation in other microbial (Beefting et al., 1990; Van Bodegom, 2007), vegetation growth (Foley et al., 1996; Cannell and Thornley, 2000; Arora, 2002; Thornley, 2011; Pretzsch et al., 2014), and ecosystem scale (Sistla et al., 2014) models. Growth respiration is applied after requirements for maintenance respirations are met. Maintenance respiration (respiration related to non-growth components) is typically proportional to microbial biomass (Van Bodegom, 2007).

2.1.4. First Order

$$\mu = \sqrt{\frac{K_P c}{S V_{max4}}} \quad (13)$$

Model 5: Traditional Decomposition (FOD) Model

The last model represents is the structure of traditional decomposition model such as CENTURY (Parton et al., 1987) or Roth-C (Coleman et al., 1996) and their derivatives, where

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~~decomposition is considered as a first order reaction~~, with the modification that carbon use efficiency changes with temperature:

$$\frac{dS}{dt} = \text{~~I − S ∗ k ∗ (1 − ε)~~} \quad \text{~~(10~~} S * k * Q_{10,k}^{\left(\frac{\Delta T}{10}\right)} * \varepsilon(\Delta T) \quad (14)$$

where k is the first order decomposition constant~~. The two major differences between our first-order~~ , and $Q_{10,k}$ is the temperature sensitivity factor of the decomposition rate. Model 5 can also be considered as a special case of model 4, where the cost of enzyme production is zero, and the microbial biomass is at an instantaneous equilibrium with the rate of decomposition ~~(FOD) model and~~ . Respiration (R) is then

$$R = S * k * Q_{10,k}^{\left(\frac{\Delta T}{10}\right)} * (1 - \varepsilon) \quad (15)$$

We note, that here – in contrast to traditional models ~~are that we consider only a single carbon pool whereas traditional models consider several quality pools that feed into each other. We also consider a temperature dependent~~ CUE ~~on top of a temperature dependent processing rate (k, see parameterisation and implementation section). This increases the fraction of carbon processed with warming to become CO_2. Respiration (R) is then~~decreases with temperature.

~~$R = S * k * (1 - \varepsilon)$ (11)~~

~~2.2 Temperature response~~

~~We implement the response of decomposition to warming by modifying the depolymerisation and the microbial respiration.~~

~~In the FWD, REV and OPT model, V_{max} is modified as~~

~~$V_{max,i}(\Delta T) = V_{max,i} * Q_{10}^{\left(\frac{\Delta T}{10}\right)}$ (12)~~

~~Where $V_{max,i}$ and $V_{max,i}(\Delta T)$ are reference and the temperature dependent maximum depolymerisation rate of the model i = (FWD, REV, OPT). Similarly, k is modified by the Q_{10} function in the FOD model.~~

~~Further, we also parameterise CUE as a linear function of the temperature change~~

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~~$\varepsilon(\Delta T) = \varepsilon_0 + \Delta T * \varepsilon_{slope}$ (13)~~

~~where ε_0 is the CUE at reference temperature, and ε_{slope} the change in CUE per °C temperature (ΔT) change. Finally, in the models where we partition growth and maintenance respiration, we formulate maintenance respiration as a Q_{10} function of temperature~~

~~$\lambda_r(\Delta T) = \lambda_{r,0} * Q_{10}^{(\frac{\Delta T}{10})}$ (14)~~

~~Where $\lambda_{r,0}$ and $\lambda_r(\Delta T)$ are maintenance respiration rate at reference and elevated temperature. Growth respiration is typically much less sensitive to warming than maintenance respiration (Frantz et al., 2004), and we therefore do not consider a temperature dependence of this particular respiration term.~~

~~In our simplified model we further neglect the weaker temperature dependence of the half saturation constants (see Davidson et al., 2012; German et al., 2012; Stone et al., 2012), and also do not consider changes in cost of enzyme production as temperature increases in the case of the OPT model.~~

~~2.3~~2.2 Parameterisation and implementation

All models are implemented in STELLA, version 10.0.3. ~~To enable comparison among the models we adjust~~We tune parameters in the ~~following way: The~~five microbial-enzyme models such that all models ~~have~~result in the ~~same initial soil organic carbon and the same initial~~equal amount of microbial biomass~~. Both CUE (ε) , ~~, substrate, and carbon use efficiency, at equilibrium for two temperatures, 15°C and ~~its temperature dependence (ε_{slope})~~ 20°C. We are ~~the same across models. Further, the temperature sensitivities of V_{max} are identical accross models so that we obtain the same increase of depolymerisation in the first time step after the temperature perturbation. We motivate this kind of parameterisation by acknowledging~~aware that many of ~~these~~the parameters are largely unknown~~, but it will provide us~~ and there is ample room for parameter adjustment. Here, we seek congruency of the models in their long-term response of 3 crucial variables, namely carbon use efficiency, soil organic matter, and microbial biomass, and evaluate their transient response instead.

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We start off with ~~the possibility of comparing the functional response to long-term warming across these models.~~

~~We~~model 1 where we use the parameters as reported in German et al. (2012), ~~with a few modification. Here~~however, we report ~~$V_{max,FWD}$~~V_{max1} and K_E by including tuning factors and by considering 15°C as our reference temperature ~~and by working their tuning factors directly into these two parameters.~~ In other words, ~~$V_{max,FWD}$~~V_{max1} and K_E are the product of the reference values in German et al. (2012), their respective tuning parameters and their adjustment to our reference temperature, 15°C. Further, we have converted the exponential temperature sensitivity of ~~$V_{max,FWD}$ into~~V_{max1} and K_E in model 1 to a Q_{10} term.

In model 2, to obtain the same equilibrium values for substrate, microbial biomass, and carbon use efficiency, we adjust g, λ_r, and $Q_{10,\lambda r}$. We first parameterised maintenance respiration, where, the coefficient for maintenance respiration is scaled to microbial turnover (Van Bodegom, 2007). We assume that carbon turnover from maintenance estimation is ca. one-third of microbial death rate such that:

$$\lambda_{r,0} = 0.334 * \lambda_d \quad (16)$$

this constrains g at reference temperature to

$$g = \frac{\lambda_d - \varepsilon_0 * (\lambda_d + \lambda_{r,0})}{\lambda_d - \varepsilon_0 * \lambda_{r,0}} \quad (17)$$

To obtain the same equilibrium values of CUE, S, and M, at 20°C as in model 1, we adjust $Q_{10,Vmax2}$ and $Q_{10,\lambda r}$ such that model 2 has the same carbon use efficiency as model 1 (which in turn results into the same microbial biomass and soil organic carbon).

In model 3, we again seek to obtain the same equilibria values for carbon use efficiency, microbial biomass, soil organic matter, and decomposition at 15°C and 20°C. To allow a diminishing return mechanism, we assumed that most of the enzyme decay/loss in a scavenging model is attributed to microbial consumption instead of denaturation. Alternatively, under conditions of limited enzyme-substrate reaction sites, we assumed that there is an excess of free enzymes, and therefore, enzyme concentrations are higher than their corresponding half saturation concentrations. Overall, these assumptions would suggest a K_M that is smaller than M (K_M <M). Here, we chose K_M to be 0.37 of M at the reference temperature. Note, that the half saturation constant in ~~the REV~~this model has ~~a~~ different formulations (unit ~~(~~:mgM cm^{-3}) than ~~in~~ the ~~FWD model (~~previous models (unit:mgS cm^{-3}~~)~~

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~~(see Appendix A for the FWD model). V_{max3}~~ and ~~Appendix B for the REV model).~~ ~~$V_{max,REV}$~~$Q_{10,Vmax3}$ are ~~then~~ tuned to yield equivalent equilibrium values of S ~~at the reference temperature~~.

In ~~the OPT~~ model ~~4~~, we adjust ~~$V_{max,OPT}$~~V_{max4} and $Q_{10,Vmax4}$ (in a similar manner ~~as in the REV~~like model ~~3~~) such that the system again yields equilibrium values for S at ~~the reference temperature (~~15°C~~) and the same initial response to warming~~ and at 20°C as in the other models~~. In the OPT model, we have to work in two additional parameters, namely the cost of enzyme production (c), and the term that contains the affinity of enzymes for the substrate (K_P). We chose to have the OPT models comparable to others~~ if ~~the cost (~~c~~)~~ is zero. Higher costs (c>0) therefore will yield different equilibrium result ~~of S and a different response to warming~~, depending on the cost of enzyme production. Both, the half saturation constant ~~(affinity parameter, K_P)~~ and the cost per enzyme produced are parameters that are hard to come by. Instead, the solution allows us to quantify these based on ~~how much of carbon depolymerised is allocated to enzyme production~~variable fractions of depolymerisation (see ~~Eq. 8 in the main text).~~Appendix).

Here, we analyse the ~~OPT~~ model ~~4~~ based on different levels of enzyme expenditures and expressed them as enzyme costs per unit carbon depolymerised ($\mu = \frac{Pc}{D}$), where μ is 0, 10, and 50 percent of the depolymerisation rate at reference temperature ~~and at steady state.~~. This yields an expression for the combined cost (c) and the half saturation constant (K_P) ~~(Y in Table 2~~):

$$K_P * c = \mu^2 * D_{Eq.,\Delta T=0} \quad \text{~~(15}* Q_{10}^{\left(\frac{\Delta T}{10}\right)}$$

Where $D_{Eq.,\Delta T=0}$ is the rate of depolymerisation at ~~zero~~0 enzyme cost and reference temperature.

~~When separating growth and maintenance respiration we sought to equalise steady state CUE, M, and S by tuning g and λ_t. We first parameterised maintenance respiration, where the coefficient for maintenance respiration is scaled to microbial turnover (Van Bodegom, 2007). We motivate the partitioning between growth and maintenance respiration based on vegetation models. LPJ (Sitch et al., 2003) and ED (Moorcroft et al., 2001) have a growth respiration factor of one third of the carbon allocated to growth. We then constrain the overall respiration by the CUE in German et al. (2012), and obtain a maintenance respiration rate by~~

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~~difference. This yields a maintenance respiration rate that is close to the microbial death rate such that:~~

$$\lambda_{r,0} = 1.25 * \lambda_d \quad (16)$$

The ~~second parameter, g is adjusted, such that the CUE at the steady state and reference temperature~~temperature ~~remains~~sensitivity of half saturation constant is the same~~. This constrains g to~~

$$g = \frac{\lambda_d - \varepsilon_0 * (\lambda_d + \lambda_{r,0})}{\lambda_d - \varepsilon_0 * \lambda_{r,0}} \quad (17)$$

~~To obtain the same equilibrium values of CUE at 20°C~~ as ~~in the base models, we adjust $Q_{10,k}$ such that models with maintenance respiration has the same CUE as in the base models.~~to other models.

Finally, in ~~the FOD~~ model 5, the traditional decomposition model, we adjust the parameters k, ε_0, and ~~ε_0~~$Q_{10,k}$ to obtain the same S, M, and CUE as in all other models at 15°C and ~~employ a $Q_{10,k}$ value identical to the Q_{10} values of V_{max} in the other models. We keep the decreasing CUE – a feature not typically set up in~~ at 20°C. The difference to a traditional ~~models.~~ formulation of first order decomposition is a variable (i.e. decreasing) carbon use efficiency.

All parameter values are given in ~~Table 3~~table 2.

2.3 Determination of apparent Q_{10}

We determined an apparent Q_{10}, $Q_{10}(t)$ by relating the changes of the respiration per unit soil organic matter to the changes in temperature (ΔT) at any given time (t):

$$\frac{R(t)}{S(t)} = \frac{R_0}{S_0} * Q_{10}^{\left(\frac{10}{\Delta T}\right)}(t) \quad (19)$$

Where R(t) and S(t) are the instantaneous rates of respiration and soil organic matter, respectively, and R_0 and S_0 the equilibrium respiration rates and equilibrium substrate at reference temperature.

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3 Results

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~~Base~~ We first analyse the equilibrium state of microbial biomass by setting the tendency for the microbial biomass to zero ($\frac{dM}{dT} = 0$), while assuming a constant soil organic matter pool. This is useful since in many cases microbial turnover is much faster than the turnover of bulk soil organic matter (Stark and Hart, 1997; Schmidt et al., 2007). In model 1 and 2 (German and modified German model), the microbial biomass would hold an unstable equilibrium (also termed a knife-edge equilibrium, see Schimel and Weintraub, 2003). The equilibrium solution is independent of M and requires thus a perfect balance of the parameters that govern growth- and death rates (Table 3). This means, that microbial biomass would thus either grow indefinitely or decay to zero. It becomes clear that the soil organic matter pool must response on a similar time scale as microbes in order to maintain microbial biomass within acceptable boundaries.

Modification of the model to allow a diminishing return with increasing enzyme production or with increasing microbial mass (models 3 and 4), will result into a stable microbial biomass under constant substrate concentration (Table 3, leftmost column). The inclusion of enzyme production costs and optimisation of microbial growth yields an equilibrium biomass where the half saturation constant (K_P) becomes important as it is, next to the direct enzyme expenditure, a central determinant of how much effort is being put into the production of enzyme. The equilibrium biomass under constant substrate allows to gauge the short-term response to a warming: All, catalytic rates, microbial respiration rates, and half saturation constants are temperature sensitive, therefore microbes will benefit from warming as depolymerisation is faster (increased V_{max}), but this benefit is reduced by the concomitant temperature response of λ_r and the half saturation constants. As a consequence, microbial biomass in models 3 and 4 can both increase or decrease with warming.

In the long term (Table 3, 3 rightmost columns) soil organic matter will adjust to the short-term microbial changes. Soil organic matter is inversely related to the maximum catalytic rate in all models. Rates of litter input are important determinants of soil organic matter in models 3 to 5. In contrast, in the microbial model based on German et al. (2012) and our derivative with maintenance respiration (model 1 and 2) the soil carbon pool is independent of the rate of new carbon added to the soil and solely a function of microbial parameters. Allowing soil organic matter to adjust to microbial growth and decay allows now a stable microbial biomass in models 1 and 2. Both, the maximum catalytic rate and the half saturation constant have no

impact on the long-term microbial biomass in models 1 to 3. Therefore, if carbon use efficiency is set to be equal in these three models, biomass, too converges to the same values. For model 4, the optimised enzyme production model, the resulting equilibria of S, M, and CUE end up being complex expressions, and we did not calculate the long-term equilibria of M and CUE, but expressed them simply as a function of soil organic matter. As expected, the effect of enzyme production cost has a negative impact on carbon use efficiency and microbial biomass and feeds back into the soil organic matter.

3.1 Model Simulations

The transient response for the different models to a temperature step from 15°C to 20°C is shown in ~~Fig.~~figure 2. We note that all models are forced through the same initial and final values of M, S, and CUE by way of parameter adjustments~~. Further, the initial response is equal across~~, and we focus on the models ~~by not allowing Q_{10}~~‘ transient behaviours (See method section). The long-term adjustments to warming are reduction in S, M, and CUE while rates of ~~V_{max} and Q_{10}~~respiration return to the initial value, equilibrating with the amount of ~~CUE to differ~~new carbon entering the system.

~~In all models, warming leads to a decline of soil organic matter and microbial biomass (Fig. 2). In this initial comparison, we assume that there is no cost associated with microbial enzyme production. Across all the models, microbial biomass first increases because of higher depolymerisation. Increased depolymerisation causes soil organic matter to decrease. In the longer term, M decreases as rates of depolymerisation decline due to a reduction in S, and due to lower CUE. We note that M becomes identical across all models in the long term, when soil organic carbon has equilibrated with the microbial processing at higher temperature (see also Table 2).~~

~~The FWD~~ Model 1 shows oscillations in M and S, as noted earlier (Wang et al., 2014). The warming triggers an increase in depolymerisation, which in turn feeds microbial biomass, causing ~~an even~~a higher rate of depolymerisation. This positive feedback experiences a break only when the substrate (S) is sufficiently depleted, such that microbial biomass begins to decline. ~~Thereafter,~~However the positive feedback takes over again, the decreasing microbial

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biomass ~~spirals down along with~~results in reduced depolymerisation until microbial biomass is low enough for soil organic matter to recover. The amplitude of the oscillations ~~dampens over time (Fig. 2). Rates of respiration oscillate along with microbial biomass, before settling at the initial rate in the long term (after ca. 200 years).~~ dampen over time (Fig. 2).

Separating out maintenance and growth respiration in model 2 increases the feedback between microbes and substrate (evidenced by higher amplitudes in M, S, and respiration). This is because part of respiration is now tied to microbial biomass, which lags depolymerisation. Carbon use efficiency initially decreases less than in model 1 (Fig. 2), because maintenance respiration lags the growing microbial biomass. The maintenance term introduces therefore also mild oscillation into the instantaneous carbon use efficiency, as microbial biomass waxes and wanes. Interestingly, including maintenance respiration decreases oscillation frequency.

The transient dynamics in ~~the REV~~ model 3 with a diminishing return as enzyme (or microbial) concentration increases, is smoother compared to ~~FWD model~~models 1 and 2 (Fig. 2). ~~The mechanism of allowing~~Allowing a finite site for enzyme-substrate reaction or microbial scavenging for enzymes curbs the growth of microbial biomass. ~~Warming still~~In contrast to models 1 and 2, warming in model 3 leads to ~~an initial increase~~ a decrease of microbial biomass, ~~owing to~~because the ~~fact that the gains of~~(curbed) carbon gain from the increase in depolymerisation ~~outweigh losses from increased~~can not balance the warming induces increase in maintenance respiration ~~(i.e. decreased CUE). As soil organic matter depletes, microbial biomass is reduced, ultimately below the initial levels~~losses.

~~The OPT model~~Model 4 considers the metabolic cost of enzyme production and allows optimising microbial growth. ~~In Fig. 2, the temporal evolution of M, S, respiration, and CUE is shown for a setup without any costs associated with~~ In absence of costs towards enzyme production~~. Among the 3 microbial models presented here (FWD, REV, OPT), the OPT model shows the strongest soil organic matter decrease in response to warming. The response in the OPT model is also almost identical with the traditional FOD model. The transient response also shows a smaller initial growth of M in the OPT vs. the REV model.~~

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3.2 Analytical steady state solutions

The analysis of equilibria helps to understand the model behaviour. We first address the "long time scale" in Table 2 where we solve for the steady state of the entire system (i.e. $\frac{dM}{dt} = 0$ and $\frac{dS}{dt} = 0$). In the long term, the steady state microbial biomass is identical in the FWD and the REV model and depends on input of fresh organic matter, the microbial CUE, and microbial turnover (Table 2, right most column). The same microbial biomass is also realised in the OPT model under zero cost (μ=0) (see Eq. 15 and Table 2, right most column). In contrast, the analytical steady state solutions of S are different among the models: For the REV and the OPT model, the input of fresh litter is a determining variable for the steady state, but not for the FWD model. In the OPT model the resulting equilibria of S and M end up being complex expressions, and we did not calculate the long-term equilibria of M, but expressed them simply as a function of soil organic matter. The OPT model has – under the assumption of marginal costs ($\mu \rightarrow 0$) the same steady state solution for M as the other models. Further, the steady states of S are the same in the traditional first order model (FOD) and the OPT model with zero cost. As expected, the effect of enzyme production cost has a negative impact on microbial biomass.

The analysis of the short-term quasi-steady state of the microbial biomass ($\frac{dM}{dt} = 0$) is useful to understand the trajectory of the coupled S-M system. Typically, microbial turnover is much faster than the turnover of bulk soil organic matter (Stark and Hart, 1997; Schmidt et al., 2007). Thus, we would expect that microbial biomass is approaching a quasi steady state given any level of S.

In the FWD model, we find that the quasi steady state for M requires a perfect balance of parameters that govern growth and death rates (Table 2, second column). In absence of such

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~~a balance (referred to as knife-edge equilibrium, see Schimel and Weintraub, 2003), M would therefore grow or decay indefinitely. It becomes clear that the soil organic matter pool must respond on a similar time scale as microbes in order to maintain microbial biomass within acceptable boundaries. In the REV and the OPT models, the short-term equilibria are a function of soil organic matter (Table 2, second column). In the REV, and the OPT model, $\bar{M}$ is strongly determined by the rate of~~ $\mu = 0$), depolymerisation ~~at a given S, the CUE and the microbial death rate. A weaker affinity for the substrate (larger half-saturation constant) and higher enzyme production cost act to reduce $\bar{M}$ in these models.~~

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~~3.3 Quasi-Steady State of Microbial Biomass~~

~~Given the equilibrium biomass, and the resulting~~ becomes a first order decomposition ~~at quasi-steady state, we set up a second line of modelling experiment, where depolymerisation rates as well as microbial respiration and death are calculated based on microbial biomass at quasi-steady state (Table 2, second and third columns). It follows that a fraction $(1 - \varepsilon)$ of depolymerisation is immediately recycled back into the soil organic matter pool, yielding the equation $\frac{dS}{dt} = (1 - \varepsilon) * D$. Depolymerisation is immediately partitioned into respiration and into a returning carbon flux, which mimics microbial death. In this modelling setup, microbial biomass is thus no longer a state variable and the models are reduced to single pool setup (Fig. 1b). $\bar{M}$ is diagnosed from S and parameters that determine depolymerisation and microbial turnover (Table 2, second column). Compared to the base models, the steady state models yield very similar results for S and respiration, but they do not reproduce the early adjustment of the microbial biomass to the temperature step. Instead of a slow adjustment to the sudden warming, $\bar{M}$ increases with the instantenous increase of depolymerisation. However, over a timescale of <1 year, $\bar{M}$ and R converge to the values of the base models in REV and the OPT model, and therefore the quasi-steady state appears to be an acceptable assumption over~~

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~~medium to long time scales. Our results further show that the depolymerisation in the OPT model at quasi-equilibrium and at marginal enzyme production cost ($\mu \rightarrow 0$) yields a depolymerisation formulation that is functionally the same as a first order decomposition model, and therefore respiration and the dynamics of S are the same for the quasi-steady state OPT model and the traditional first order model.~~

~~3.4. Partitioning~~ process. The transient behaviour of S and M is similar between ~~maintenance and growth respiration~~

~~In the third modification of our base models, we partition respiration in our models into a temperature independent growth respiration and a temperature (and biomass) dependent maintenance respiration. This affects the transient pattern of the FWD in that it increases the feedback between microbes and substrate (evidenced by higher amplitudes in M, S, and respiration). This is because part of respiration is now tied to microbial biomass, which lags depolymerisation. CUE initially decreases less than in the base model, because maintenance respiration lags the growing microbial biomass. The maintenance term introduces also a mild oscillation into CUE, as microbial biomass waxes and wanes. Interestingly, including maintenance respiration decreases oscillation frequency. In the REV and the OPT model, microbial biomass is slightly higher and respiration is slightly below the values of the base models shortly after the step increase, however, this difference diminishes over time. The nuanced consideration of microbial respiration causes CUE to declines in 2 stages. The~~model 3 and model 4 (without respiratory costs of enzyme production). However, the absence of a half saturation constant in model 4 (Equation 10) yielded a quicker adjustment of microbial biomass to temperature, a slightly slower degradation of soil organic matter initially, and a much more pronounced initial drop ~~occurs via the~~in CUE. Decomposition in model 4 without enzyme costs behaves the same way as decomposition in the traditional linear model (model 5), therefore, values of soil organic matter are almost equal with an indistinguishable difference that stems from an immediate ~~increase in maintenance respiration. This drop is followed by further changes in CUE as M oscillates (FWD model), or as M net growth is diminishing (REV and OPT). Similar as in the case with equilibrium microbes, differences disappear within < 1 year after the step warming. We note that in our modelling setup, we~~

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~~adjusted the temperature sensitivity of the maintenance respiration such that CUE is the same at reference (15°C) and elevated (20°C) temperature.~~return of dead microbial biomass in model 5.

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~~3.5. Enzyme production expenditures~~

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~~Finally, we analyse how levels of costs associated with enzyme production affects soil carbon storage and response to temperature (Fig. 4). Because of largely unknown parameters we express enzyme expenditures as the fraction of respiratory carbon for enzyme production per unit carbon depolymerised at the reference state (see Eq. 8). We tested 3 levels of enzyme production cost:~~Next, we employed different levels of enzyme production costs in model 4. That is, we set cost per enzyme production such that total enzyme expenditure is 0%, 10%, and 50% of equilibrium depolymerisation at our reference condition (i.e. 15°C). As expected, increasing enzyme production cost reduced the rate of depolymerisation, and S is therefore maintained at a higher level. The increasing costs also resulted into a smaller ~~relative decline of S in~~ response of soil organic matter to warming~~, whereas the absolute loss is larger, indicated by higher rates of respiration~~. Similarly, the response of CUE to warming is smaller and the decline of M is less pronounced if enzyme production costs are considered. —Initial hikes in respiration rates are lowest under the highest costs of enzyme production.

We calculated an apparent Q_{10} by relating respiration per unit soil organic matter to its value at 15°C. Q_{10} values would converge as the system reaches a new steady state, since we adjusted relevant parameters such that equilibrium values of microbial biomass, S, and CUE are the same across all models and for both temperatures. The initial change of respiration Q_{10} was highest in model 2, followed by model 1. In both models transient Q_{10} oscillates while oscillation amplitude is dampening over time. All models which consider microbial dynamics show higher Q_{10} with a downward adjustment over time. Initial hikes in respiration and apparent Q_{10} occur because of increased growth and associated growth respiration (model 1 and 2). Immediately after warming, the higher than equilibrium microbial biomass causes increased maintenance respiration (models 3 and 4) driving up the apparent Q_{10}. In the enzyme production optimisation model (model 4) Q_{10} decreases under higher enzyme production costs while later attenuation is smaller (Fig. 4). Finally, in the traditional model with no (or implicit) microbial biomass Q_{10} does not change over time.

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4 Discussion

4.1 Key differences between Models

Recently developed microbial decomposition models (Schimel and Weintraub, 2003; Allison et al., 2010; German et al., 2012) highlight the importance of microbial processes and microbial physiology during decomposition. ~~Their~~The application of these models specifically highlights the role of extracellular enzymes during decomposition and how these constraints will further affect the release of soil organic matter as a consequence of warming. Further, it has been shown that carbon use efficiency and microbial turnover are central parameters in the prediction of soil carbon storage to warming (Hagerty et al., 2014). While microbial decomposition models are able to improve prediction of organic carbon stock globally, and can successfully recreate litter decomposition dynamics, the long-term trajectory of a warming response needs further evaluation (Wang et al., 2014). In particular, a positive feedback between depolymerisation and microbes can only be curbed via the longer term adjustment of soil organic matter and therefore lead to oscillation in both microbial biomass and soil organic matter (Wang et al., 2014). ~~The oscillation is the consequence of a positive feedback between depolymerisation and microbial growth, and is evidenced by a knife's edge or unstable equilibrium under constant substrate condition (Schimel and Weintraub, 2003). A break in this feedback only occurs via interplay with the reduction of soil organic matter.~~ Here, we build on recent advances of microbial decomposition models and ask how nuanced representation of CUE (in the form of maintenance respiration and enzyme production cost), and how mechanisms that constrain the depolymerisation at high enzyme or microbial biomass concentration would affect model behaviour and response to warming.

~~Such interplay occurs on a longer timescale than that of microbial turnover, causing the swings in M and S. We note that some attenuation of the oscillation may occur via direct input into a DOC pool that does not require depolymerisation (Allison et al., 2010), a feature not considered here.~~

~~The display of oscillation in the FWD model~~Models 1 and 2, i.e. the microbial decomposition model as proposed by German et al. (2012) and our variation that includes a partitioning between growth and maintenance respiration show qualitatively similar characteristics. Most importantly, the equilibrium solution under a constant substrate concentration (S) shows a

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knife's edge or unstable equilibrium (Schimel and Weintraub, 2003). As a consequence, changes in microbial biomass result in a positive feedback between depolymerisation and growth. That is, in the case of a temperature increase, depolymerisation picks up, feeds microbe, which produce more extracellular enzymes causing faster rates of depolymerisation. A break in this feedback only occurs via reduction of soil organic matter. The positive feedback in conjunction with a break in a slower responding soil carbon pool leads to oscillation in M, S, and respiration. Separating respiration into growth and maintenance terms changes the model behaviour marginally. In fact, the positive feedback between microbial biomass and soil organic matter depolymerisation in model 2 is slightly amplified compared to model 1 because maintenance respiration lags depolymerisation.

While the partitioning between growth and maintenance respiration in the microbial pool is slightly more realistic (Sinsabaugh et al., 2013), the changes between models 1 and 2 are small overall. For example, changes in frequency and amplitude can easily be introduced by other parameter changes (Wang et al., 2014). Although it is more mechanistic to separate growth and maintenance respiration, it remains open whether the addition of extra parameters is justified at this point, particularly since this requires knowledge of climate sensitivity of these different respiration terms.

The oscillatory behaviour arising from the spiraling between microbial growth and depolymerisation in models 1 and 2 has been a point of critique as it has not been observed in laboratory and field incubation studies (Wang et al., 2014). Here, we introduce mechanisms that ~~curb~~introduce a break in the positive feedback between substrate and microbial biomass.We portray two scenarios, where each increment in microbial biomass or enzyme concentration yields a smaller increase in depolymerisation than the previous increment (i.e. diminishing return). The scenarios we worked out are 1) microbial biomass feeds on active extracellular enzymes, 2) limited sites for substrate/enzyme reactions (see Appendix ~~B~~). We derived the forms of depolymerisation from the original Michaelis-Menten kinetics and the resulting formulations presented in the method section are simplified and more illustrative versions of more complex functions~~. Wang and Post (2013) arrived at the same function for depolymerisation of the reverse Michaelis-Menten model, where an enzyme only adsorbs to a fraction of binding sites because of complex substrates.~~ (see Appendix). The simplified formulation of depolymerisation and microbial consumption we arrived at has been dubbed reverse Michaelis-Menten formulation (Schimel and Weintraub, 2003), because microbial

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biomass (or enzyme concentration) instead of the substrate concentration is now occurring in the denominator of the depolymerisation term, invoking ~~the~~ diminishing return. Our analysis shows that the positive feedback between decomposition and microbial growth is removed, as ~~our REV~~ model 3 has now a stable equilibrium.

Limited sites may play a role if the substrate has a high volume to surface ratio, or if the substrate is associated with minerals (Davidson and Janssens, 2006; Gillabel et al., 2010; Conant et al., 2011; Davidson et al., 2012, 2014; Cotrufo et al., 2013; Wagai et al., 2013; Benbi et al., 2014; Wieder et al., 2014a; Tang and Riley, 2015). Our implementation of limited substrate causes a surplus ~~of~~ free enzymes that compete among themselves for binding to substrates similar to the Langmuir adsorption isotherm theory (Vetter et al., 1998; Schimel and Weintraub, 2003~~, Wang and Post, 2013,~~ and see Appendix ~~B, Model with limited available substrate).~~). Effects of microbial scavenging for enzymes cause a ~~diminishing return~~negative feedback because more microbial biomass will lead to an increased probability of enzymes being consumed before they interact with soil organic matter. Other mechanisms of diminishing return as enzyme increase may be stabilisation of enzymes into organic matter-humate complex (Allison, 2006), or sorption to minerals, soil organic matter, or microbes (Tang and Riley, 2015). Diminishing returns also occur with rate-yield tradeoffs (Allison, 2014).

Many microbial decomposition models work under the assumption that enzyme production is proportional to microbial biomass. It is conceivable, that microbes are adjusting production to maximise return or growth (Cooney, 2009; Merchant and Helmann, 2012). ~~In our OPT model, we relax the proportionality~~We consider such an optimisation of microbial ~~enzyme production and microbial biomass but instead allow a best possible return, given~~growth under the consideration of an acquisition cost in the ~~cost of~~ form of respiratory expenditures for enzyme synthesis~~.~~ (Model 4). While the exact cost of enzyme ~~production~~synthesis is not known, we fixed parameters (the product of K_P and c) that they relate to the fractional expense of carbon depolymerised upon initialization (i.e. at steady state and reference temperature, ~~Eqs. 8 and 15~~Equation 13). Importantly, enzyme ~~production~~ optimisation is not possible for some of the models presented here. Higher enzyme production would always lead to further microbial growth in ~~the FWD model~~models 1 and 2 and the highest yield would occur ~~with~~ infinite enzyme production. Similarly, in the case of microbial scavenging for enzymes, additional investments into enzymes always increases depolymerisation.

The ~~response to temperature in our OPT~~ model with no-cost enzyme production closely resembles the traditional first order decay model ~~(FOD). In~~with the ~~limit~~variation of ~~enzyme production cost is zero~~an explicit microbial pool and variable carbon use efficiency. In this model, depolymerisation occurs ~~-~~at the maximum reaction rate (V_{max}~~*S), confirming~~). Fixing the ~~resemblance to the first order model. This~~ steady state values of S, M, and CUE of the no-cost model ~~shows~~4 to the ~~strongest response~~respective values of Model 1 required us to choose Q_{10} for V_{max4} close to ~~warming in the long term because the temperature dependence of~~ 1, indicating no change in maximum depolymerisation ~~is not reduced via a half saturation constants (K_E in forward, K_M in OPT, and K_P in OPT model) as in~~ with warming, which confirms the lower climate sensitivity found in microbial decomposition model (Allison et al., 2010). Therefore, the response to warming for the ~~FWD or REV~~no-cost model~~. We note that half saturation constants in our models combine several parameters such as enzyme productivity relates to microbial biomass, and turnover of the enzyme pool. In the REV and the OPT model, smaller the half saturation constant is, the closer we arrive at the formulation of decomposition in a first order model, this occurs via an~~ 4 (Fig. 2) mainly stems from the increase ~~of enzyme concentration by way of higher production or reduced enzyme turnover. Both, parameter are hard to come by.~~

~~The response of decomposition to warming can be viewed as a response ocurring on multiple timescale. For example, enzyme activity produces likely an immediate response, microbial respiration responses may also be triggered quickly, although longer term acclimation may occur (Frey et al., 2013). It may take longer for microbial biomass to respond to the changes (weeks to months). Finally, because the rate of decomposition is slow compared to the overall abundance of soil oganic matter, discernible changes in this pool occur on timescales of months to years. Based on the distinct rates of adjustements, timescales can – in principle – be separated by assuming a quasi steady state of pools that turn over fast.~~

~~The assumption that both enzyme concentrations and DOC (i.e. the depolymerisation products) are at quasi steady state cuts across all models presented here (FWD, REV and OPT, see Appendix A). When we extend our assumption of steady state to the microbial timescale (quasi-steady state of microbial biomasss), we find that for both the REV and the OPT model, the short term response of microbial biomass and respiration is influenced by the adjustment of microbial dynamics to the warmer temperature. Because microbial biomass jumps immediately to higher level after the temperature increase in such an equilibrium assumption, depolymerisation and thus respiration are affected. However, the equilibrium assumption does not affect the trajectory of the soil carbon pool, S. At timescales that allow microbes to turn over a couple of times (several months), the quasi steady state poses a suitable approximation to represent respiration and microbial biomass, even after a sharp perturbation in form of a step change. Perhaps more intruiging is the fact that a traditional first order model is the special case of the OPT model with microbial quasi-steady state and with marginal enzyme production costs ($\mu \rightarrow 0$). Here, we maintain reduction of CUE under increasing temperature in the FOD, a feature typically not include in traditional first order models.~~

~~CUE ultimately is the result of different microbial respiration terms. Here, we considered 3 processes that may affect microbial respiration under a warming scenario. We first considered a partitioning into growth and~~ in maintenance respiration ~~across our 3 models. Growth respiration was simply assumed to be a proportion of carbon allocated to microbial growth. In contrast, maintenance respiration scales in our models to microbial biomass, where the proportionality factor increases with temperature. We motivate the partitioning by formulations of plant respiration in terrestrial biosphere models. We find that this separation affects the short term responses of respiration, because microbial biomass lags the increase of depolymerisation. The temperature response of CUE is thus delayed. The partitioning of the respiration terms has particularly also an impact on the transient dynamics of the FWD model, in that the lag in maintenance respiration amplifies the oscillation. However, in the REV and~~

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~~the OPT model, effects of separation are only discernible on the microbial time scale, before microbial biomass is approaching quasi steady state values.~~and the associated decline in carbon use efficiency.

~~In~~Perhaps the ~~OPT model, we introduce an additional respiration term, namely~~ most intriguing feature of the ~~cost of~~optimised enzyme production~~, which we allow microbes to adjust in order to optimise growth. It~~ model is ~~interesting~~ that increasing costs lead to a smaller immediate response in respiration and more resilient soil organic matter pool in the long term, when subject to warming. The ~~early~~immediate respiration response ~~in~~can be attributed to the ~~OPT model is both a product of~~ higher ~~rates of depolymerisation, but also a higher rate of enzyme production. However, the enhancement relative to the rates at reference temperature is smaller, the higher the enzyme production cost.~~microbial biomass that can be maintained if enzyme expenditures are low. A warming then increases maintenance respiration much more in the low-cost scenario. In the long term~~,~~ soil organic matter decreases much less when enzyme production costs are considered. ~~This yield tradeoff thus act~~The decrease in the soil organic matter pool in the high cost scenario ($\mu = 0.5$) is a mere 3 % under a 5°C warming, compared to ~~buffer respiration increases that could be expected from physiological responses alone (V_{max}), although~~12.5 % if costs are negligible.

Model 4 (at low cost) is among our suite of models, the one that most closely resembles the ~~effects are smaller~~traditional first order decomposition model. Here, we modified a traditional model by a variable carbon use efficiency and ~~may be well within~~we obtain a qualitative similar result as in model 4. The nuances are small and mainly caused by the ~~uncertainty of the temperature~~lag of carbon returned, as it passes through the microbial biomass. Even if the enzyme production costs are higher, the functional form of the response to warming can easily be captured by a first order decomposition model.

4.2 Short-term and long-term response to temperature

Because many of ~~any~~the parameters ~~considered here.~~in these models are hard to come by, we chose the strategy to start off with a previously used set and adjust the different models such that their equilibrium values of microbial biomass, soil carbon storage, and carbon use efficiency are the same at the reference temperature (15°C) and at the warmed temperature (20°C). We obtained this mainly by adjusting first V_{max} (maximum depolymerisation), and λ_r (per M maintenance respiration rate) to obtain a match at the reference temperature, followed

by tuning temperature sensitivity (Q_{10}) for V_{max} and λ_r to obtain identical values across models for M, S, and CUE at the warmed equilibrium. The tuning of V_{max} and the Q_{10} of V_{max} and λ_r yield different values across the models.

We investigate the consequence of this tuning by analysing the transient changes in the „apparent" Q_{10}. We define apparent Q_{10} as the Q_{10} response of the relative respiration (respiration per unit substrate, see method section). While the apparent Q_{10} converges over time, the differences in physiological temperature responses (Q_{10} for V_{max} and λ_r) have different impact in the short term. These differences in physiological responses are evident immediately after the temperature increase, as they are displaying very disparate responses in respiration, and consequently in the apparent Q_{10} (Fig. 4). Models 1 and 2 show the strongest initial response before the apparent Q_{10} adjusts to its long-term value. In the models with diminishing return (models 3 to 5) the long-term temperature response is much closer to the short-term (physiological) response. But also the models with diminishing return show considerable differences. The major difference in the model structure between model 3 and model 4 (assuming where costs of enzyme synthesis are 0) is a non-negligible half saturation constant (K_M = 0.37 of microbial biomass at reference temperature). The respiration in model 3 increases much more dramatically than in model 4, causing Q_{10} to increase to a higher level, before slowly adjusting down. A sizeable cost for enzyme synthesis with optimisation of microbial growth, further reduces a long-term adjustment of the temperature sensitivity. Similar to the first order decomposition model, the initial response to a temperature increase is quasi-locked in and does not change much over time.

The difference in the apparent Q_{10} critically shows, that understanding the mechanisms, how microbial biomass acquires its building blocks, insights in what limits this acquisition, and also how the microbial community responses to limitation are central to our understanding of how soil organic matter responds to warming.

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We acknowledge that we used a simplified set-up of our model suite. For example, we assumed that depolymerised carbon in soil solution (~~DOC~~dissolved organic carbon) is always at steady state with the microbial biomass. We justified this simplification by assuming fast and efficient scavenging ~~of microbes. Further sensitivity analysis may shed further light on the dynamics across the full parameter space, while using the simplified linear terms (Appendices B and C, Tang, 2015), particularly also because many of the parameters are hard to come by.~~for microbes. We further did not include nutrient requirements of microbes.

Considering the stoichiometric requirements can in particular change the allocation of resources to optimise enzyme synthesis. ~~Finally, our model does not include interaction that may occur with adsorption to mineral surfaces, which may occur with the substrate, the enzymes and microbial biomass, and which has important short and long term consequences to temperature flctuations and changes (Wieder et al., 2014a; Tang and Riley, 2015).~~ Nevertheless, our suite of models show the importance of how the depolymerisation step is formulated in mathematical models when evaluating the response of decomposition under warming~~, and it provides ecosystem modelers a mechanistic handle when expanding microbial frameworks into to more complex, models with multiple substrates of different quality and different propensities to microbial processing~~.

5 Conclusions

Our findings suggest that different formulation of how microbes acquire substrate will have significant impact on the short vs. long-term consequences of warming. Here, we present simple, yet feasible mechanisms of microbial dynamics. We show that substrate limitation in the form of decreasing marginal return can create a break in the positive feedback between microbial biomass and depolymerisation, ~~turning a forward Michaelis-Menten model into a reverse model. We further seperate out 3 types of respiration, that possibly have consequences on the temporal trend of CUE in response to warming. Although such seperation is more mechanistic, it remains open whether the addition of extra parameters is justified at this point, given the uncertainty in models, and because much of the effects of this separation diminishes on timescales longer than the microbial lifespan. Finally, our OPT model is among our suite of models, the one that most closely resembles the traditional first order decomposition model, and can be converted to such a model by applying a series of tangible mechanisms and simplfication. These include 1) mechansims of dimishing returns that breaks the feedback between substrate and microbes 2) relaxing the proportionality of enzyme production and microbial biomass, 3) small cost associated with enzyme synthesis, 4) assumption of microbial quasi-steady state.~~but also opens the possibility of microbes to optimise carbon uptake. We find that decreasing marginal return leads to apparent temperature responses that are closer to the physiological responses, even more so when microbes adjust enzyme production to optimise growth. Carefully designed long-term experiments, can therefore, provide insights and can further help with the interpretation of short-term incubations.

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Appendix ~~A~~

Michaelis-Menten kinetics with enzyme denaturation

The dynamics of the enzyme-substrate complex ~~are~~is

$$\frac{d[E]}{dt} = P - K_S[S][E] - \lambda_{E1} * [E] \text{ ~~+ } K_r \text{ ~~+ } K([ES]} \quad \text{(A1)}$$

$$\frac{d[ES]}{dt} = -(K_{cat} + K_r + \lambda_{E2})[ES] + K_S[S][E] \quad \text{(A2)}$$

Where P is the ~~microbial~~ production of new enzymes, [S] ~~is~~are the concentration of ~~the~~free sites available for enzyme substrate complexation, [E] the concentration of enzymes, [ES] the substrate-enzyme complex, K_s, K_{cat}, and K_r are reaction constants that denote substrate-enzyme binding, actual depolymerisation rate, the reversibility of the enzyme-binding process. λ_{E1} and λ_{E2} are enzyme decay parameters that lead to enzyme denaturation or render enzymes inactive in the free enzyme pool or in the enzyme-substrate complex, respectively. ~~In the FWD and REV model, P is proportional to microbial biomass. The Michaelis Menten approximation for depolymerisation assumes that the system is in quasi steady state in which the tendency $\frac{d[ES]}{dt}$ and $\frac{d[E]}{dt}$ are zero. This implies also that tendency of the total enzyme concentration $\frac{d[Et]}{dt}$ (with [Et] = [ES] + [E]) becomes zero.~~

~~Setting Eq. (~~We are mostly interested in total enzyme concentration

$$[E_t]=[ES]+[E] \quad \text{(A3)}$$

The Michaelis-Menten approximation for depolymerisation assumes that the system is in quasi steady state in which the total enzyme concentration $[E_t]$. Here we include also that the total available sites do not change (S is constant) within the timescale of enzyme reactions. This implies that Equation A2~~) to~~ becomes zero~~, and substituting [Et] = [ES] + [E], it follows~~ as the different reactants will approach a steady state

And thus

$$[E] = \frac{[E_t]\,K_E}{([S]+K_E)} \quad \text{(~~A3~~A4)}$$

$$[ES] = \frac{[E_t]\,[S]}{([S]+K_E)} \quad \text{(~~A4~~A5)}$$

And the rate of depolymerisation

$$D = \frac{[E_t] * V_{max} * [S]}{([S] + K_E)} \qquad (\text{~~A5~~}A6)$$

where D is the familiar Michaelis-Menten equation with $K_E = \frac{K_{cat} + K_r + \lambda_{E2}}{K_S}$ and V_{max} is equivalent to K_{cat}.

DOC and enzyme dynamics

We assumed ~~that,~~ DOC concentrations are in equilibrium with substrate and microbial uptake. In microbial decomposition models, the only DOC sink is microbial consumption, which by way of mass conservation leads to microbial consumption being equivalent to the rate of depolymerisation.

Similarly, we estimate the equilibrium total enzyme concentration by setting its tendency to zero:

$$\frac{dE_t}{dt} = P - \lambda_{E2}[ES] - \lambda_{E1}[E] = 0 \quad \text{(A7)}$$

where P is the production of enzymes. Substituting Equation A4 and Equation A5 for E and ES yields

$$E_t = \frac{P([S]+K_E)}{\lambda_{E1}K_E+\lambda_{E2}[S]} \quad \text{(A8)}$$

And the overall depolymerisation yields

$$D = \frac{P*K_{cat}*[S]}{\lambda_{E1}K_E+\lambda_{E2}[S]} \quad (\text{~~Previous~~A9})$$

We note, that previous models (Allison et al., 2010; German et al., 2012) assumed a general decay of the total enzyme pool, where

$$\frac{\text{~~d[E$_t$]~~}}{\text{~~dt~~}} \frac{dE_t}{dt} = P - \lambda_E * \text{~~[E$_t$]~~} \quad (\text{~~A6~~}E_t \quad \text{(A10)}$$

~~Because enzyme turn over fast, we can assume a quasi steady state of the total enzyme pool by setting Eq. A6 to zero. We obtain~~

~~[E$_t$] = $\frac{P}{\lambda_E}$~~This is the special case of $\lambda_{E1} = \lambda_{E2} = \lambda_E$. This case leads to an equilibrium concentration of

$$E_t = \frac{P}{\lambda_E} \quad (\text{~~A7~~A11})$$

And depolymerisation as:

$$D = \frac{\frac{P}{\lambda_E}*K_{cat}*[S]}{[S]+K_E} \quad (\text{~~A8~~A12})$$

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Finally, microbial decomposition models assume that enzyme production is proportional to the microbial biomass ~~(M):~~: P = b*M, hence, in the special case of a general decay of enzymes

$$D = \frac{V_{max} * M * [S]}{[S] + K_E} \quad (\text{~~A9~~A13})$$

With $V_{max} = \frac{b * K_{cat}}{\lambda_E}$

~~Yet, it is conceivable, that the enzyme substrate complex,~~We used A13 in models 1 and ~~free enzymes~~ 2.

More generally (with specific decay ~~at differeent~~ rates ~~see also Eqs A1~~for free enzyme and ~~A2.~~ enzymes associated with the substrate)

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$$\frac{d[E_t]}{dt} = P - \lambda_{E2}[ES] - \lambda_{E1}[E] \quad (\text{~~A10~~})$$

~~Substituting Eq. A3 and Eq. A4 for [E] and [ES], and applying a quasi-steady state as before yields~~

$$[E_t] = \frac{P([S] + K_E)}{\lambda_{E1} K_E + \lambda_{E2}[S]} \quad (\text{~~A11~~})$$

~~And the overall depolymerisation is thus~~

$$D = \frac{P * K_{cat} * [S]}{\lambda_{E1} K_E + \lambda_{E2}[S]} \quad (\text{~~A12~~})$$

~~Which can be converted into a Michaelis-Menten form~~

$$D = \frac{V_{max} * M * [S]}{[S] + K_S} \quad (\text{~~A13~~A14})$$

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where $V_{max} = \frac{b * K_{cat}}{\lambda_{E2}}$ and $K_S = K_E \frac{\lambda_{E1}}{\lambda_{E2}}$

~~**Appendix B**~~

Microbial consumption of ~~enzymes~~Enzymes

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Microbes feeding on free enzymes can be represented as:

$$F = \lambda_{E,M} * [E] * M \quad (\text{~~B1~~A15})$$

Where F is microbial enzyme consumption and $\lambda_{E,M}$ the feeding rate. We can then represent the decay of the free enzymes with

$$[E]^* \lambda_{E1} = [E](\lambda_{E1,0} + \lambda_{E,M}*M) \quad (\text{~~B2~~A16})$$

where the total $\lambda_{E,0}$ is the spontaneous enzyme decay rate.

Substituting the new enzyme decay formulation into the depolymerisation (~~Eq. A12~~A9) yields

$$D = \frac{P*K_{cat}*[S]}{\lambda_{E2}*[S] + \lambda_{E1,0}*K_E + \lambda_{E,M}*M*K_E} \quad (\text{~~B3~~A17})$$

~~For the REV model, we simplify Eq. B3 and assume~~Assuming that enzymes associated with substrate do not undergo denaturation (λ_{E2}=0~~), which yields~~)

$$D = \frac{P*K_{cat}*[S]}{\lambda_{E1,0}*K_E + \lambda_{E,M}*M*K_E} \quad (\text{~~B4~~A18})$$

And in the case where enzyme production scales to microbial biomass (P = b*M)

$$D = \frac{M*V_{max}*[S]}{K_M + M} \quad (\text{~~B5~~A19})$$

~~Which is again the familiar Michaelis-Menten function with~~Where $V_{max} = \frac{b*K_{cat}}{\lambda_{E,M}*K_E}$ and $K_M = \frac{\lambda_{E1,0}}{\lambda_{E,M}}$

Model with limited available substrate

Access to substrate might be finite, for example, if organic matter is associated with mineral soil or if the rate of depolymerisation is constrained by the surface area. In this case, the relationship between the total available substrate and the free sites can be calculated as

$$\text{~~[~~}S\text{~~]~~} = \theta * ([S_f] + [ES]) \quad (\text{~~B6~~A20})$$

Where S_f are the available sites for enzyme reaction, θ a scalar relating the total amount of substrate to the total potentially free sites (e.g. a surface to mass conversion), and [ES] represents the sites with enzyme-substrate complexes. ~~We note that [S] in this case is not the available substrate anymore, but reduced by a fraction θ.~~

Substituting [ES] from ~~Eq. A4~~Equation A5, but knowing that ~~[~~S~~]~~ has now become ~~[~~S_f~~],~~ we obtain:

$$[S_f] = \frac{\text{~~[S]~~}}{\text{~~θ~~}} \frac{S}{\theta} - \frac{[S_f][E_t]}{K_E + [S_f]} \quad (\text{~~B7~~A21})$$

$[S_f]$ is thus the solution of a quadratic polynomial:

$$[S_f] = \frac{1}{2}\left\{-\left([E_t] + K_E - \frac{[S]}{\theta}\right) \pm \sqrt{\left([E_t] + K_E - \frac{[S]}{\theta}\right)^2 + 4 * \frac{[S]}{\theta} * K_E}\right\}\left\{-\left([E_t] + K_E - \frac{S}{\theta}\right) \pm \sqrt{\left(E_t + K_E - \frac{S}{\theta}\right)^2 + 4 * \frac{S}{\theta} * K_E}\right\} \quad (B8A22)$$

The scenario ofAs we assume there are limited reaction site is relevant if $\frac{[S]}{\theta}$ is small (i.e. $\frac{[S]}{\theta} << [Et]$).Under this scenario,sites ($\frac{S}{\theta}$), we simplify Eq. B8this function using a Taylor expansion around ($\frac{[S]}{\theta}\frac{S}{\theta} = 0$)

$$[S_f] = \frac{[S]}{\theta} * \left(\frac{K_E}{[E_t] + K_E}\right) S_f = \frac{S}{\theta} * \left(\frac{k_E}{E_t + k_E}\right) + O[(\frac{[S]}{\theta})^2 \frac{S}{\theta})^2] \quad (B9A23)$$

Plugging this into the depolymerisation

$$D = \frac{K_{cat} * [E_t] * \frac{[S]}{\theta}}{[E_t] + K_E + \frac{[S]}{\theta}} \cong \frac{K_{cat} * [E_t] * \frac{[S]}{\theta}}{[E_t] + K_E} \quad (B10)$$

$$D = \frac{K_{cat} * E_t * \frac{S}{\theta}}{E_t + K_E + \frac{S}{\theta}} \cong \frac{K_{cat} * E_t * \frac{S}{\theta}}{E_t + K_E} \quad (A24)$$

which has a Michaelis-Menten form with a saturating enzyme concentration. This particular solution is for a small amount of binding sites, and enzymes compete for free sites. Thus $[E_t] >> \frac{[S]}{\theta}$, and it can be dropped from within the denominator. On a side note: we obtain the same expression if we approximate from Eq. B7:

$$[S_f] = \frac{[S]}{\theta} - [S_f]\frac{[E_t]}{[S_f] + K_E} \quad (B11)$$

$$[S_f] \cong \frac{[S]}{\theta} - \frac{[S_f][E_t]}{K_E} \quad (B12)$$

Which assumes very few free sites ($[S_f] >> K_E$). Therefore

$$[S_f] = \frac{[S]}{\theta}\frac{K_E}{[E_t] + K_E} \quad (B13)$$

We can also include equationsEquations for enzyme turnover (Eq.Equation A7) to calculate $[E_t]$:E_t:

However, we need to substitute [S] in this equationEquation with $[S_f]$, thus

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~~$\frac{d[E_t]}{dt} = P - \frac{\lambda_{Ez}*[E_t]*\frac{[S]}{\theta}}{[E_t]+K_E+\frac{[S]}{\theta}} - \frac{\lambda_{E1}*[E_t]*([E_t]+K_E)}{[E_t]+K_E+\frac{[S]}{\theta}}$ (B14)~~

$$\frac{dE_t}{dt} = P - \frac{\lambda_{E2}*[E_t]*\frac{S}{\theta}}{E_t+K_E+\frac{S}{\theta}} - \frac{\lambda_{E1}*[E_t]*[E_t+K_E]}{E_t+K_E+\frac{S}{\theta}} \quad (A25)$$

Maintaining ~~$\frac{[S]}{\theta} << ([E_t]+K_E)$~~ $\frac{S}{\theta} << [E_t + K_E]$ we obtain

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~~$\frac{d[E_t]}{dt}$~~ $\frac{dE_t}{dt} \cong P - \frac{\lambda_{E2}*[E_t]*\frac{S}{\theta}}{[E_t]+K_E} - \lambda_{E1} * [E_t]$ (~~B15~~A26)

The ~~quasi~~ equilibrium solution ($\frac{d[E_t]}{dt}\frac{dE_t}{dt} = 0$) yields a quadratic expression for ~~$[E_t]$~~E_t, however, we can evaluate ~~the following scenarios:~~end member

a) suppose $\frac{\lambda_{Ez}*[E_t]*\frac{S}{\theta}}{[E_t]+K_E}\frac{\lambda_{E2}*[E_t]*\frac{S}{\theta}}{E_t+K_E} \gg \lambda_{E1} * [E_t]$, this assumes that enzyme decay occurs mainly when bound to the substrate.

setting ~~$\frac{d[E_t]}{dt}$~~ $\frac{dE_t}{dt} = 0$, we obtain

~~$[E_t] = \frac{K_E*P}{\frac{\lambda_{Ez}*\frac{S}{\theta}}{P}}$ (B16)~~

$$E_t = \frac{K_E*P}{\lambda_{E2}*\frac{S}{\theta}-P} \quad (A27)$$

and with P proportional to microbial biomass (M)

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$$D = \frac{K_{cat}*P}{\lambda_{E2}} = V_{max} * M \quad (~~B17~~A28)$$

Where $V_{max} = \frac{K_{cat}*b}{\lambda_{E2}}$

In this case, depolymerisation and microbial consumption is independent of the substrate but is determined by the relative rate of catalysis and irreversible destruction of the enzyme-substrate complex.

b) suppose $\frac{\lambda_{E2}*[E_t]*\frac{S}{\theta}}{~~[E_t]+K_E~~E_t+K_E} \ll \lambda_{E1} * [E_t]$

This implies that ~~enzymes~~enzyme mainly decay if they are not associated with the substrate and that there is an appreciable amount of free enzymes. This is realistic under substrate limiting conditions, as there will be a sizeable amount of free enzymes compared to enzyme substrate complexes.

We then obtain: ~~$[E_t] = \frac{P}{\lambda_{E1}} E_t$~~ $= \frac{P}{\lambda_{E1}}$

And

$$D = \frac{K_{cat} * P * \frac{S}{\theta}}{P + \lambda_{E1} * K_E} \quad (\text{~~B18~~A29})$$

With P = b*M, we have

$$D = \frac{M * V_{max} * S}{K_M + M} \quad (\text{~~B19~~A30})$$

Where $V_{max} = \frac{K_{cat}}{\theta}$, and $K_M = \frac{\lambda_{E1} * K_E}{b}$

~~**Appendix C**~~

Optimising depolymerisation

Microbes may be able to optimise their growth, and thus depolymerisation becomes a function of the metabolic costs of enzyme production. Depolymerisation based on enzyme production, assuming fixed turnover of free enzymes yields:

$$D(P) = \frac{P * V_{max} * [S]}{K_P + P} \frac{P * V_{max} * S}{K_P + P} \quad (\text{~~C1~~A31})$$

Where P ~~is~~ the amount of new enzyme produced, V_{max} ~~is~~may be $\frac{K_{cat}}{\theta}$ and $K_P = \lambda_{E1} K_E$, based on the model with limited available substrate.

Microbial growth (G) will be

$$G = (1-g) * (D - Pc - \lambda_r * M) \quad (\text{~~C2~~A32})$$

Where g is the growth respiration factor, c the respiratory cost per unit enzyme production, and λ_r the maintenance respiration factor.

Enzyme production (P) can be optimised by substituting ~~Eq. C1~~Equation A31 into ~~Eq. C2~~Equation A32 and setting $\frac{dG}{dP} = 0$. This yields:

$$Pc = -K_P c + \sqrt{V_{max} * [S] * K_P c} \sqrt{V_{max} * S * K_P c} \quad (\text{~~C3~~A33})$$

The proportion of carbon expended for enzyme production relative to depolymerisation (μ) is

$$\frac{Pc}{D} \mu = \frac{Pc}{D} = \frac{\sqrt{K_P c}}{\sqrt{[S] V_{max}}} \sqrt{\frac{K_P c}{S V_{max}}} \quad (\text{~~C4~~A34})$$

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Instead of specifying c, we used ~~Eq. C4~~Equation A34 to express overall microbial carbon expenditure for enzyme production. After assigning a value to μ, we calculate c based on equilibrium S at reference temperature.

In contrast, the microbial scavenging scenario does not provide an optimum enzyme production. In this case depolymerisation is

$$D = \frac{P*V_{max3}*[S]}{(K_M+M)*\lambda_E} \frac{P*V_{max3}*S}{(K_M+M)*\lambda_E} \quad (\text{~~C5~~A35})$$

And thus $\frac{dG}{dP}$ will yield a constant where growth scales with the rate of enzyme production.

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Table 1. Key features of the five microbial decomposition models.

~~FWD~~ Model	Description
Model 1	German et al., 2012
~~*FWD Model with maintenance respiration*~~	
Model 2	As ~~FWD~~ model 1 but microbial respiration is partitioned into temperature insensitive growth and temperature sensitive maintenance respiration terms.
~~REV Model~~	
Model 3	Depolymerisation and uptake relative to microbial biomass decreases with increasing M (diminishing return mechanism).
~~*REV Model with equilibrium microbes*~~	
~~As REV model but fast microbial adjustments.~~	
~~*REV Model with maintenance respiration*~~	
~~As REV model but maintenance respiration added.~~	
~~OPT Model~~	
Model 4	Optimisation of microbial enzyme production to maximise microbial growth, and consideration of carbon costs associated with enzyme synthesis.
~~*OPT Model with equilibrium microbes*~~	
~~As OPT model but fast microbial adjustments.~~	
~~*OPT Model with maintenance respiration*~~	
~~As OPT model but maintenance respiration added.~~	
~~FOD Model~~	
Model 5	First order decomposition model, modified to account for temperature

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sensitive carbon use efficiency.

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Table 2. ~~Quasi-steady-state values for for~~Parameters used in the five microbial ~~biomass (M), and~~ decomposition ~~at the short/fast timescale (at any given S) and "true" long-term equilibria for M and S across the~~ models~~. Note, for simplicity~~ (In models 2 to 5, we ~~did not substitute S in the long-term microbial equilibrium for OPT model.~~provide only those parameters where modifications have been made).

Model	~~Short/Fast time scale~~Parameter	~~Long time scale~~Unit	Value	Description	Source
	~~M~~I	~~Decomposition~~mg cm^{-3} hr^{-1}	~~S~~0.001	~~M~~ Input of fresh litter	
Model 1					German et al., 2012

~~FWD~~	~~no solution *~~	~~no solution *~~	$\frac{\lambda_d K_E}{V_{max,FWD}\,\varepsilon - \lambda_d}$	$\frac{I\,\varepsilon}{(1-\varepsilon)\,\lambda_d}$
~~REV~~	$\frac{V_{max,Rev}\,S\,\varepsilon - K_M\,\lambda_d}{\lambda_d}$	$(V_{max,REV}\,S - K_M\,\lambda_d/\varepsilon)$	$\frac{I}{V_{max,REV}\,(1-\varepsilon)} + \frac{K_M\,\lambda_d}{V_{max,REV}\,\varepsilon}$	$\frac{I\,\varepsilon}{\lambda_d\,(1-\varepsilon)}$

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~~OPT~~	$\frac{(X-Y)^2\,\epsilon}{\lambda_d}$	X^2-XY	$\frac{1}{2\,V_{max,OPT}\,(1-\epsilon)^2}[-Y\,(2\epsilon-1)\sqrt{4IY\,(1-\epsilon)+Y^2}+(1-\epsilon)\,(2I-2\epsilon Y^2)+Y^2]$	$\frac{(X-Y)^2\,\epsilon}{\lambda_d}$

1 $X=\sqrt{S\,V_{max,OPT}}$, $Y=\sqrt{K_P*\epsilon}$

2

3 * requires $\lambda_d=\frac{V_{max,FWD}\,S\,\epsilon}{S+K_P}$

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~~Table 3. Parameters used in microbial decomposition models (In subsequent models, we provide only those parameters where modifications have been made.)~~

	~~Parameter~~	~~Unit~~	~~Value~~	~~Description~~	~~Source~~
~~FWD Model~~					
	~~I~~	~~mg cm^{-3} hr^{-1}~~	~~0.001~~	~~Input of fresh litter~~	~~German et al., 2012~~
	λ_d	hr^{-1}	0.0005	Death rate of microbes	
	~~V_{max}~~ ~~,FWD~~ $V_{max1,0}$	mg cm^{-3} hr^{-1}	0.0049	Maximum catalytic rate @ 15°C	
	$Q_{10,}$ ~~$_{Vmax}$~~ ~~,FWD~~ $Vmax1$	-	1.9	Q_{10} of maximum catalytic rate	
	$K_{E,0}$	mg S cm^{-3}	270	Half-saturation constant @ 15°C	
	$Q_{10,KE}$	-	1.07	Q_{10} of half-saturation constant	
	ε_0	-	0.39	Microbial growth efficiency @ 15°C	
	ε_{slope}	°C^{-1}	-0.016	Microbial growth efficiency temperature slope	
~~FWD~~ Model ~~with maintenance respiration~~ 2	$V_{max2,0}$	mg^{-1} M cm^{-3} hr^{-1}	0.0049	Maximum catalytic rate @ 15°C	This study
	$Q_{10,Vmax2}$	-	1.9	Q_{10} of maximum catalytic rate	
	$\lambda_{r,0}$	hr^{-1}	0.~~0006~~000 17	Maintenance respiration @ 15°C	~~This study~~

	$Q_{10,\lambda r}$	-	~~2.28~~	Q_{10} of maintenance respiration	
	g	-	0.~~24~~55	Growth respiration coefficient	
~~REV Model~~					
Model 3	~~$V_{max,REV}$~~$V_{max3,0}$	mg^{-1} M cm^{-3} hr^{-1}	$2.61*10^{-5}$	Maximum catalytic rate @ 15°C	This study
	$Q_{10,Vmax3}$	-	1.33	Q_{10} of maximum catalytic rate	
	$K_{M,0}$	mg M cm^{-3}	0.68	Half-saturation constant @ 15°C	
~~OPT Model~~					
Model 4	~~$V_{max,OPT}$~~$V_{max4,0}$	mg^{-1} M cm^{-3} hr^{-1}	$1.71*10^{-5}$	Maximum catalytic rate @ 15°C	This study
	$Q_{10,Vmax4}$	-	1.0	Q_{10} of maximum catalytic rate	
	~~$\mu \frac{Pc}{D}$~~		0 , 0.1, 0.5	Enz production cost (as % of decomposition ~~@ 15°C steady state~~)	
	~~$K_p * c$~~	~~mg M cm^{-3}~~	~~0, $1.64*10^{-5}$ 0.0004~~	~~combined cost and the half saturation constant~~	
~~FOD Model~~					
Model 5	k~~*~~	hr^{-1}	$1.71*10^{-5}$	First order decay constant @ 15°C	This study
	$Q_{10,k}$	-	1.0	Q_{10} of k	

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Table 3. Equilibrium solutions for microbial biomass, soil organic carbon, and CUE at short/fast time scale and long time scale.

Model	Short/Fast time scale	Long time scale		
	M	S	M (when, S=Eq. S)	CUE (when, S=Eq. S)
Model 1	no solution *	$\frac{\lambda_d K_E}{V_{max1}\,\varepsilon - \lambda_d}$	$\frac{I\,\varepsilon}{(1-\varepsilon)\,\lambda_d}$	ε (T)
Model 2	no solution **	$\frac{K_E\,b}{V_{max2}\,(1-g) - b}$	$\frac{I\,(1-g)}{b - \lambda_d\,(1-g)}$	$\frac{\lambda_d\,(1-g)}{b}$
Model 3	$\frac{V_{max3}\,S\,(1-g) - K_M\,b}{b}$	$\frac{b\,[I\,(1-g) + K_M\,\{b - \lambda_d\,(1-g)\}]}{V_{max3}\,(1-g)\,\{b - \lambda_d\,(1-g)\}}$	$\frac{I\,(1-g)}{b - \lambda_d\,(1-g)}$	$\frac{\lambda_d\,(1-g)}{b}$
Model 4	$\frac{(1-g)\,(X-Y)^2}{b}$	$\frac{1}{2\,V_{max4}\,(1-\eta)^2}[-Y\,(2\eta-1)\sqrt{4IY\,(1-\eta)+Y^2} + (1-\eta)\,(2I-2\eta Y^2) + Y^2]$	$\frac{(1-g)\,(X-Y)^2}{b}$	$\frac{(1-g)\,(X-Y)\,\lambda_d}{b\,X}$

$X = \sqrt{S\,V_{max4}}, Y = \sqrt{K_P c}, b = [(1-g)\,\lambda_r + \lambda_d], \eta = \frac{(1-g)\,\lambda_d}{b}$

* requires $\lambda_d = \frac{V_{max1} S \varepsilon}{S + K_E}$ ~~* k in FOD model is identical to $V_{max,OPT}$ in OPT model.~~

** requires $\lambda_d = (1-g)\,(\frac{V_{max2} S}{S+K_E} - \lambda_r)$

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Figure Captions

Figure 1. Conceptual diagrams for the microbial-enzyme models ~~applied.~~used in this study. Solid lines represent material flow (in ~~FWD and FWD~~ model ~~with maintenance respiration~~1 and model 2) and dashed lines represent information flow (in ~~Rev~~model 3 and ~~OPT models~~model 4). E, S, E-S, ~~D,~~ DOC, M represent enzyme, substrate, enzyme-substrate complex, ~~depolymerisation,~~ dissolved organic carbon, and microbial biomass carbon, respectively. ~~We analyse the different models in three ways: a) Base models of forward vs reverse formulation of depolymerisation. In the forward version, depolymerisation scales microbial biomass via enzyme production. In the reverse formulation the decreasing marginal return curbs rates of depolymerisation. This decreasing marginal return can partly be overcome by enzyme production optimisation. b) For all models we introduce partitioning between maintenance and growth respiration. c) Microbes are instantaneously in steady with substrate delivery (reverse models only).~~

Figure 2. Responses of a) soil organic carbon, b) microbial biomass carbon, c) CUE, and d) respiration to a 5°C warming ~~in base~~for all models ~~(forward vs reverse). The black.~~ Black line represent initial values, ~~which are model~~ where equilibria ~~at15~~@ 15°C. ~~We chose logarithmic axis to better highlight the differences in short-term responses.~~ (Note: ~~Differences in simulated~~Simulated soil organic carbon and respiration by ~~OPT and the FOD are almost equal, and therefore not discernible. In the OPT~~ model 4 are superimposed with the model 5 results. For model 4, simulations are carried out at zero enzyme production cost, i.e. $\mu = 0$~~).~~)

~~Figure 3. Responses of a) soil organic carbon, b) microbial biomass carbon, c) CUE, and d) respiration to a 5°C warming for all models, if separation of maintenance and growth respiration are considered, and if microbial biomass is assumed to be at quasi-steady state. Black thin line represent initial values, where equilibria @ 15°C. Colored thin lines represent base models. Dashed lines (growht and maintenance) and dotted lines (quasi-steady state) represent modifications for REV and OPT models respectively. (In the OPT model, simulations are carried out at zero enzyme production cost, i.e. $\mu = 0$).~~

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~~Figure 4. Long-term responses of optimized enzyme production (OPT)~~Figure 3. Long-term responses of optimized enzyme production model to a 5°C warming in a) soil organic carbon, b) microbial biomass carbon, c) CUE, and d) respiration operating at different relative enzyme production costs (μ), see Equation 13. Thick lines represent warming response and thin lines represent corresponding equilibrium at reference temperature.

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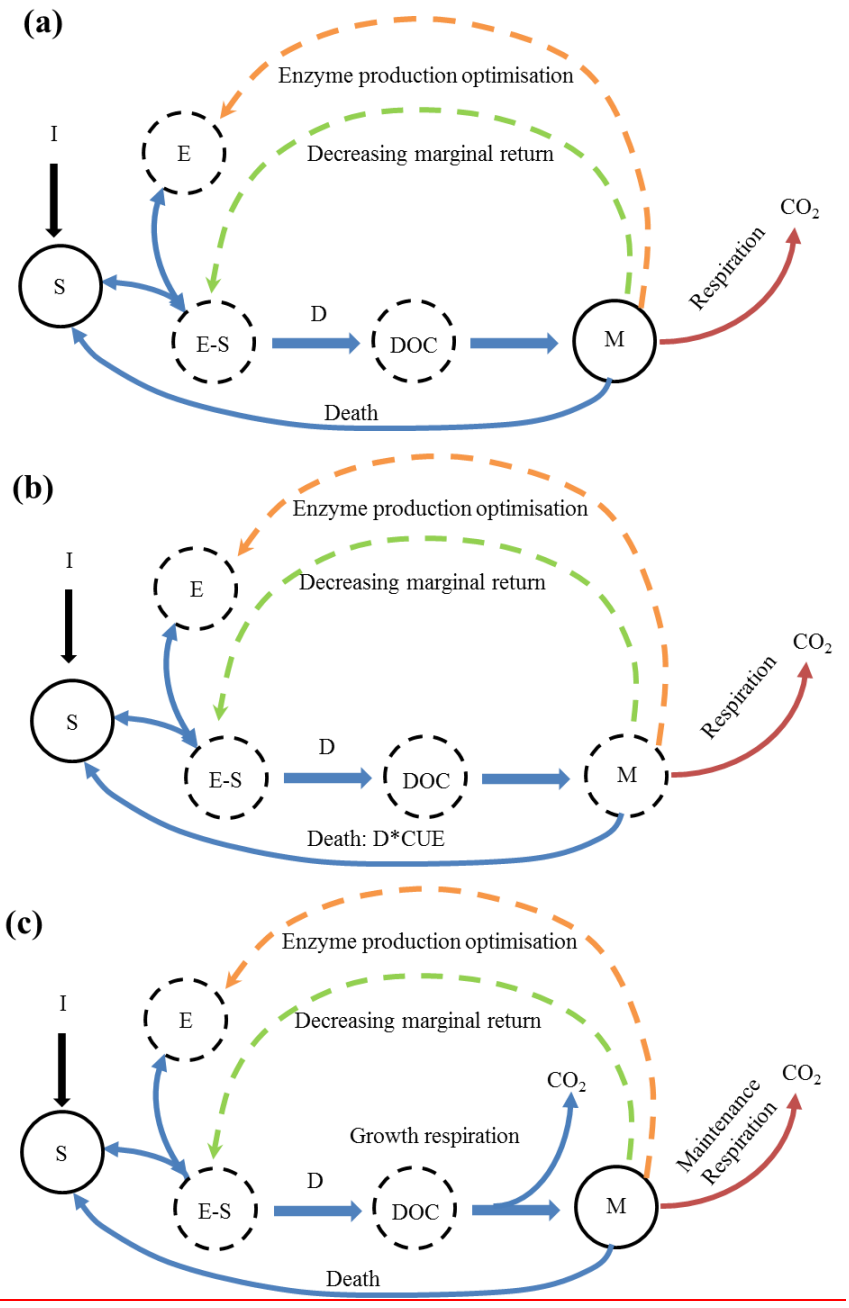
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Fig. 1

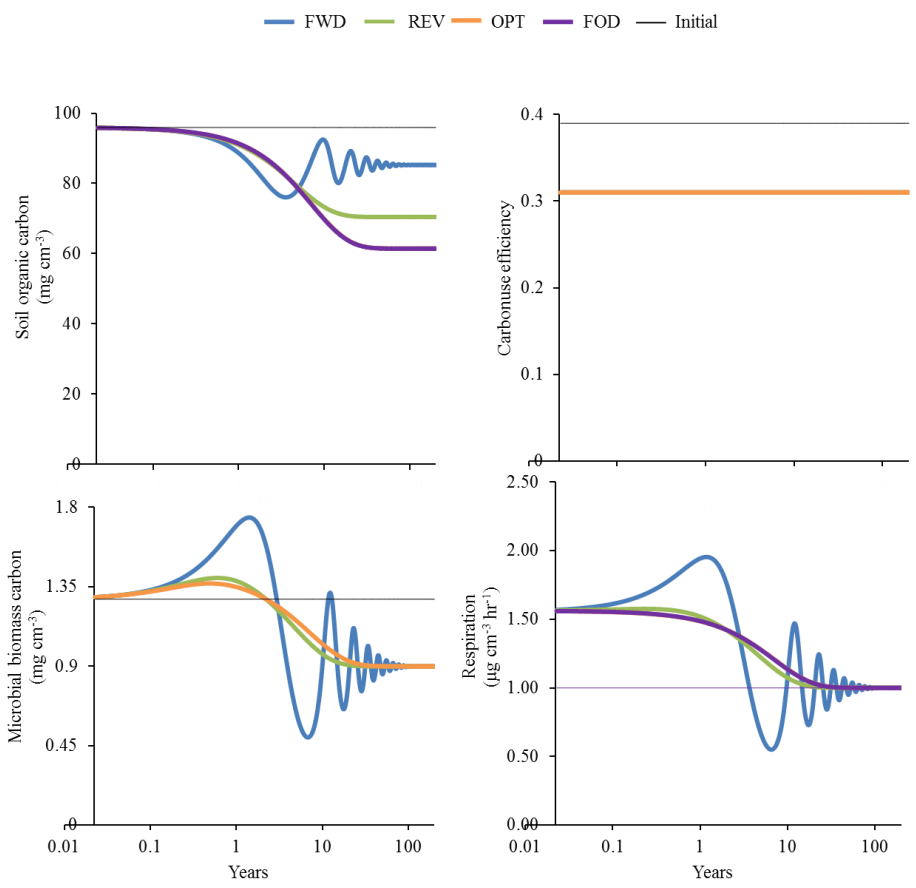

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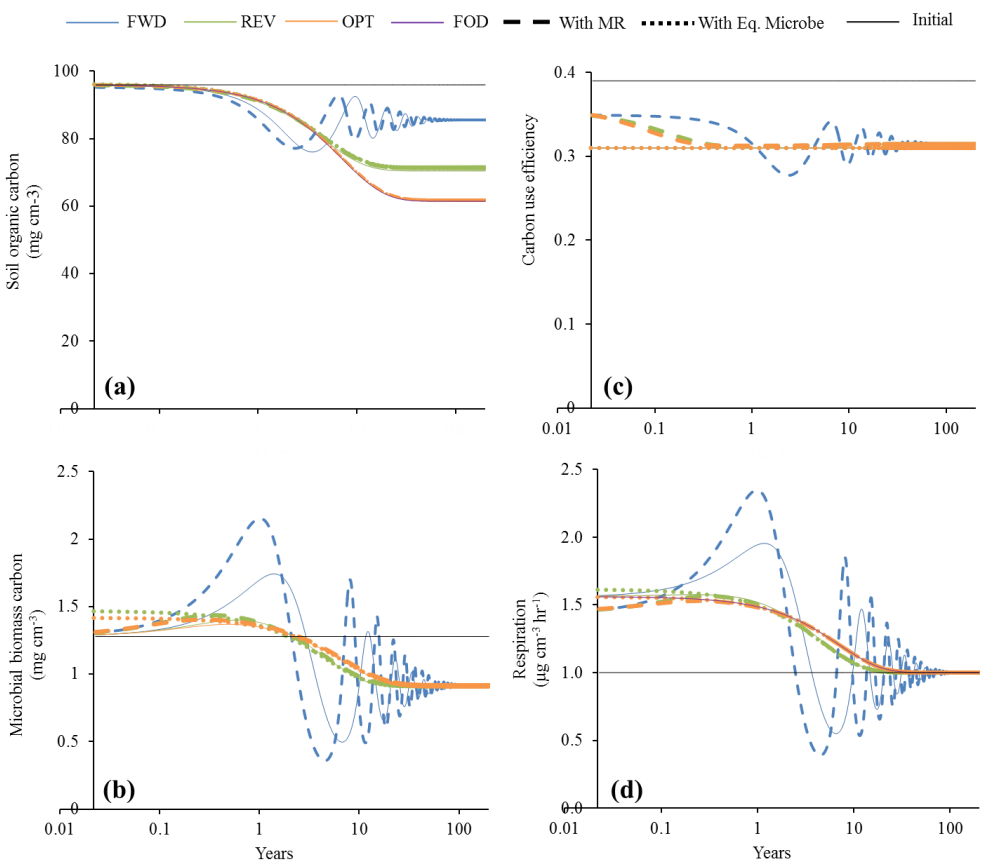
Fig. 2


FWD
REV
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With MR
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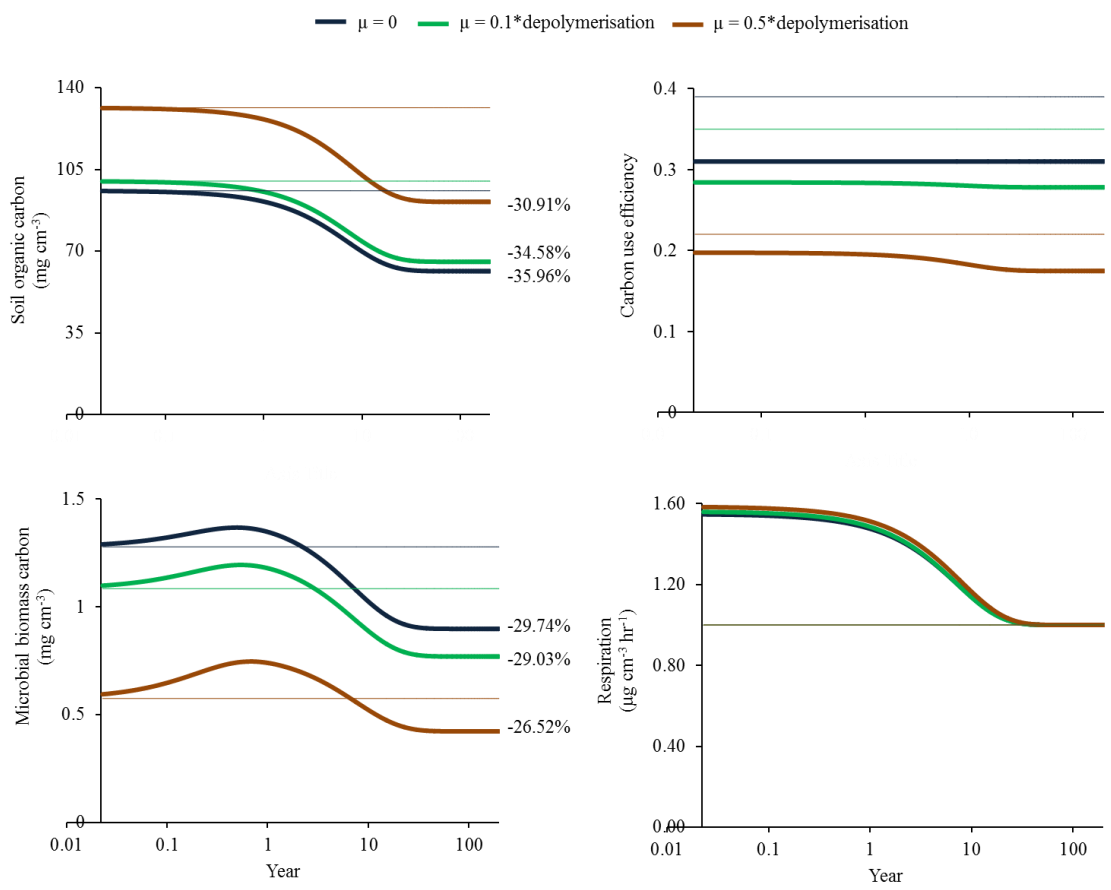
Initial
Soil organic carbon (mg cm-3)
(a)
Carbon use efficiency
(c)
Microbial biomass carbon (mg cm-3)
(b)
Respiration (µg cm-3 hr-1)
(d)
Years
Years

Fig. 3



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2 ~~Fig. 4~~Figure 4. Apparent Q_{10} of respiration over time, $Q_{10}(t)$ a) in our five microbial

3 decomposition models, and b) under different levels of enzyme expenditure cost in model 4.

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