Dear Editor,

We have carried out a thorough revision of our manuscript. Most importantly, this included a recalibration of all models following a well documented procedure. The methods have been updated to reflect this and provide all steps to reproduce the study. In the new calibration we carried out a well documented first step to explore parameter spaces using Latin hypersquare sampling. In addition, we changed the following:

- Activation energy parameters for all reaction rates were merged into one parameter E_V (not for enzyme and microbial decay).
- Parameter f_ug was fixed at 0.7 following Hagerty et al. 2014.
- V_D was separated into different parameters, one for each reaction kinetics type (Eq. 6-9). The latter was done after calculating that the ranges required for these parameters differ by several orders of magnitude, and thus require separate calibration (our previous calculations had been incorrect).

Recalibration generally resulted in a better model fit (an exception was model M2-sat which we took anyways for consistency). In particular, first and second order decomposition models now resulted in good fits, so that we were able to make a relevant comparison between them and Michaelis-Menten models. The manuscript was thus generally and significantly improved. The main results remained unchanged, with diffusion and MM kinetics performing the best. All figures and numbers were updated. A second temperature sensitivity figure was added. Several figures and tables were also added to the supplementary material.

The improved results allowed us to better address the comments from the reviewers (as discussed in our respective answers to each). Thus, we added a new model version with reverse Michaelis-Menten kinetics and expanded the results and discussion sections on temperature responses as well as several other sections.

We also changed the ms title to reflect the broader focus of the study: "Diffusion limitations and Michaelis-Menten kinetics as drivers of combined temperature and moisture effects on carbon fluxes of mineral soils"

Here follow specific responses to editor comments and our previous responses to reviewers added below. (Note that pages and lines of modifications are respect to the non-revised manuscript. Positions may differ in the new version).

1. When measuring the result of the cumulated CO2 evolution in differing time intervals, i.e. in sealed samples, the O2 concentration will fall under the experiment, accordingly.
Was the reduction in O2 concentrations considered and if not, what were the consequences for the interpretation of the results?

According to the accumulated CO2, minimum O2 levels were over 15 %. We now discuss O2 limitations in the manuscript in light of previous literature and our observations. P16 L9-14 (revised ms)

2. In your comment to Robert Grant's report, you mention (page 3 at the end of the second paragraph):
"Adding further complexity would introduce parameter identifiability problems and add no relevant information."
If you intend to use this argument in the discussion, I would ask you, to consider the situation that two or more drivers affect the same process in a similar way. Examining only one of them, will

include the risk of falling for a "wrong" one in the empirical analysis. So, one should be careful when going for simplicity and discuss the risks of doing so.

We agree. With "no relevant information" we meant functions with variables that in our experiment do not change. While O2 is a changing factor, we now discuss why it may not have been limiting. See response to comments below.

3. In your comments to Thomas Wutzler's report, you mention on page 6 that you based your parameter space exploration and choice of initial parameters on preliminary work ('manual'...) . I would rather recommend using the appropriate statistical methods (as suggested in the report) and document the robustness of the approach. Then give the corresponding parameter correlation matrix (appendix) and discuss the main parameter correlations in the text also in the light of model complexity and equifinality (see point mentioned above). Please make sure to use a methodology that is reproducible (the 'manual' is not).

As stated above, models were all recalibrated following a documented procedure. Using a Latin hypercube of parameter spaces as a first step allowed a better exploration of parameter spaces. See manuscript for details. We now added the parameter correlation matrix as well as kernel density estimation plots to indicate which parameters were better constrained.

Response to comments by R. Grant

We thank R. Grant for a critical evaluation of our work. Below we address each comment individually.

"General comments This is an interesting model study that makes a key point that higher order kinetics are needed to model respiration responses to changes in temperature and soil water content. This point is important because many SOC models still retain first order kinetics when making projections of climate change effects on SOC and hence on climate feedbacks, with a possible risk of error. Of particular interest to me but not often considered in modelling was the reduced sensitivity to temperature of microbial decay vs that of uptake and growth, and the point raised in the Discussion about density-dependent microbial decay, as both these processes affect microbial biomass and hence decomposition rates in higher order models."

We agree. We found it particularly interesting that from a process perspective we can demonstrate that respiration dynamics do not necessarily reflect decomposition dynamics in the short and mid-term.

"The authors have done a good job in testing model performance against experimental results from soil incubations. However a more constrained testing of its performance would be achieved by comparing the actual time courses of CO2 emissions measured during the incubations, rather than just the totals measured over the duration of each treatment as done in this study."

Soil respiration was determined by measuring the accumulated CO_2 in the flask's headspace at irregular intervals. As such, the data represent a time course, although irregular and of low frequency (we unfortunately did not have the capability to measure high frequency respiration rates). This actual measured data was what we used for modelling purposes. While this is already

described in the methods, we added text in order to make the procedure clearer.

Changes in manuscript: added text near P.4 L.28-29, P.8 L.10-14

"Two key areas need to be developed for this model to be capable of more robust performance and hence wider application: the coupling of C with N for all transformations, because kinetics of decomposition and respiration are strongly affected by SOC quality, and the simulation of O2 limitations on microbial activity rather than fitting declines in activity with higher soil water contents. Both these areas are already well developed in some ecosystem models."

We fully agree that these two aspects are potentially important for building predictive soil carbon models for general application. There are other processes that should be included in predictive models as well, such as oregano-mineral associations and vertical transport of SOM/DOC. However, this study has a defined focus and purposefully ignores many process that could be relevant under a variety of situations. This study does not present a generally valid predictive model. On the other hand, we believe the model showed a robust performance for the purpose of simulating our observations. Diffusion limitations as implemented here can, in future studies, be integrated in more complex predictive models and validated against larger sets of data.
The variability in SR at high water content was captured by our diffusion-based model without a representation of O2 limitation. We note that this model does not include an empirical decline at higher moisture (as we understand is suggested in the comment). Only the saturation function in the alternative model we compare it with included such an empirical function. This does not imply that O2 limitations are not an important limiting factor in saturated soils and mechanistic simulations in that area are useful.
In summary, our approach was to start from a simple model and add complexity until the observations could be reproduced, specifically testing the effect of adding diffusion. Adding further complexity would introduce parameter identifiability problems and add no relevant information. That said, we understand the comment addresses the "*wider application"* of the model and we now further address this by extending the discussion.

Changes in manuscript: added text near P.13 L.1-7

"P.2. L.15: a strong effects ? Introduction P.2 L.29: But note more rapid soil N mineralization, uptake, NPP and litterfall that may offset this feedback. N cycling very much needs to be included in any soil SOC model and the authors need to acknowledge this."

We now acknowledge this in the discussion making the model limitations more explicit.

Changes in manuscript: added text near P.13 L.15-18

"P.4 L.16: Soil grinding and mixing will increase microbial access to SOC from that in a natural soil, likely raising decomposition rates."

Agreed but unavoidable in this setup. To minimize such effects, we allowed samples to rest during pre-incubation.

"P.4 L.18: A BD of 1.8 is inconsistent with a porosity of 0.45. One or the other must be checked."

Bulk density was incorrect and was changed.

Changes in manuscript: bulk density corrected to 1.4 g cm-3. P.4 L.18

"P.4 L.21: The saturated value of 0.25 is less than the porosity of 0.45."

Please note these are gravimetric moisture values, so 0.25 in g/g is close to the 0.45 volumetric content.

"P.5 L.6: This statement is valid as long as the model simulates experimental protocol (e.g. duration of treatments)."

Agreed. Our simulations reproduced the exact incubation protocols we used.

Modelling approach
"P.7 L.3: Although if diffusion limitations reduce FPD you will also reduce CD and hence uptake, so make sure there isn't a duplicated effect caused by direct diffusion limitation to CD."

Since in the model diffusion affects enzyme pools and the availability of CD for uptake, there is a double effect on uptake, one indirect and one direct. This is intended. However, because CE decays and CD does not, long term effects will result from the former but not from the latter limitation. Unless CD is lost through another path such as leaching (here not considered).

"P..7 L.7: Check variable names."

Changes in manuscript: corrected the variable name.

"P.7 L.13: But temperature sensitivity of fmr is different from that of growth."

Equations 15 and 16 had not been updated to the latest model version and were incorrect. They have been corrected. (But note that other manuscript sections were correct, e.g. parameter table). r_mr is in fact temperature dependent, as expected. Temperature dependencies were calibrated, since we did not find strong evidence for fixing these parameters.

Changes in manuscript: corrected a wrong version of equations 15 and 16. Now F_MP= C_M * r_md and F_MRM = C_M * r_mr

---

"P.8. eq.19: Low and high temperature inactivation terms are often used with Arrhenuis equations to give greater Q10 at low temperature and must lower Q10 at higher while using biologically realistic values of Ea (typically ca 65 kJ mol-1)."

With a simple Arrhenius function, we found that the observed Q10 can vary between low and high temperatures (see supplementary figures). For the range we used, between 4 and 35 degC, a more complex temperature function was not justified.

Model Calibration
"Was a spinup run used to enable key state variables to stabilize at values independent of those initialized? This is standard modelling protocol."

A spinup would be valid only if a steady state at initial conditions is assumed. Our soil was from arable fields and pre-processes in the lab. Because of this we did not assume steady state at time 0 and instead estimated the initial pool sizes through calibration, as done in other studies (Menichetti, et al., *Biogeosciences,* 2016). This is stated in P.8 L.14-16.

"P.8 L.25: This is a commendable objective because some SOC models still retain first order algorithms."

Agreed.

"P.9 eq. 20, 21: Reductions of f (theta) and f(psi) at higher theta and psi are caused by O2 deficiency as noted later in the text, and are better modelled as such because these reductions are temperature-dependent."

We commented on the O2 limitations above and in the paper discussion.

"P.10 L.4: MPa Results"

Changes in manuscript: spelling corrected

"P.11 L.6: How were C inputs evaluated, as in natural ecosystems these also vary with temperature and swc. In fact, these inputs are the most important part of a SOC model as they are the main drivers of microbial activity."

We used a fixed value of 1.2 g d^{-1} C, which we found to be realistic for cultivated temperate soils. Steady state was calculated analytically (supplementary equations) so inputs, temperature and swc needed to be constants, as described.

"P.11 L.13-14. This is a nice test of the model. Describe how values for initialization of C pools and threshold swc were determined for this study. How did these values affect model results, particularly without model spinup? Ideally you should just change total SOC as determined from the soil measurement, and develop rules for allocating total SOC to initial C pools depending on site conditions, and then spinning up the model to equilibrium before comparison with observed values. An even better test of the model would be against the actual time course of CO2 effluxes measured during each incubation, as has been done in earlier modelling studies (e.g. Soil Sci. Soc. Amer. J. 58:1681-1690). This test lets you see whether the model is really simulating the temporal dynamics of respiration at different water contents under changing temperatures."

As stated in P.9 L.23 – P.10 L.4., the C pools were initialized not by spinup but by calibration, given that also in the validation case we could not determine if initial conditions were in steady state. The reason to calibrate the swc threshold during validation is that this parameter is expected to change between soils but we do not currently have a reliable means to estimate it, as stated in P.10 L.1-4. We expanded the discussion where we address the issue of how pool sizes may be

affecting the modelled and observed values

Changes in manuscript: text added near P.14 L.2–8

Discussion
"P.12 L.10. Specify these changes as noted in Results to establish how robust the model really is."

P.12 L.10 reads "We note that few studies were found with data on moisture and temperature interactions and this was the only validation attempt carried out." It is not clear what changes are referred to.

"P.12 L.19-20. Would this problem be addressed by a cold temperature inactivation term in eq. 19?"

A further decrease in activity using an inactivation term, while realistic, would probably exacerbate the problem here, since it seemingly already is the result of the lower rates under colder conditions. A solution to this problem is however out of the scope of this study.

"P.12 L.29-30. The absence of O2 limitations is likely causing the reductions in Ea and Q10 at higher swc in Fig. 5. Modelling these limitations should be a key next step in model development. These limitations are already simulated in some other ecosystem models."

We believe it is likely not a O2 limitation for two reasons. First, the decrease occurs sharply at ca. 50% saturation. At this water content and in small samples O2 should not be limiting. Second, our model reproduced this decrease quite well without O2 limitations, showing that it is the result of pool dynamics.

"P.13 L.4-5: The reduced temperature sensitivity of microbial and enzyme decay needed to model realistic biomass at different temperatures is an important finding of this study."

Yes, our results are compatible with such lower values.

Discussion paper
"P.13 L.15: Experimental determinations of Ea are often in the 65 kJ mol-1 range. The larger value modelled here may have been required in the absence of a cold temperature inactivation term in eq. 19."

If 4 degC is "cold" then this may be the case. It should be noted, however, that experimental determinations are "apparent" values. Apparent values given by our model are also in the lower range.
However, our focus is more on the distinction between the prescribed values (parameter values) and the apparent ones and how these may interact with moisture. A detailed analysis of temperature effects is outside this study's scope.

Changes in manuscript: added text near P.14 L.2-8

"P.13 L.16-18. Lower values probably arise from O2 limitations. The authors realistically address the current limitations of the model."

We do not discard a O2 effect, especially near saturation. But see our responses above.

"P.13 L.32: models"

Changes in manuscript: spelling corrected.

"P.14 L.8-9. Why not make the percolation threshold depend on soil water potential (e.g. -15 MPa)? This might improve model robustness by reducing reparameterization for each soil."

The reason is that the published value was not valid for this soil, as explained in the following lines P.14 L.22-25.

"P.14 L.30-21: Would CUE decline at higher temperatures if Rm (fmr in (16)) increased exponentially with temperature, as it is known to do?"

As noted above, the equations using fmr were outdated and have been corrected. The parameter r_mr is temperature dependent and determines the Rm flux. If CUE changes with T may depend on its definition. Here we define it as f_ug, so it remains constant.

We thank T. Wutzler for a constructive review. Below we address each comment individually.

Reviewer comments are in quotations followed by the author response and, where relevant, changes to the manuscript.

Response to comments by T. Wutzler

"Moyano et al. compare several versions of SOM turnover models with a comprehensive set of observations of varying temperature and soil moisture. They show that explicitly accounting for diffusion, compared to using empirical formulation of the temperature/moisture rate modifiers, improves fit and understanding of SOM decomposition. This result is interesting to the soil model developers and biogeoscientists studying SOM turnover and consequences at soil core to larger scales. The paper contains a strong validation by a good agreement with independent data. The clarity of the discussion on reasons for the good validation fit, interactions with initial pools, and different resulting temperature sensitivities can be improved. With an extension of discussion and some more clarifications in the discussion, the paper could be published. Nevertheless, I suggest several additional tasks with this model and data, that would help the community."

Please see responses to comments by R. Grant for changes already made to the discussion.

"First, while the paper already contains three different structural versions of decomposition, I suggest including another version of an inverse Michaelis-Menten dynamics for depolymerization (but not for DOM uptake), where the non-linear term is in enzymes instead of the substrate (F = V

*C_P * C_E/(K + C_E)). This would broaden the application of conclusions of this study, because the inverse formulation is used by many microbial models since suggested by Schimel and Weintraub 2003."*

Reverse MM kinetics assume that enzyme concentrations can increase enough that they start to compete for binding sites on SOM and thus saturate at some point. Schimel and Weintraub used this approach to deal with a problem of model instability driven by the dynamics of the microbial pool. However, we think a general saturation of the available SOM by enzymes in soils is unlikely to be the norm, as it would imply a large and likely unsustainable production of enzymes and very rapid decomposition of all polymeric C. We did not have stability issues in our model that would justify using this MM form and find it an unlikely explanation of soil C dynamics. However, we will test the effect of using the reverse MM and, if relevant, include information in the revised manuscript.

Changes in manuscript: inclusion of any relevant results following model calibration using reverse-MM and comparison with other versions.

"Second, the study describes a decoupling between depolymerization and microbial uptake at low diffusion rates, I assume by accumulating OM in the dissolved pool. Are the fluxes correlated again for the same treatment, if you aggregate over say two weeks? The decoupling is a challenging fact for upscaling studies, that often assume the DOM pool in quick quasi steady state with decomposition and microbial uptake. For low moisture the decomposed flux was almost not taken up and respired (Fig. 9). Would this also be true with two separate DOM pools after longer time? I would appreciate an extended discussion on this topic."

As well pointed out, Figure 9 shows that for some samples at lower moisture decomposition did not equilibrate with uptake in the 6 months of the simulated incubation. The plot also gives a good idea of how fast soil at higher moisture content return to equilibrium.
We kept the model simple where possible. The current form where there is only one DOC pool is a simplification that assumes microbes have access to an amount equivalent to the concentration in the bulk soil times a conductivity value. If conductivity is not 0, this amount will increase if the concentration increases, until the input from decomposition equals output from uptake. The reason this is done differently for enzymes is that enzymes have a decay rate, which means that the pool decreases with time. So even if equilibrium is reached, the flux of enzymes from microbes to the decomposition site will be lower if conductivity is lower, simply because a larger fraction is lost before diffusing. Further analysis of the model could indeed go more into detail looking at such dynamics. We take this as a suggestion future research.

Changes in manuscript: discussion extended under section 7.2 Moisture effects and diffusion limitation

---

"The model used enzyme pools split to locations but a simplified diffusion limited rate multiplier for DOM. What is the reasoning for this decision, and what are the expected consequences for using a rate modifier for enzymes too?"

See response to previous comment and addition to discussion.

"There is an interesting differentiation between parameterized temperature sensitivity (E_a) and an apparent predicted one, the latter one also depending on partitioning of the pools (P13l20ff). What are the reasons and consequences here. The paper would profit from an extended discussion here."

We have extended the discussion on this topic. See also responses to R. Grant.

Changes in manuscript: discussion extended. P.14 L.2-18

"p5l10: The choice of the wording "particulate" suggests to OM floating together with the DOM. I assume instead that C_P comprises litter and residues also sitting on surfaces. When using "polymeric" it conveys a different connotation and still the "P" can be used as acronyms."

We followed the advice and changed to "polymeric". (For the record: according to Wikipedia "Particulate organic matter is defined as soil organic matter between 0.053 mm and 2 mm in size".)

Changes in manuscript: the term "particulate" was changed to "polymeric"

"p7l10: The model assumes enzyme production to be modeled similar to growth respiration as a fraction of uptake, instead similar to maintenance respiration as determined by microbial biomass. What are the reasons for this formulation ? "

From a practical side, initial testing of model structure resulted in this approach fitting the data best (data not shown). From a theoretical side, is would be logical that microbes produce enzymes mostly when C becomes available and save resources otherwise. A continued enzyme production would lead to an unnecessary depletion of resources. We now note this in the same paragraph.

Changes in manuscript: text added near P.7 L.10-12

"p8l4ff: The wording here suggests, that all the processes have the same temperature sensitivity, i.e. same E_a. I suggest adding another index to E_a that this parameter varies between processes."

We followed the suggestion and added a subindex.

Changes in manuscript: E_a in equation 19 changed to E_ap

"P8l10: I assume there is only one set of parameters fitted to the entire data of all temperature and moisture treatments. Would be nice to state that here. Please, also state the number of fitted parameters, and add the initial partitions to Table 1.

That is correct. This is now clarified. Because of space limitations, initial parameter values and their lower and upper bounds were added in a table in the supplementary material.

Changes in manuscript:

- text added at P.8 L.10-11.
- table with initial parameter values and boundaries added to supplementary material.

The fitted parameter vector in a 20 dimension space is quite challenging for a gradient based search. Did you check global convergence by starting from more states, maybe more random distributed as just the one described for p11l30. What are the most important correlations in

parameter estimates?"

We agree. A global minima can of course not be guaranteed. In preliminary work we explored parameter spaces manually and using latin hyper square methods. The initial parameter values used here are already the result of these tests. Parameters in such model are often correlated and this was also the case in our study. High, but not very high, correlations occurred between some parameters, e.g. V_U_ref and g_0 (0.89), V_U_ref and E_r (0.83), V_D_ref and g_0 (0.84), f_CD and E_K (0.83) and V_D_ref and V_U_ref (0.8), f_ug and f_ge (0.83). We therefore do not make conclusions on how well constrained our estimates are, as this information is not obtained with gradient or deterministic algorithms. However, we now added these remarks and a correlation plot in the supplementary material for extra information.

Changes in manuscript:

- text added in the discussion P.12 L. 13-16
- correlation plot of parameter sensitivities added to the supplementary material

---

"P8l10: model calibration open questions: How were enzyme pools initialized? What were the values of fractions for particulate, dissolved and microbial pool, how do they compare to usual concentration of DOM and microbial biomass? I assume they were equal for all moisture and temperature treatments, right?"

As clarified in response to comments by R. Grant, all C pools were initialed by fitting them similarly to other model parameters (as initial steady state was not assumed). Upper and lower bounds were set (see Table S1) to assure they stayed in a realistic range (text added in P.8 L.23-24). The initial values of these fractions are found in Table 1. f_M with 0.07 is in particular on the upper range of observed values.

"P3l20: Citation of the kinetic respiration analysis (Wutzler 2011) is not appropriate in this context. I assume you wanted to refer to: Wutzler T & Reichstein M (2008) Colimitation of decomposition by substrate and decomposers - a comparison of model formulations. Biogeosciences, 5, 749-759 10.5194/bg-5-749-2008"

Thanks for pointing this out.

Changes in manuscript: reference changed to Wutzler T & Reichstein M (2008)

"Fig 4: There seem to be two groups of observations, a higher branch and a lower one. Why is this? Is it ok to fit a single smoother to this data?"

Figure 4 is mainly meant as a visual aid since it is not possible to mark which model point corresponds to which data point. The smooth lines ignore the variability along the y axis, caused mainly by the time effects resulting from two incubation cycles, but they help visualize the general resulting relationship between moisture content and respiration fluxes. We added this clarification in the results section.

Changes in manuscript: added text near P.11 L.2-4

Technical comments:

The grammar of the paper needs to be re-checked, e.g. p9L18, p9L25, p13L19."

Changes in manuscript: spelling and grammar mistakes were corrected.

Diffusion ~~based~~ limitations and Michaelis-Menten kinetics as drivers ~~of modelling of~~ combined temperature and moisture effects on carbon fluxes of mineral soils

Fernando E. Moyano[1]*, Nadezda Vasilyeva[2,3], Lorenzo Menichetti[4]

[1]University of Goettingen, Bioclimatology, Göttingen, 37077, Germany

[2]UPMC-CNRS-INRA-AgroParisTech, UMR 7618, Bioemco, Thiverval-Grignon, 78850, France

[3]V.V. Dokuchaev Soil Science Institute, Moscow, Russia

[4]Sveriges Lantbruksuniversitet (SLU), Ecology Department, Ulls Väg 16, 756 51 Uppsala, Sweden

Correspondence to: Fernando E. Moyano (fmoyano@uni-goettingen.de)

Abstract. ~~While~~ CO_2 production in soils ~~strongly~~ responds strongly to changes in temperature and moisture but~~,~~ the magnitude of such responses at different time scales remains difficult to predict. ~~In particular, little is known~~Knowledge of the mechanisms leading to the often observed interactions in the effects of these drivers on soil CO_2 emissions is especially limited.~~, even though such observations are common. Here we compare a number of modelling approaches to test which underlying mechanisms best~~ Here we test the ability of different soil carbon models to simulate ~~the interactive~~ responses measured in soils incubated ~~under combined levels of temperature and~~ at a range of moisture levels and cycled through 5, 20 and 35 °C. We applied parameter optimization methods while modifying two structural components of models: 1. the reaction kinetics of decomposition and uptake and 2. the functions relating fluxes with soil moisture. We found that ~~two model components were critical for reproducing~~ the observed interactive patterns were best simulated by a model using ~~: 1. : 1.~~ Michaelis-Menten ~~reaction~~ decomposition kinetics~~, which strongly improved the model fit when applied to decomposition reactions, and 2.~~ combined with diffusion of dissolved C and enzymes. ~~The latter replaces c~~In contrast, conventional empirical functions ~~as a mechanism relating moisture content with C fluxes~~that scale decomposition rates directly~~.. Indeed, empirical functions failed to~~ were unable to properly simulate ~~capture~~ the main observed interactions. ~~After model calibration we were able to~~Our best model was able to explain 87 % of the variation in the data. Model simulations revealed ~~resulted in~~ a central role of Michaelis-Menten kinetics as a driver of temperature sensitivity variations as well as a decoupling of decomposition and respiration C fluxes in the short and mid-term, with ~~interaction effects and~~ general sensitivities to temperature and moisture being more pronounced for respiration. Sensitivity to different model parameters was highest for those affecting diffusion limitations, followed by activation energies, the Michaelis-Menten constant, and carbon use efficiency. ~~Model validation resulted in a high fit against independent data~~Testing against independent data strongly validated the model (R^2 = 0.99) and highlighted the importance of initial soil C pool conditions.~~). The same underlying model parameters resulted here in different apparent temperature sensitivities compared to the calibration step, demonstrating a strong effects of initial soil conditions. With t~~ Our~~these~~ results ~~we could~~ demonstrate the importance of model structure and the central role of diffusion and reaction kinetics for simulating and understanding complex dynamics in soil C.~~ dynamics related to temperature and moisture interactions. Future studies should further validate this mechanistic approach and extend its use to a larger range of soils.~~

1. Introduction

Soils are a main component of the global carbon (C) cycle, storing ca. 2200 Pg of C in the top 100 cm ~~alone~~ according to recent estimates (Batjes, 2014). This soil C pool is dynamic, and often exists in a non-equilibrium state as the result of an imbalance between input and output C fluxes, in which case it will act either as a C sink or source over time. Changes in the speed at which soil organisms decompose soil organic matter (SOM) and mineralize soil organic carbon (SOC) into CO_2 are one way in which an imbalance can occur, producing a net sink or source of atmospheric CO_2.

It is well known that SOC mineralization and resulting CO_2 fluxes are highly sensitive to variations in soil temperature and moisture (Hamdi et al., 2013; Moyano et al., 2013). As a result, feedback~~s~~ effects, either positive or negative, are expected to

occur from the interaction between climate change and global soil C stocks (Crowther et al., 2016; Davidson and Janssens, 2006; Kirschbaum, 2006). However, the direction and magnitude of such feedbacks at the global scale remain uncertain. Increased soil respiration with a resulting net loss of soil C, and thus a positive climate feedback, is expected with the warming of permafrost soils and the drying of wetland soils. But there is still ~~much~~ large uncertainty and a lack of consensus regarding the long term response to climate variability of soils that are non-saturated, non-frozen, and dominated by a mineral matrix (Crowther et al., 2016), i.e. soils found under most forests, grasslands and agricultural lands.

Future predictions of soil C dynamics require the use of mathematical models. Early soil C models~~,~~ and most still in use~~,~~ are based on first order decay of multiple C pools, with temperature and moisture having ~~a~~ general non-interactive effects on decay rates (Rodrigo et al., 1997). When appropriately calibrated t~~T~~hese models~~, when appropriately calibrated,~~ do well at simulating soil respiration fluxes of soils under relatively stable conditions. They ~~were~~ are often developed to approximate long term steady~~–~~state conditions under specific land uses. They are also capable of fitting long term trends of soil C loss, such as data from long-term bare fallow where all litter input has stopped (Barré et al., 2010). However, they lack a theoretical basis justifying their basic assumptions of pool partitioning and decay mechanisms. They also generally need calibration for specific soil types or land cover types, and often fail to properly simulate observed short and mid-term variability in soil respiration.

Some of the most relevant observations these models have failed to reproduce include: changes (typically a dampening) of temperature sensitivities of decomposition over time (Hamdi et al., 2013), non-linear responses to soil moisture content (Borken and Matzner, 2009), and changes in decomposition rates in response to variations in concentrations of organic matter (Blagodatskaya and Kuzyakov, 2008). Such model shortcomings, which reflect missing or wrongly simulated processes, create a difficult to quantify uncertainty in global long term predictions of soil C and its feedback to climate change. It is therefore unclear if first order models can predict long term changes in C stocks under more dynamic (and therefore realistic) environmental conditions.

Second order models have a more realistic basic structure compared to conventional first order models, since they simulate organic matter decomposition as a reaction between ~~two pools, one of these being~~ SOC and ~~the~~ decomposers ~~pool~~ (i.e. a microbial ~~pool~~ or enzyme pool). This single but fundamental change in decomposition kinetics strongly affects predicted long term changes in soil C, largely as a result of the dynamics of the decomposer pool, which itself can respond to temperature in a number of ways (Wutzler and Reichstein, 2008). Second order models also lead to more complex dynamics of short to mid-term soil respiration, with apparent temperature sensitivities that vary over time, more in line with many observations.

The temporal variability in the response of decomposition to moisture is most evident in the strong respiration pulses after dry soils are re-wetted, known as the Birch effect (Birch, 1958). But studies have shown that a successful simulation of ~~the soil respiration pulses associated to re-wetting events~~ such pulses requires the incorporation of additional mechanisms, namely the explicit representation of a bio-available C pool, such as dissolved organic matter (DOC), and a moisture regulation of decomposer's access to this pool that may differ from the moisture regulation on the decomposition reaction itself (Lawrence et al., 2009; Zhang et al., 2014).

The response of soil respiration to temperature and moisture is highly dynamic, both spatially and temporally (Hamdi et al., 2013; Moyano et al., 2012). Moisture and temperature interactions have been observed in a number of experimental studies (Craine and Gelderman, 2011; Rey et al., 2005; Suseela et al., 2012; Wickland and Neff, 2008), but neither consistent trends nor general explanatory theories have been identified. Improving our understanding of these interactions is a crucial step in increasing confidence in models and for interpreting modelling and experimental results (Crowther et al., 2016; Tang and Riley, 2014). Identifying the model structures and parameterizations that can best represent these interactive effects has been attempted by very few studies (Sierra et al., 2017, 2015).

The objectives of this study are~~, first,~~ to compare the ability of different soil C modelling approaches to reproduce temperature and moisture interactive effects on soil carbon fluxes and thus to ~~, and second, to~~ gain insight into ~~the underlying~~ mechanisms underlying the observed responses ~~from the model comparison~~. With the hypothesis that a more mechanistic model will be better capable of simulating such interactions, we compare ~~variations of a~~ different model structures, ~~model based on a microbial model with an explicit representation of a dissolved C pool. We~~ testinged first order, second order, and Michaelis-Menten reaction kinetics in combination with an explicit simulation of diffusion fluxes. We then ~~, and then~~ compare the best diffusion model with versions based on common empirical moisture relationships.

2. Observational data

Measurements of the interaction effects of temperature and moisture on soil respiration fluxes were obtained by incubating a crop field soil at several fixed levels of soil moisture and variable levels of temperature over a period of ca. 6 months, as detailed in the following.

Soils from 0-20 cm depth were sampled at Versailles, France, from the 'Le Closeaux' experimental field plot, cultivated with wheat until 1992 and with maize since 1993. Mean annual temperature and rainfall are 10°C and 640 mm. The soil is classified as Luvisol (FAO) silt loam (26 % sand, 59 % silt, 15 % clay) containing no carbonates. Organic carbon contents at the start of the incubation were 1.2 % in weight. Soil samples were prepared for elemental analysis (C, N) using a planetary ball mill (3 min at 500 rpm). C concentrations were measured using a CHN auto-analyzer (NA 1500, Carlo Erba).

Sampled soils were thoroughly mixed, sieved at 2 mm and stored moist at 4 °C in plastic bags with holes for aeration for 10 days. Soils were then put in small plastic cylinders containing the equivalent of 90g dry soil. To ensure a high and equal water conductivity, all samples were compacted to a bulk density of 1.4~~8~~ g cm^{-3}. The resulting soil porosity was 0.45.

All samples were brought to a pF of 4.2 corresponding to about 7 % mass basis moisture. Three replicate samples were then adjusted to each of the moisture levels 1, 3, 7, 9, 11, 13, 15, 17, 19, 21, 25 % by weight by adding water or air drying. These values range from air-dry to saturation, with saturation reached at 25 %. Immediately after, the plastic cylinders were put in 500 ml jars containing a small amount of water on the bottom (except for the 1 and 3 % moisture) to prevent soil drying, and equipped with a lid and a rubber septum for gas sampling. Because of the extremely low respiration rates, samples with 1 % moisture were placed in 125 ml jars containing 170 g of soil.

To minimize post-disturbance effects, samples were pre-incubated at 4 °C during 10 days. The samples were then cycled through incubation temperatures following the sequence 5-20-35-5-20-35 °C, thus applying two temperature cycles to each sample. This was done in order to capture possible hysteresis of temperature effects and to reduce~~reduces~~ the covariance between a temperature response and substrate depletion (helping constrain model parameters). Soil respiration was calculated at every temperature step by measuring the amount of CO_2 accumulated in flask headspaces. For this, samples were ~~Samples were~~ flushed with CO_2 free air and left to accumulate CO_2 for 3 to 74 days. The variable accumulation time~~amount of days~~ was chosen so that sufficient CO_2 accumulated for the micro gas chromatographer measurements (at least 100 ppm), thus depending on the soil temperature and moisture content. After the accumulation time, an~~An~~ air sample was ~~then~~ taken from each soil sample headspace and respiration rates calculated as the accumulated amount over the accumulation time. Samples were incubated for~~This process was performed repeatedly over successive temperature steps over~~ a total ~~incubation~~ period of ca. 6 months (Figure 1).

As shown in Figure 1, the timing of temperature treatments was not equal for all samples, with some temperature steps missing at low moisture levels. This was partly due to the time required for CO_2 concentrations in the flask headspace to reach detectable limits, the time necessary for carrying out measurements and human error. However, while important for a statistical comparison between treatments, such differences are of little consequence when looking at model performance and the fit between model and data, which is~~constitute~~ the focus of this study~~results presented here~~.

3. Modelling approach

3.1. Structure and state variables

We started with a basic soil C model with the following state variables: a bio-unavailable polymeric~~particulate~~ C pool (C_P), a bio-available dissolved C pool (C_D), a microbial C pool (C_M) and two extracellular enzyme C pools, one representing the enzyme fraction at the decomposition site (C_{ED}) and one the fraction at the microbial site (C_{EM}). With this model we assume two conceptual soil spaces that are separated by a diffusion barrier, one being the site of decomposition and the other the site of microbial uptake and enzyme production (Figure 2). This model thus closely follows Manzoni et al. (2016), and otherwise builds on other published microbial models (Allison et al., 2010; Schimel and Weintraub, 2003). We refer to those studies for general assumptions and application of this type of model. Aspects specific to this study are described below.

The rates of change of the model state variable were defined as:

$$\frac{dC_P}{dt} = F_{L_S P} + F_{MP} - F_{PD} \quad (1)$$

$$\frac{dC_D}{dt} = F_{L_M D} + F_{PD} + F_{E_D D} + F_{E_M D} - F_{DM} - F_{DR_G} - F_{DE_M} \quad (2)$$

$$\frac{dC_M}{dt} = F_{DM} - F_{MP} - F_{MR_M} \quad (3)$$

$$\frac{dC_{ED}}{dt} = F_{E_M E_D} - F_{E_D D} \quad (4)$$

$$\frac{dC_{EM}}{dt} = F_{DE_M} - F_{E_M E_D} - F_{E_M D} \quad (5)$$

where F represents the flux of C from one pool to another as indicated by the subscripts, so that F_{PD} is the flux from the polymericparticulate pool to the dissolved pool. The subscripts L_S and L_M denote input of structural and metabolic litter (as defined by Parton et al., 1987), which for simulating the incubated soils were set to zero, and R_M and R_G are microbial growth and maintenance respiration.

3.2. Decomposition and microbial uptake

The flux of C_P to C_D, F_{PD}, represents decomposition of organic matter, a process that in soils is largely driven by the activity of microorganisms. The latter produce exo-enzymes that catalyse the decomposition reaction. U_D, represents the total uptake flux by microbes of the water soluble decomposed pool C_D (microbes being the reaction "catalysers"). Conventional soil C models simulate decomposition as a first order decay reaction. However, more realistic models can be built by using either simple second order or Michaelis-Menten reaction kinetics. Thus, optional ways of modelling both F_{PD} and U_D include:

$$F = V[R] \quad (6)$$

$$F = V[R][C] \quad (7)$$

$$F = \frac{V[R][C]}{K + [R]} \quad (8)$$

$$F = \frac{V[R][C]}{K + [C]} \quad (9)$$

where F is the flux, V is a base reaction rate, K is the half saturation constant, R the reactant and C the catalyst. The 'reverse' Michaelis-Menten (Eq. 9) was applied by Schimel and Weintraub (2003) as an alternative for improving model stability and is included here for completeness.

In the case of decomposition, The value for V is not equivalent among these equations, differing by several orders of magnitude. As a result, different parameters were used for V in each case, namely V_{Dm}, V_{Dmr}, V_{D1}, and V_{D2}. Similarly, are V_D and parameters K_D and K_{De} were used for K in Eq. (8) and (9), respectively. The terms [R] and [C] are concentrations of C_P and C_{ED}. In the case of uptake, the parametersesthese are respectively V_U, K_U, C_D and C_M. The three four approaches for reaction kinetics were tested in order to find the best fit between model and data, as described in Sect. 4.

3.3. Diffusive fluxes

Diffusion fluxes depend on a concentration difference, a diffusivity term, and the distance over which diffusion occurs (Manzoni et al., 2016). For the purpose of modelling diffusion in soils, values of diffusivity and diffusion distances are required that best average or represent the actual underlying soil complexity. For practical purposes, we combined these two values into a single calibrated parameter, a conductance (g_0), representing the compound effects of diffusivity and distance. This was done because the values of the latter are unconstrained (from lack of information), and their effects are inversely correlated, so simultaneous calibration would lead to a problem of parameter identifiability. The moisture-scaled conductance (g), which in our model is assumed equal for the C_D and C_E pools, is then given by:

$$g = g_0 d_\theta \quad (10)$$

where d_θ is a function of soil volumetric water content (VWC or θ):

$$d_\theta = (\phi - \theta_{th})^m \left(\frac{\theta - \theta_{th}}{\phi - \theta_{th}}\right)^n \quad (11)$$

where ϕ is pore space, and n and m are calibrated parameters (Hamamoto et al., 2010; Manzoni et al., 2016), which are variable and were also calibrated in this study. θ_{th} is the percolation threshold for solute diffusion, for which ~~,~~Manzoni and Katul (2014)~~, was here set to 0.063 VWC, corresponding to~~ reported a value of -15MPa. This value was not optimal in our case, so θ_{th} was also calibrated. The diffusive flux of enzyme C between the microbial and the decomposition spaces is then calculated as:~~where ϕ is pore space, and n and m are calibrated parameters (Hamamoto et al., 2010; Manzoni et al., 2016), which are variable and were also calibrated in this study. θ_{th} is the percolation threshold for solute diffusion (Manzoni and Katul, 2014), which was here a calibrated parameter. The diffusive flux of enzyme C between the microbial and the decomposition spaces is then calculated as:~~

$$F_{E_M E_D} = g(C_{EM} - C_{ED}) \quad (12)$$

Diffusion limitations also affect the amount of the dissolved pool (C_D) available for microbial uptake. Instead of dividing C_D into a pool for each space, the conductance, g, was used as a multiplier of the base uptake rate, V_U (Eq. (6-~~8~~9)). This served to reduce the number of model pools and parameters while still retaining a diffusivity limitation on this flux.

3.4. Microbial and enzyme dynamics

U_D is split into F_{DM}, F_{DR_G} ~~F_{DE_M}~~ and F_{DE_M}, representing the fluxes of C_D going to C_M, R_G and C_{EM}, respectively. These fluxes are defined as:

$$F_{DM} = U_D f_{ug}(1 - f_{ge}) \quad (13)$$

$$F_{DR_G} = U_D(1 - f_{ug}) \quad (14)$$

$$F_{DE_M} = U_D f_{ug} f_{ge} \quad (15)$$

where f_{ug} represents the fraction of uptake going to growth, otherwise known as microbial growth efficiency or carbon use efficiency, and f_{ge} is the fraction of growth going to enzyme production. Enzyme production thus depends here on uptake rather than on microbial biomass. This approach follows the assumption that microbes produce enzymes only when new carbon is available and save resources otherwise. C_M goes to either maintenance respiration or the C_P pool according to:

$$F_{MP} = C_M r_{md}(1 - f_{mr}) \quad (16)$$

$$F_{MR_M} = C_M r_{mr} r_{md} f_{mr} \quad (17)$$

where r_{md} is the rate of microbial death~~decay~~ and $r_{mr} f_{mr}$ is the rate~~fraction~~ of microbial ~~that decay that is lost as respiration. f_{mr} thus determines the amount of~~ maintenance respiration. ~~and is here assumed to be constant (but note that r_{md} is temperature dependent).~~ The breakdown of enzymes going to the C_D pool, is determined by the rate of enzyme decay, r_{ed}, as:

$$F_{E_D D} = C_{ED} r_{ed} \quad (18)$$

$$F_{E_M D} = C_{EM} r_{ed} \quad (19)$$

3.5. Temperature effects

Reaction rates (V_U, V_D, K_U, K_D in Eq. (6-~~9~~8)~~,8)) and~~ decay and respiration rates (r_{ed}~~, and~~ r_{md}, r_{mr}) are temperature sensitive and calculated from their reference values following an Arrhenius type temperature response:

$$r = r_{ref}\, exp\left(-\frac{E_a}{R}\left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right) \quad (20)$$

where r i~~e p~~ is the temperature modified ~~value~~ rate~~for the respective parameter~~, r_{ref} ~~p_{ref}~~ the reference ~~value~~ rate at temperature T_{ref}, T temperature in Kelvin, E_a the activation energy, and R the universal gas constant. Three parameters were used for E_a: E_{a_m} and E_{a_e} for microbial and enzyme decay rates, respectively, and E_{a_V} for other reaction rates.~~Volumetric water content, θ ($m^3 m^{-3}$) and temperature, T, are model input variables.~~

Temperature thus affects the rates of decomposition and uptake, the half saturation constant in the Michaelis-Menten equation, as well as the rates of microbial and enzyme decay. Apparent activation energies – describing the observed temperature relationship, both in measurements and model data – were obtained by fitting an Arrhenius equation to the temperature-flux relationship at each level of moisture~~ and separately for 5-20 °C and 20-35 °C~~. E_a was calculated for measured respiration, modelled respiration (R_G+R_M) and modelled decomposition (F_{PD}).

4. Model calibration and comparisons

Calibrated and non-calibrated parameters for all models are given in the supplementary material (Tables S1, S2 and S3). Whenever possible, fixed parameters as well as lower and upper bounds for calibrated parameters (Table S1) were set according to values reported in literature (e.g. Hagerty et al., 2014; Li et al., 2014; Price and Sowers, 2004). Equilibrium conditions were not assumed at the start of the experimental procedure. Therefore, initial conditions were obtained by also optimizing the fractions of initial carbon pool sizes (f_P, f_D, f_M). Total organic C was set equal to the measured value. Models were calibrated by optimizing ~~a set of~~ parameters to best fit the measured soil respiration data described in section 2. Each model was calibrated by fitting a single set of parameters simultaneously to all the incubation data (Table S3).~~.~~ For this, the model was run to reproduce each sample treatment, i.e. the applied incubation times and temperatures for each level of moisture (Figure 1). Accumulated soil respiration amounts were then calculated to match those from the observed data. Measured and simulated data from all samples were then combined and ~~the~~ an overall model cost calculated using the root mean square error (RMSE) and a weighting term, as described below~~calculated~~. ~~Calibrated and non-calibrated parameters are shown in *Table 1*. Equilibrium conditions were not assumed at the start of the experimental procedure. Therefore, initial conditions were obtained by also optimizing the fractions of initial carbon pool sizes (f_P, f_D, f_M). Total organic C was set equal to the average measured value.~~

~~For p~~Parameter optimization was carried out in two steps. We first explored parameter spaces using a Latin Hypercube of parameter values. For this we randomly selected unique parameter sets from a uniform distribution over each parameter range (R function randomLHS, package lhs, Stein, 1987) to obtain 30000 parameter sets. Model costs were then obtained by running models with each set. In the second step we used the Nelder-Mead algorithm (as implemented in the function modFit in package FME of the R programming language, R Development Core Team, 2016; Soetaert and Petzoldt, 2010) with initial parameter values being the set from the previous step with the lowest model cost. For the cost calculations ~~W~~we used an error term ('err' argument to FME function modCost) to weight the residuals. The error was calculated as the normalized (0-1) standard deviation of measured values at each combination of temperature and moisture, with 0.1 added ~~T~~to avoid an unreasonable weighting of measurements with near zero errors~~, 0.1 was added to the normalized value. For parameter optimization we used the Nelder Mead algorithm, as implemented in the function modFit in package FME of the R programming language (R Development Core Team, 2016; Soetaert and Petzoldt, 2010). We used an error term ('err' argument to FME function modCost) to weight the residuals. The error was calculated as the normalized (0-1) standard deviation of measured values at each combination of temperature and moisture. To avoid an unreasonable weighting of measurements with near zero errors, 0.1 was added to the normalized value.~~

For a visual inspection of the model-data fits, we plotted both the measured and model relationship between soil respiration vs. moisture, soil respiration vs. temperature, and apparent activation energy (E_a) vs. moisture content.

4.1. Comparison of reaction kinetics

Models were named according to their decomposition kinetics followed by the uptake kinetics and the moisture function, using the abbreviations: 1 = first order, 2 = second order, M = Michaelis-Menten, M_r = reverse Michaelis-Menten, dif = diffusion, psi = water potential function, sat = water saturation function. ~~Models with a~~Alternative~~alternative~~ reaction kinetics leading to fluxes F_{PD} and U_D were compared ~~by calibrating versions~~ in diffusion based models using ~~using~~ different~~all~~ combinations of ~~fluxes F_{PD} and U_D using~~ Eq. (6-98)~~. Thus, we tested all combinations of~~. Specifically, we compared first order for decomposition and uptake (11-dif),~~,~~ second order for decomposition and uptake (22-dif), and~~,~~ ~~and~~ Michaelis-Menten ~~kinetics reaction kinetics for both~~ decomposition ~~and uptake~~with all combinations of uptake (M1-dif, M2-dif and MM-dif). In addition, we tested reverse Michaelis-Menten decomposition with second order uptake (M_r2-dif).~~.~~ We then evaluated the model-data fit based on RMSE values as well as on a visual inspection of the plotted relationships. A "best" model was then selected for further analysis.

4.2. Comparison of moisture regulations: diffusion versus empirical

A second model comparison was carried out to test the impact of different approaches for modelling moisture effects. For this we modified the model M2-dif (*Table 2*) removing diffusion fluxes and adding empirical moisture functions. This consisted in removing all diffusion effects (so that C_{EM} and C_{ED} were replaced by a single C_E pool and the uptake rate, V_U, was no longer modified by g) and adding a function to scale (i.e. multiply) the decomposition flux, F_{PD}. This approach is equivalent to the conventional way used to model moisture effects on soil C fluxes. Two alternative moisture scaling functions were tested (Moyano et al., 2013), one based on relative water saturation (M2-sat) and the other on water potential (M2-wp):

$$f(\theta_S) = a\theta_S - b{\theta_S}^2 \quad (21)$$

$$f(\Psi) = \max\begin{cases} min\begin{cases} 1 - \dfrac{\left[\log_{10}(\Psi) - \log_{10}(\Psi_{opt})\right]}{\left[\log_{10}(\Psi_{th}) - \log_{10}(\Psi_{opt})\right]} \\ 1 \end{cases} \\ 0 \end{cases} \quad (22)$$

were θ_S is relative water saturation, Ψ is soil water potential and a, b, Ψ_{opt} and Ψ_{th} are fitted parameters. The latter two represent the optimal water potential for decomposition and a ~~percolation~~ threshold water potential ~~(equivalent to θ_{th} in Eq. (10)), and have~~ with values of ~~close to~~ -0.03 and -15 MPa, respectively. Water potential was calculated based on Campbell (1974) and Cosby et al. (1984). a and b are empirical parameters and were calibrated.~~were θ_S is relative water saturation, Ψ is soil water potential and a, b, Ψ_{opt} and Ψ_{th} are fitted parameters. The latter two represent the optimal water potential for decomposition and a percolation threshold water potential (equivalent to θ_{th} in Eq. (10)), and have values close to 0.03 and 15 MPa, respectively. Water potential was calculated based on Campbell (1974) and Cosby et al. (1984).~~

5. Model steady state, sensitivity analysis and validation

Equations for steady state were derived by setting the rate of change in the state variables to zero in Eqs. 1-5 (where the flux terms are replaced by their respective equations), and then solving for the state variables. This was performed in Python using the "sympy" package (Meurer et al., 2017).~~Equations for steady state were derived by setting the rate of change in the state variables to zero in Eqs. 1-5 (where the flux terms are replaced by their respective equations), and then solving for the state variables. This was performed in Python using the "sympy" package (Meurer et al., 2017).~~

A sensitivity analysis was carried out on ~~all~~ model parameters~~. For this we simply used~~ using the ~~default~~ "sensFun" function from the R package FME, which perturbs each parameter individually by a small amount. We ran the model as above, i.e. simulating the incubation~~,~~ and using daily output. Daily sensitivities were then averaged to obtain an overall value. Sensitivity values were~~The sensitivity was~~ calculated for the C_P pool alone, as this pool represents the largest fraction of soil C.

For model validation, we used soil respiration data from the study by Rey et al. (2005) where a Mediterranean oak forest soil was incubated for one month in a full factorial design at 100, 80, 60, 40 and 20 % of water holding capacity and at 30, 20, 10 and 4 °C. This soil differed from the one used for model calibration in at least 3 aspects: the amount of organic C (7 %), soil pore space (65 %), and texture (classified as silty clay loam). The optimized set of parameters from model M2-dif was used with the exception of the initial fraction of C pools (f_P, f_D, f_M) and the percolation threshold (θ_{th}),which we ~~chose to~~ calibrated against the new data~~-~~ (Nelder-Mead calibration)~~with the same procedure as above~~. The former was required since we had no information to estimate the microbial, dissolved, and enzyme C for this study and information regarding an initial soil steady-state was also lacking.~~.~~ In the case of θ_{th}, we assumed that this parameter is especially sensitive to variations in soil texture and structure. Although~~Calibration was then necessary as we did not have a formula to derive it for the new soil (although~~ in previous studies it has been determined to be~~as~~ equal to a water potential of -15MPa (Manzoni and Katul, 2014)~~15MP,~~ this value did not provide a~~give~~ good fit when applied to the validation data.~~. results in our analysis).~~

6. Results

6.1. Reaction kinetics

The calibrated values for all models are shown in Table S3. Using different reaction kinetics resulted in ~~a strong~~ variations~~variation~~ in model performance as measured by RMSE (Table 2). Changes in RMSE were ~~most~~ more sensitive to the kinetics of decomposition (F_{PD}), with models using ~~Michaelis-Menten~~M and M_r decomposition kinetics resulting in ~~distinctly~~ lower RMSE values compared to 1st and 2nd order kinetics.~~On the other hand,~~ ~~different~~ In terms of uptake ~~reaction~~kinetics~~reaction kinetics~~, both ~~for the uptake flux, U_D, had a much smaller impact on the RMSE, being slightly lower for~~ 1st and 2nd order kinetics~~.~~ performed better than Michaelis-Menten kinetics.

Models M1-dif, M2-dif and M$_r$2-dif all showed a good fit to the data with the first two having a slightly higher R^2. Thus, selecting a "best" model necessarily remains partially subjective. A visual comparison shows some weaknesses and strengths in each case. M1-dif and M$_r$2-dif better captured the variability in the data along the respiration axis at 35 °C (Figure S1) while M2-dif more closely captured the relationship at 20 °C and thus the temperature sensitivities (Figure S2). ~~Given the small difference in performance between different uptake reaction kinetics,~~ We selected model ~~we chose to work with 2nd order kinetics for~~ M2-dif (R^2 = 0.87, Figure 3) as the "best" model, ~~further analysis (model M2-dif, R^2 = 0.84, Figure 3), as~~ since ~~this~~ it better ~~is a closer representation~~ represents ~~of the underlying mechanisms~~ the actual mediation of uptake ~~actually driving this flux, i.e. uptake does not occur without~~ by microbial ~~mediation~~biomass when compared to model M1-dif.~~.~~ ~~It also requires less parameters than Michaelis-Menten and is thus a compromise in complexity. (We note~~ We also had no theoretical reason to prefer M$_r$ to M decomposition. ~~(We note that the choice between 1st and 2nd order uptake had a small impact on this study's results. On the other hand, Michaelis-Menten uptake kinetics had the poorest agreement with observations when comparing the plotted relationships. Plots for the three models based on Michaelis-Menten decomposition can be found in the Supplement material, Fig S1 and S2)~~ The decomposition and uptake equations of ~~the~~ model M2-dif are then ~~thus~~:

$$F_{PD} = V_D C_{ED} C_P / (K_D + C_P) \quad (23)$$

$$U_D = C_D C_M V_U g \quad (24)$$

~~6.1.~~6.2. Moisture regulation

Replacing diffusion effects with empirical moisture scalars~~,~~ followed by re-calibration~~,~~ decreased model performance compared to a diffusion based model, both when using relative water saturation (M2-sat) and water potential (M2-wp) functions (Table 2). Although empirical functions were ~~it was possible~~able to ~~simulate~~ approximate the shape of the respiration-moisture relationship ~~for a specific temperature~~at 20 °C, they~~,~~ ~~empirical functions~~ were unable to capture the variation of this response ~~across~~ at higher and colder temperatures, as seen in the measurements and ~~best~~ simulated by ~~the~~ diffusion base models~~model~~ (Figure 4). ~~The d~~Diffusion~~diffusion~~ based models~~model~~ more accurately simulated a linear relationship between respiration and moisture at lower temperatures and a steep increase followed by a plateau at high temperatures, with an ~~. An~~ intermediate response ~~was~~ seen at 20 °C.

6.3. Temperature sensitivities

Figure 5 show the apparent temperature sensitivities fitted to observations and modelled fluxes at different moisture levels and for two temperature ranges, 5-20 °C and 20-35 °C. Figure 5 compares different reaction kinetics and Figure 6 different moisture functions. Michaelis-Menten decomposition outperformed 1st and 2nd order kinetics when simulating the variability in E_a observed along the moisture axis as well as the differences observed between colder (5-20 °C) and warmer (20-35 °C) temperature ranges. Model M2-dif closely followed the observed E_a values, which were near 100 kJ at colder temperatures

and in the 30-70 kJ range at warmer temperatures. Models M2-sat and M2-wp captured the large differences between temperature ranges but did not simulate the variability along the moisture axis as well as diffusion based models.

~~6.2.~~6.4. Model steady state, sensitivity analysis and validation

Model steady state equations are provided in the Supplement material. For 20 °C, 30 % VWC, 1.2 g d^{-1} C input, and 30 cm soil depth (z), the equilibrium sizes of the model pools are: 2560~~800~~, 375~~0~~, 120~~2800, 50, 7~~ and 40~~.4~~ g C for the C_P, C_D, C_M and C_{ED} pools respectively. These values are stable over most of the moisture range and increase exponentially only at very low soil moisture (data not shown). A similar pattern was observed for temperature, with the C_P pool increasing towards high values only at temperatures near 0 °C. The same pool showed little sensitivity to changes in C input.

Table 1 shows the averaged values from the sensitivity analysis done on the model C_P pool. ~~The h~~High~~esthighest~~ sensitivities ~~were~~ were found for g_0 ~~parameters~~ and n, indicating the importance of ~~parameters that affect the~~ diffusion fluxes~~, with the n exponent in Eq. (10) having the largest effect, followed by the base conductance, g_0~~. Large effects were also seen for ~~most~~ the activation energy parameters, denoting a strong general effect of temperature. Also high were the sensitivities to K_D and f_{ug}, reflecting the importance of Michaelis-Menten kinetics for decomposition and carbon use efficiently, respectively. ~~Notably h~~Low sensitivities were found for rates of microbial and enzyme decay.

Simulation of the incubated soil from the study of Rey et al. (2005) resulted in a very high fit to the validation data after calibration of initial SOC fractions and θ_{th}, with an RMSE of 0.09 in fluxes that were almost an order of magnitude higher than those used for calibration, and a model R^2 of 0.99 (Figure 7). This was reflected in a generally good agreement between the relationships of model and observations with moisture (Figure 8) and temperature (Figure 9).

7. Discussion

The interaction often observed in the effects of temperature and moisture on the cycling of soil C is an indicator of the complex nature of soil systems. Such responses are often ignored, particularly by modellers trying to minimize model complexity and derive functions that are easy to parameterize, but also by experimentalists focusing on finding an invariable response to a single factor. But a careful consideration of the nature of soils suggests that interactions should be expected, something that becomes evident in multi-factorial experiments as well as in field measurements. Here we found clear interactive effects in our experimental observations, adding to the evidence that fixed empirical temperature and moisture scalars, as used in conventional soil C models, are inappropriate for simulating the variability often found in natural conditions.

~~When fitting models to the data, we were unable to attain a close fit when using first or second order reactions kinetics for decomposition. In fact, the resulting R^2 values were highly negative, meaning that the models were worse predictors than the simple mean of the data.~~ Since the total amount of soil C ~~in our samples~~ was equal among samples and its relative change in the six months of incubation was small, we expected that ~~simple~~ second order kinetics would do as well as Michaelis-Menten kinetics~~, assuming that the parameter values are adjusted to compensate for the different equation forms~~. ~~The poor fits we saw~~

~~suggested that the optimization algorithm remained in a local minima of the cost function. However, better fits were not attained with 2nd order kinetics even after starting optimization with a higher initial V_{D_ref} to compensate for the Michaelis-Menten effects of K_D. This, combined with the fact that~~ But using Michaelis-Menten increased the R^2 by ca. 5 % compared to second and first order kinetics. This~~This,~~ combined with the fact that the model was ~~more~~ highly ~~than twice as~~ sensitive to a change in K_D, ~~more than to compared to~~ V_D, would indicate that Michaelis-Menten kinetics are in fact important for explaining soil C flows. Indeed, even in this case where the C_P pool is relatively invariant, the outcome of a strong temperature effect modifying K_D (E_a of 94 k~~89k~~J) cannot be reproduced by ~~simple~~ 2nd order kinetics.

The relative importance of different processes was also shown by the model parameter sensitivity values. It is perhaps not surprising that ~~the~~ some of the highest values were related to diffusion and temperature, since these were the two factors that varied in our experiment. However, these factors also vary considerably in natural ecosystems~~, so the values remain informative~~ and largely drive changes in decomposition rates~~. The high sensitivity found for f_{ug} also demonstrated the importance of C use efficiency of microbes, with the optimized value of 0.7 coinciding with that obtained by Haggerty et al. (2014)(2014)~~. No strong correlations between the effects of different parameters were found, with most being below 0.6 (Figure S4), thus giving a degree of confidence in the estimated values. While we did not obtain statistical confidence intervals, kernel density estimations (Figures S5-S12) suggest differing degrees of likelihood for different parameters. Activation energies in particular showed narrow ranges of optimal values with a strong dependence on model structure.

Since optimizing all parameters against our data resulted in an R^2 of 0.87~~484~~, it was surprising ~~that model validation gave an~~to obtain an R^2 of 0.99 during model validation. We note that few studies were found with data on moisture and temperature interactions under controlled conditions, and this was the only validation attempt carried out. This~~The~~ very high R^2 is ~~largely~~ partially thanks to the~~a~~ recalibration of initial pool sizes ~~and probably also~~ and may have to do with~~to~~ the reduced amount of data coming from a simpler experimental design compared to our study. There were only 20 data points in the validation data, one for each temperature and moisture combination. In contrast, we had ~~With~~ 3 replicates, 11 moisture levels and 2 temperature cycles, and therefore~~,~~ ~~we had~~ more data and associated~~more~~ variability. Despite these points~~the above~~ and this being ~~just~~ a first validation step, such a close agreement using independent data and a soil that differed considerably in C content, provides strong support to the model structure we used.

Model steady state or equilibrium is attained when the rate of change of all state variables equals zero, reflecting the state towards which the system will tend under invariant input and forcing conditions. Even though ~~A steady state is never attained in natural systems, where~~ external drivers are in constant change in natural systems, ~~but~~steady state information ~~, but they~~ can indicate the approximate model~~help evaluate how the~~ behaviour ~~model behave~~ under specific average conditions. Results here showed that ~~the~~ model M2-dif gives realistic values in the range of temperature for which it was calibrated, but leads to unrealistic values under colder conditions. In addition, the C_P pool shows little sensitivity to changes in C input. ~~Clearly, w~~While~~while~~ the model fitted well the validation data~~,~~, it ~~should not be~~ may not be suitable when applied ~~extrapolated~~ outside the ~~used ranges~~ conditions used for development and ~~should not~~ may need further changes for ~~be applied for~~ field ~~simulations~~applications. The limitations encountered are characteristic of non-linear microbial models and mark their current

limitations as predictive tools. However, such limitations are most likely the result of missing processes that still need to be adequately represented. For example, ~~R~~recent~~Recent~~ work has shown~~, e.g.,~~ that a density dependent mortality rate of the microbial pool can lead to much more realistic long term simulations (Georgiou et al., 2017).

It is important to point out~~(Georgiou et al., 2017). Leaching of C_D is another example~~ that our approach was to use a simple model with few processes and~~could significantly affect~~ C pools and modify only those components we tested. This allowed us to distinguish the effects of each modification and minimize parameter identifiability problems arising from having too many parameters with effects that may correlate. While this allowed us to focus on specific processes, it also meant that important~~dynamics. Such~~ mechanisms where left out. Some ~~not essential for simulating our observations but will need to be assimilated for extending the application~~ of these mechanisms are oxygen limitations in saturated conditions, leaching of C_D, the coupling of the C and N cycles (introducing SOC quality and microbial stoichiometry limitations) and organo-mineral interactions. Our model thus needs further development to extend its application and general predictive capacity. In its current form, it cannot be extended to litter decomposition (Cotrufo et al., 2015) or organic soils, which will be much more dependent on substrate quality and less affected by carbon diffusion (Manzoni et al., 2012b). Also, peatlands and other saturated soils (Clymo, 1984; Frolking et al., 2001) will show different dynamics, reflecting the critical role of oxygen as a limiting factor. We did not include mineral adsorption of carbon as an active mechanism in this study. This is contrary to recent studies that used adsorption-desorption fluxes to explain the variability in temperature responses (Tang and Riley, 2014). However, some values of mineral desorption rates found in the literature (Ahrens et al., 2015) suggest that these rates, although important in the long term, are too slow to have a noticeable impact on the time scale of this or similar experiments, and thus on most estimates of soil respiration temperature sensitivities. Finally, nitrogen requirements will impose limits on the growth of microbial communities, which in models with microbial driven uptake and/or decomposition, will also regulate C fluxes (Grant et al., 1993; Manzoni et al., 2012a). Despite such limitations, we demonstrated the effects and relevance of combining Michaelis-Menten kinetics with diffusion in mineral soils, with model results being well supported by the data.~~models.~~

~~Other limitations for simulating soil C cycling using this type of model can be pointed out. Our results cannot be extended to litter decomposition (Cotrufo et al., 2015) or organic soils, which will be much more dependent on substrate quality and less affected by carbon diffusion (Manzoni et al., 2012). Also, oxygen supply, which is critical in peatlands and other soils (Clymo, 1984; Frolking et al., 2001), was not taken into account. Finally, we did not include mineral adsorption of carbon as an active mechanism in this study. This is contrary to recent studies that used adsorption-desorption fluxes to explain the variability in temperature responses (Tang and Riley, 2014). However, values of mineral desorption rates found in the literature (Ahrens et al., 2015) suggest that these rates, although important in the long term, are too slow to have a significant effect on the time scale of this or similar experiments, and thus on most estimates of soil respiration temperature sensitivities.~~

7.1. Temperature effects

Unlike other calibrated parameters, the activation energy values for microbial (E_{a_m}) and enzyme (E_{a_e}) decay were fixed at 10 kJ, representing a positive but low temperature sensitivity. This value was used in order to be consistent with two main observations:

a) *The effect of E_{a_m} on the amount of microbial carbon.* A high E_{a_m} results in large changes of microbial biomass C with temperature. However, observations often show a negative but moderate effect of temperature on microbial biomass (Grisi et al., 1998; Salazar-Villegas et al., 2016).~~*The effect of E_{a_m} on the amount of microbial carbon.* A high E_{a_m} results in large changes of microbial biomass C with temperature. However, observations often show a negative but moderate effect of temperature on microbial biomass (Grisi et al., 1998; Salazar-Villegas et al., 2016).~~

b) *The effect of E_{a_e} on carbon decomposition rates.* High E_{a_e} values result in increasing accumulations of soil C with warming (Allison et al., 2010; Tang and Riley, 2014) as a consequence of a decrease in the enzyme pool caused by accelerated turnover. This is a critical aspect of enzyme driven soil carbon models and largely determines simulated responses to long term warming. Experimental evidence for E_{a_e} is lacking, but the latest observations of mid-term responses to warming are compatible with low values (Crowther et al., 2016).~~*The effect of E_{a_e} on carbon decomposition rates.* High E_{a_e} values result in increasing accumulations of soil C with warming (Allison et al., 2010; Tang and Riley, 2014) as a consequence of a decrease in the enzyme pool caused by accelerated turnover. This is a critical aspect of enzyme driven soil carbon models and largely determines simulated responses to long term warming. Experimental evidence for E_{a_e} is lacking, but the latest observations of mid-term responses to warming are compatible with low values (Crowther et al., 2016).~~

The~~All~~ optimized E_{a_V} ~~E_a~~ value~~s~~ of models with first and second order decomposition kinetics were in the range 40-50 kJ, translating to a Q_{10} of ca. 2. In contrast, for all but one model using M decomposition, values were above ~~came close to~~ 90 kJ~~90kJ~~, which translate~~s~~ to a ~~fairly high~~ Q_{10} ~~range of 3~~ of nearly 4. This high value ~~Interestingly, however~~was apparent in the modelled respiration fluxes only at lower temperatures, while at temperatures higher than 20 the apparent Q_{10} ~~, the actual relationship with temperature of both the observed and modelled CO_2 production indicated a much lower sensitivity,~~ approximated~~ingapproximating~~ the more commonly ~~measured~~ observed ~~Q_{10}~~ value of 2. Such results followed closely our observations and agree well with general trends in Q_{10} along the temperature axis reported by Hamdi et al. (2013).~~Q_{10} value of 2.~~ These values were mostly stable at high levels of soil moisture, but increased sharply under drier conditions. This moisture relationship, however, is not necessarily the norm and seems to depend on initial conditions and/or pool dynamics, as demonstrated by the validation step (Figure 9), where the apparent E_a remained close to 90 kJ and thus near the parameterized value. Also the change in E_a with moisture content followed a different trend in the validation data, although again values increased with lower moisture.

The difference between prescribed and observed temperature sensitivities may be related to two factors. First, the apparent sensitivities do not represent the instantaneous sensitivities dictated by the prescribed values but reflect also the effects of other limiting factors that change with time. Pool sizes, including C_M and C_E, may differ from the initial conditions as time

progresses, making measurements at different temperatures not strictly comparable. The observation that Q_{10} values from studies using short incubation times (hours to days) are higher compared to those using longer incubation times (Hamdi et al., 2013) is consistent with this idea. The second factor is related to the temperature sensitivity of the K constant of Michaelis-Menten kinetics. Our results are well in line with the theory discussed by Davidson and Janssens (2006), who stated that "because the K_m of most enzymes increases with temperature, the temperature sensitivities of K_m and V_{max} can neutralize each other, creating very low apparent Q_{10} values". Indeed, this seems to be the most important effect of introducing Michaelis-Menten kinetics in our simulations; not, as first assumed, the effects of concentrations of either the C_P or C_{ED} pools, since the choice of M or M_r kinetics had only a small impact on the results.

The above results demonstrate how different apparent sensitivities can be measured when soil pool dynamics change (e.g. through changes in diffusion limitations) even when~~On~~ the underlying temperature sensitivities are the same. ~~heother hand, the relationship we observed and were able to simulate seems by no means to be general, but rather to depend strongly on the system's initial conditions. This became evident in the validation step, where the apparent temperature sensitivities, both in the observations and the model, remained close to an E_a of 90 kJ and thus much closer to the parameterized E_a values. Also the change in E_a with moisture content followed a different trend in the validation data, although again values increased with lower moisture. We thus could see that, with the same underlying temperature sensitivities but different soil pool sizes (and possibly different diffusion limitations).~~Much of the variability in reported temperature sensitivities of soil respiration, and in particular its relationship with soil moisture (Craine and Gelderman, 2011), may be the result of the changing dynamics in microbial, enzyme and dissolved C pools during measurement times. Clearly, misleading ~~), different~~ conclusions regarding an intrinsic~~on the~~ temperature sensitivity of soil C decomposition are often~~can be~~ reached by the usual procedure of simply fitting a function to~~when looking only at measured~~ respiration vs. temperature data~~fluxes~~.

Decomposition, which was only modelled, consistently showed a lower apparent temperature sensitivity than respiration, with a Q_{10} between 1-2 for our experiment and just below 3 for the validation study. ~~Arguably, t~~These values ~~are~~ may be the most relevant for predicting long term changes, since uptake and respiration ultimately depend on C made available by decomposition. ~~Why these values remained especially low and how they may change in the long term remains to be explored, but~~ These rather low sensitivities are consistent with some integrative studies at the ecosystem level (Mahecha et al., 2010) and again likely respond to the temperature sensitivities of K_m and V_{max} neutralizing each other.~~(Mahecha et al., 2010).~~ Such results raise the question of what E_a or Q_{10} values – i.e. the apparent for respiration, apparent for decomposition, or the parameterized – are ~~adequate when applying~~ best suited for conventional ~~conventional~~ first order empirical soil models. Since these models~~model~~ will tend to have similar apparent and intrinsic behaviour, the answer is not clear and will require further research. Ultimately, the ~~better~~ best option may be to abandon such models and develop better validated mechanistic alternatives for prediction purposes.

7.2. Moisture effects and diffusion limitations

~~8~~Diffusion fluxes are a function of water content, diffusivity coefficients and pool concentrations. Different equations have been used to calculate diffusion as a function of water content in soils (Hamamoto et al., 2010; Hu and Wang, 2003). All these equations generally predict a strong positive near exponential effect of water content on diffusion. Following previous studies (Manzoni et al., 2016), we chose the function from Hamamoto et al. (2010). This equation allows for an adjustment of the percolation threshold (θ_{th}) in different soils. We note that when using the θ_{th} obtained during calibration (0.063) we also obtained a high fit to the validation data (R^2 = 0.9~~8~~7, data not shown), so the recalibration of θ_{th} led to a noticeable but small improvement. While the value 0.063 for our soil came close to the water potential of -15 MPa found in previous studies (Manzoni and Katul, 2014), this relationship did not hold for the validation soil, where we assumed a higher clay and silt content from its classification. Thus, a prerequisite for applying our model to other soils is finding a relationship between θ_{th} and soil type that holds in all cases.

Diffusion regulations can be implemented either by simulating two separate pools between which diffusion takes place or by determining the available amount of a pool as a function of diffusivity (or conductance in our case) at each time step. In our model we used a combination, simulating a diffusion flux between enzyme pools and calculating ~~the~~ how much C_D is available for uptake at each time step. We did not assume a diffusion regulation of available polymeric~~particulate~~ C, an approach that is closer to empirical functions scaling the decomposition flux directly and that has been implemented in other microbial models (Davidson et al., 2012).

In our study especially, but also in the validation data, the moisture response tended to become less linear and have a larger plateau at higher temperatures. The mechanisms leading to such interactions are still unclear, but our model comparison indicates that solute diffusion limitations play a central role. The plateau behaviour, a decrease near saturation, and even near linear responses, all contrast with the near exponential relationship between moisture and conductance given by Eq. (11~~010~~) and with the fact that no oxygen limitations at high moisture were modelled. They may, however, result from a faster depletion of available carbon at high moisture and at high temperatures, driving down the accumulated fluxes over time.

While a low supply of O_2 usually limits respiration rates in saturated soils under field conditions, O_2 seemed to have a negligible effect in our study. At 35 °C, where fluxes were highest, no clear drop in respiration was observed near saturation, as is expected when O_2 becomes limiting. Rather, the general behaviour was well simulated by our models using solute diffusion limitations only. Schurgers et al. (2006) found that the anaerobic fraction in soils with air O_2 concentrations over 10 % is low until very close to saturation. The minimum flask air O_2 concentrations in our samples, according to the maximum accumulated CO_2 (56000 ppm), was over 15 % O_2, which next to the small samples sizes would not indicate an O_2 limitation.

In models where decomposition and respiration are separated processes, these fluxes can show different responses. This decoupling is especially evident when diffusion limitations come into play. Plots of modelled fluxes against temperature and moisture (Fig. S3) showed a different relationship when comparing respiration and decomposition. Figure 10 shows modelled decomposition against respiration (using M2-dif) as accumulated values, each line being a sample at a different water content.

Without any diffusion limitation, the relationship follows a slope of ca. 0.3, determined by $1\text{-}f_{ug}$, where f_{ug} is the fraction of uptake ~~going~~ to growth (the C use efficiency). This slope, however, changes as diffusion becomes limiting, ~~and~~ with temperature ~~seems~~ also ~~to~~ playing~~play~~ a role as evidenced by the shifts in the slope occurring at various intervals. With time these fluxes will tend to equilibrate as the C_D and C_{ED} pools adjust. But the proportionality between these fluxes is not constant and~~in~~ will depend on moisture, temperature, and time, even after months of incubation. These results show that, without a proper modelling framework and when assuming a constant proportionality, interpretations based only on respiration activity may lead to wrong conclusions about the dynamics of organic matter decomposition, especially at low moisture contents and in short and mid-term experiments.

Conclusions

As the main mechanism linking water content with the movement of substrates, microbes and enzymes, diffusion plays a central role in soil organic matter decomposition. We here showed that integrating it into models can significantly improve our understanding of soil C dynamics. ~~Not only was our d~~Diffusion~~diffusion~~-based models were~~model~~ better at simulating the effects of moisture ~~variability, it also~~ and improved the simulated temperature responses, thus allowing for a better interpretation of the observed temperature sensitivities. This and similar studies indicate that measured temperature sensitivities cannot be generalized or correctly interpreted without having a full understanding of the relevant ~~soil factors involved~~mechanisms, their interactions, and the state of soil carbon and microbial pools.

We also found evidence that Michaelis-Menten kinetics plays an important role in soil C dynamics, explaining the strong differences in temperature sensitivities across temperature ranges. Our results are consistent with relatively high activation energies for both the V and K Michaelis-Menten parameters and generally lower apparent values.

Creating models that capture the variability in the response of C dynamics across different soils and at different levels of driving factors remains challenging. However, process based models are of central importance for establishing confidence in C cycle predictions and soil-climate feedbacks. As seen here, the structure and process representation of models can be critical for simulating the complex response of soil C fluxes to combined changes in temperature and moisture. Diffusion as a moisture regulation of soil C fluxes has not been used in large scale predictions, which still rely on empirical scaling functions. Evidence of interactions seen in experiments and presented ~~here~~ from a mechanistic model perspective indicate that these simpler approaches do not always hold. Further research should focus on more extensive~~ly~~ validation~~ngvalidating these approaches~~ and finding the relationships necessary for extending the application of ~~such~~ models to diverse soil types.

Code availability

All code used for this analysis is available at https://doi.org/10.5281/zenodo.1290716 ~~https://doi.org/10.5281/zenodo.1208756~~

Data availability

Data used for this analysis is available at https://doi.org/10.5281/zenodo.1208756

Author contribution

F.M. developed the model, analysed the data and wrote the paper. N.V. carried out the lab experiments and revised the paper. L.M. contributed to model optimization, discussions and revisions.

Competing interests

The authors declare no competing interests.

Acknowledgements

References

Ahrens, B., Braakhekke, M.C., Guggenberger, G., Schrumpf, M., Reichstein, M., 2015. Contribution of sorption, DOC transport and microbial interactions to the 14C age of a soil organic carbon profile: Insights from a calibrated process model. Soil Biol. Biochem. 88, 390–402. https://doi.org/10.1016/j.soilbio.2015.06.008

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

Barré, P., Eglin, T., Christensen, B.T., Ciais, P., Houot, S., Kätterer, T., van Oort, F., Peylin, P., Poulton, P.R., Romanenkov, V., Chenu, C., 2010. Quantifying and isolating stable soil organic carbon using long-term bare fallow experiments. Biogeosciences 7, 3839–3850. https://doi.org/10.5194/bg-7-3839-2010

Batjes, N.H., 2014. Total carbon and nitrogen in the soils of the world. Eur. J. Soil Sci. 65, 10–21. https://doi.org/10.1111/ejss.12114_2

Birch, H.F., 1958. The effect of soil drying on humus decomposition and nitrogen availability. Plant Soil 10, 9–31.

Blagodatskaya, E., Kuzyakov, Y., 2008. Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review. Biol. Fertil. Soils 45, 115–131. https://doi.org/10.1007/s00374-008-0334-y

Borken, W., Matzner, E., 2009. Reappraisal of drying and wetting effects on C and N mineralization and fluxes in soils. Glob. Change Biol. 15, 808–824. https://doi.org/10.1111/j.1365-2486.2008.01681.x

Campbell, G.S., 1974. A simple method for determining unsaturated conductivity from moisture retention data. Soil Sci. 117.

Clymo, R.S., 1984. The Limits to Peat Bog Growth. Philos. Trans. R. Soc. B Biol. Sci. 303, 605–654.

Cosby, B.J., Hornberger, G.M., Clapp, R.B., Ginn, T.R., 1984. A Statistical Exploration of the Relationships of Soil Moisture Characteristics to the Physical Properties of Soils. Water Resour. Res. 20, 682. https://doi.org/10.1029/WR020i006p00682

Cotrufo, M.F., Soong, J.L., Horton, A.J., Campbell, E.E., Haddix, M.L., Wall, D.H., Parton, W.J., 2015. Formation of soil organic matter via biochemical and physical pathways of litter mass loss. Nat. Geosci. 8, 776–779. https://doi.org/10.1038/ngeo2520

Craine, J.M., Gelderman, T.M., 2011. Soil moisture controls on temperature sensitivity of soil organic carbon decomposition for a mesic grassland. Soil Biol. Biochem. 43, 455–457. https://doi.org/10.1016/j.soilbio.2010.10.011

Crowther, T.W., Todd-Brown, K.E.O., Rowe, C.W., Wieder, W.R., Carey, J.C., Machmuller, M.B., Snoek, B.L., Fang, S., Zhou, G., Allison, S.D., Blair, J.M., Bridgham, S.D., Burton, A.J., Carrillo, Y., Reich, P.B., Clark, J.S., Classen, A.T., Dijkstra, F.A., Elberling, B., Emmett, B.A., Estiarte, M., Frey, S.D., Guo, J., Harte, J., Jiang, L., Johnson, B.R., Kröel-Dulay, G., Larsen, K.S., Laudon, H., Lavallee, J.M., Luo, Y., Lupascu, M., Ma, L.N., Marhan, S., Michelsen, A., Mohan, J., Niu, S., Pendall, E., Peñuelas, J., Pfeifer-Meister, L., Poll, C., Reinsch, S., Reynolds, L.L., Schmidt, I.K., Sistla, S., Sokol, N.W., Templer, P.H., Treseder, K.K., Welker, J.M., Bradford, M.A., 2016. Quantifying global soil carbon losses in response to warming. Nature 540, 104–108. https://doi.org/10.1038/nature20150

Davidson, E.A., Janssens, I.A., 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature 440, 165–173.

Davidson, E.A., Samanta, S., Caramori, S.S., Savage, K., 2012. The Dual Arrhenius and Michaelis-Menten kinetics model for decomposition of soil organic matter at hourly to seasonal time scales. Glob. Change Biol. 18, 371–384. https://doi.org/10.1111/j.1365-2486.2011.02546.x

Frolking, S., Roulet, N.T., Moore, T.R., Richard, P.J.H., Lavoie, M., Muller, S.D., 2001. Modelling northern peatland decomposition and peat accumulation. Ecosystems 479–498.

Georgiou, K., Abramoff, R.Z., Harte, J., Riley, W.J., Torn, M.S., 2017. Microbial community-level regulation explains soil carbon responses to long-term litter manipulations. Nat. Commun. 8, 1223. https://doi.org/10.1038/s41467-017-01116-z

Grant, R.F., Juma, N.G., McGill, W.B., 1993. Simulation of carbon and nitrogen transformations in soil: Mineralization. Soil Biol. Biochem. 25, 1317–1329. https://doi.org/10.1016/0038-0717(93)90046-E

Grisi, B., Grace, C., Brookes, P.C., Benedetti, A., Dell'abate, M.T., 1998. Temperature effects on organic matter and microbial biomass dynamics in temperate and tropical soils. Soil Biol. Biochem. 30, 1309–1315. https://doi.org/10.1016/S0038-0717(98)00016-9

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

Hamamoto, S., Moldrup, P., Kawamoto, K., Komatsu, T., 2010. Excluded-volume expansion of Archie's law for gas and solute diffusivities and electrical and thermal conductivities in variably saturated porous media. Water Resour. Res. 46. https://doi.org/10.1029/2009WR008424

Hamdi, S., Moyano, F., Sall, S., Bernoux, M., Chevallier, T., 2013. Synthesis analysis of the temperature sensitivity of soil respiration from laboratory studies in relation to incubation methods and soil conditions. Soil Biol. Biochem. 58, 115–126. https://doi.org/10.1016/j.soilbio.2012.11.012

Hu, Q., Wang, J., 2003. Aqueous-phase diffusion in unsaturated geologic media: A review. Crit. Rev. Environ. Sci. Technol. 33, 275–297.

Kirschbaum, M.U.., 2006. The temperature dependence of organic-matter decomposition–still a topic of debate. Soil Biol. Biochem. 38, 2510–2518.

Lawrence, C.R., Neff, J.C., Schimel, J.P., 2009. Does adding microbial mechanisms of decomposition improve soil organic matter models? A comparison of four models using data from a pulsed rewetting experiment. Soil Biol. Biochem. 41, 1923–1934.

Li, J., Wang, G., Allison, S.D., Mayes, M.A., Luo, Y., 2014. Soil carbon sensitivity to temperature and carbon use efficiency compared across microbial-ecosystem models of varying complexity. Biogeochemistry 119, 67–84. https://doi.org/10.1007/s10533-013-9948-8

Mahecha, M.D., Reichstein, M., Carvalhais, N., Lasslop, G., Lange, H., Seneviratne, S.I., Vargas, R., Ammann, C., Arain, M.A., Cescatti, A., Janssens, I.A., Migliavacca, M., Montagnani, L., Richardson, A.D., 2010. Global Convergence in the Temperature Sensitivity of Respiration at Ecosystem Level. Science 329, 838–840. https://doi.org/10.1126/science.1189587

Manzoni, S., Katul, G., 2014. Invariant soil water potential at zero microbial respiration explained by hydrological discontinuity in dry soils. Geophys. Res. Lett. 41, 7151–7158. https://doi.org/10.1002/2014GL061467

Manzoni, S., Moyano, F., Kätterer, T., Schimel, J., 2016. Modeling coupled enzymatic and solute transport controls on decomposition in drying soils. Soil Biol. Biochem. 95, 275–287. https://doi.org/10.1016/j.soilbio.2016.01.006

Manzoni, S., Philip, T., Richter, A., Amilcare, P., I., Å.G., 2012a. Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. New Phytol. 196, 79–91. https://doi.org/10.1111/j.1469-8137.2012.04225.x

Manzoni, S., Schimel, J.P., Porporato, A., 2012b. Responses of soil microbial communities to water stress: results from a meta-analysis. Ecology 93, 930–938. https://doi.org/10.1890/11-0026.1

Meurer, A., Smith, C.P., Paprocki, M., Čertík, O., Kirpichev, S.B., Rocklin, M., Kumar, Am., Ivanov, S., Moore, J.K., Singh, S., Rathnayake, T., Vig, S., Granger, B.E., Muller, R.P., Bonazzi, F., Gupta, H., Vats, S., Johansson, F., Pedregosa, F., Curry, M.J., Terrel, A.R., Roučka, Š., Saboo, A., Fernando, I., Kulal, S., Cimrman, R., Scopatz, A., 2017. SymPy: symbolic computing in Python. PeerJ Comput. Sci. 3, e103. https://doi.org/10.7717/peerj-cs.103

Moyano, F.E., Manzoni, S., Chenu, C., 2013. Responses of soil heterotrophic respiration to moisture availability: An exploration of processes and models. Soil Biol. Biochem. 59, 72–85. https://doi.org/10.1016/j.soilbio.2013.01.002

Moyano, F.E., Vasilyeva, N., Bouckaert, L., Cook, F., Craine, J., Curiel Yuste, J., Don, A., Epron, D., Formanek, P., Franzluebbers, A., Ilstedt, U., Katterer, T., Orchard, V., Reichstein, M., Rey, A., Ruamps, L., Subke, J.-A., Thomsen, I.K., Chenu, C., 2012. The moisture response of soil heterotrophic respiration: interaction with soil properties. Biogeosciences 9, 1173–1182. https://doi.org/10.5194/bg-9-1173-2012

Parton, W., Schimel, D., Cole, C., Ojima, D., 1987. Analysis of Factors Controlling Soil Organic Matter Levels in Great Plains Grasslands. Soil Sci Soc Am J 51, 1173–1179.

Price, P.B., Sowers, T., 2004. Temperature dependence of metabolic rates for microbial growth, maintenance, and survival. Proc. Natl. Acad. Sci. U. S. A. 101, 4631–4636.

R Development Core Team, 2016. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Rey, A., Petsikos, C., Jarvis, P.G., Grace, J., 2005. Effect of temperature and moisture on rates of carbon mineralization in a Mediterranean oak forest soil under controlled and field conditions. Eur. J. Soil Sci. 56, 589–599. https://doi.org/10.1111/j.1365-2389.2004.00699.x

Rodrigo, A., Recous, S., Neel, C., Mary, B., 1997. Modelling temperature and moisture effects on C-N transformations in soils: comparison of nine models. Ecol. Model. 102, 325–339.

Salazar-Villegas, A., Blagodatskaya, E., Dukes, J.S., 2016. Changes in the Size of the Active Microbial Pool Explain Short-Term Soil Respiratory Responses to Temperature and Moisture. Front. Microbiol. 7. https://doi.org/10.3389/fmicb.2016.00524

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

Schurgers, G., Dorsch, P., Bakken, L., Leffelaar, P., Haugen, L., 2006. Modelling soil anaerobiosis from water retention characteristics and soil respiration. SOIL Biol. Biochem. 38, 2637–2644. https://doi.org/10.1016/j.soilbio.2006.04.016

Sierra, C.A., Malghani, S., Loescher, H.W., 2017. Interactions among temperature, moisture, and oxygen concentrations in controlling decomposition rates in a boreal forest soil. Biogeosciences 14, 703–710. https://doi.org/10.5194/bg-14-703-2017

Sierra, C.A., Trumbore, S.E., Davidson, E.A., Vicca, S., Janssens, I., 2015. Sensitivity of decomposition rates of soil organic matter with respect to simultaneous changes in temperature and moisture. J. Adv. Model. Earth Syst. 7, 335–356. https://doi.org/10.1002/2014MS000358

Soetaert, K., Petzoldt, T., 2010. Inverse Modelling, Sensitivity and Monte Carlo Analysis in R Using Package FME. 2017-12-21 161648.

Stein, M., 1987. Large Sample Properties of Simulations Using Latin Hypercube Sampling. Technometrics 29, 143–151. https://doi.org/10.1080/00401706.1987.10488205

Suseela, V., Conant, R.T., Wallenstein, M.D., Dukes, J.S., 2012. Effects of soil moisture on the temperature sensitivity of heterotrophic respiration vary seasonally in an old-field climate change experiment. Glob. Change Biol. 18, 336–348. https://doi.org/10.1111/j.1365-2486.2011.02516.x

Tang, J., Riley, W.J., 2014. Weaker soil carbon–climate feedbacks resulting from microbial and abiotic interactions. Nat. Clim. Change 5, 56–60. https://doi.org/10.1038/nclimate2438

Wickland, K.P., Neff, J.C., 2008. Decomposition of soil organic matter from boreal black spruce forest: environmental and chemical controls. Biogeochemistry 87, 29–47. https://doi.org/10.1007/s10533-007-9166-3

Wutzler, T., Reichstein, M., 2008. Colimitation of decomposition by substrate and decomposers - a comparison of model formulations. BIOGEOSCIENCES 5, 749–759.

Zhang, X., Niu, G.-Y., Elshall, A.S., Ye, M., Barron-Gafford, G.A., Pavao-Zuckerman, M., 2014. Assessing five evolving microbial enzyme models against field measurements from a semiarid savannah-What are the mechanisms of soil respiration pulses? Geophys. Res. Lett. 41, 6428–6434. https://doi.org/10.1002/2014GL061399

Figures

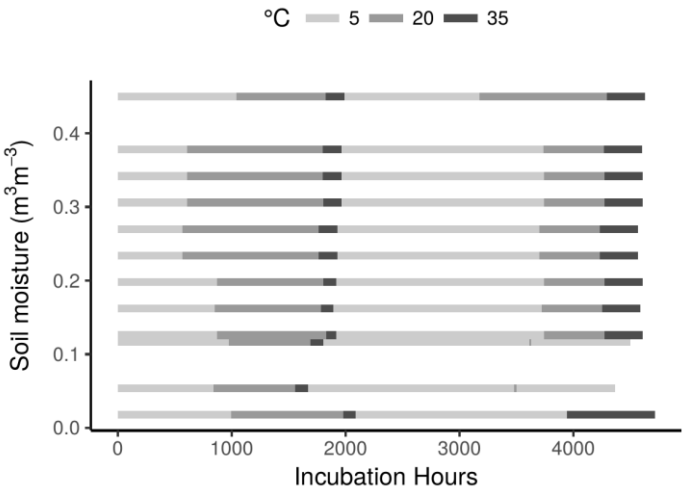


Figure 1: Graphical representation of the incubated soil samples showing the fixed levels of moisture content and the times at different temperatures.

5

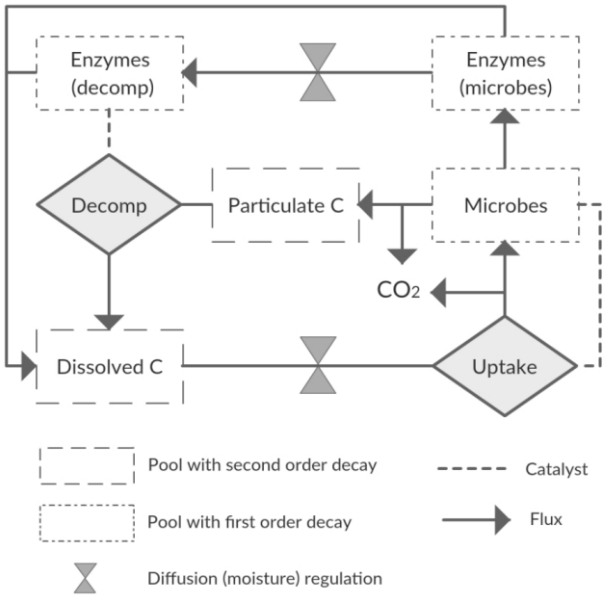


Figure 2: Diagram showing C pools and fluxes, as well as the points of diffusion limitations. Second order decay may refer also to Michaelis-Menten reaction kinetics. Variations of this scheme were tested in this study.

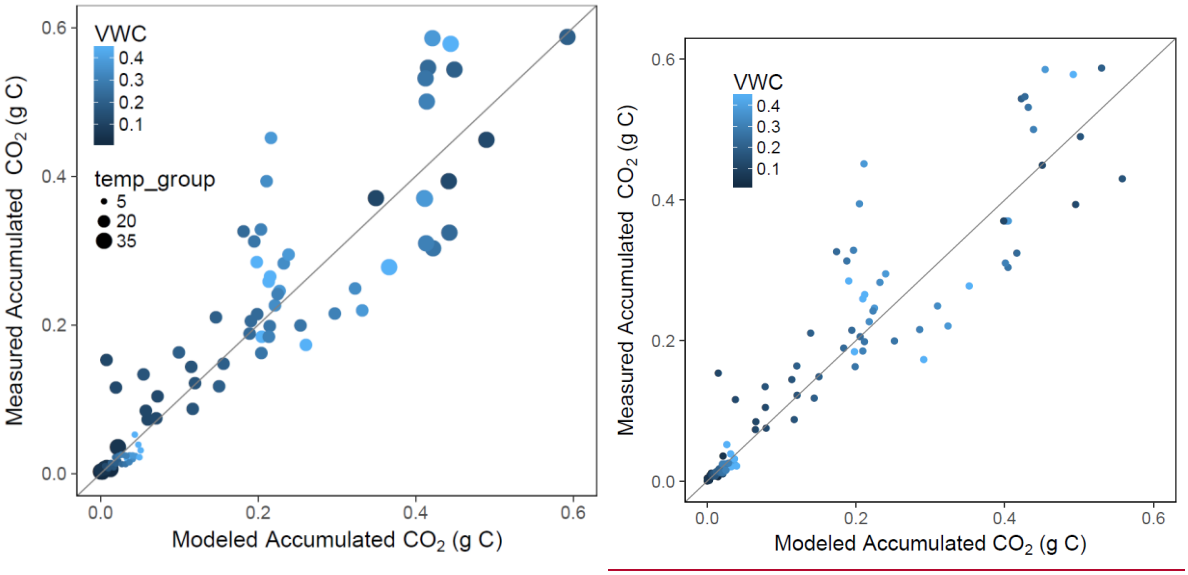


Figure 3: Model vs measured accumulated CO_2 of incubated soil samples. Colour depicts the range of volumetric water content (VWC) ~~**and size the temperature group**~~**. The model R^2 is 0.847.**

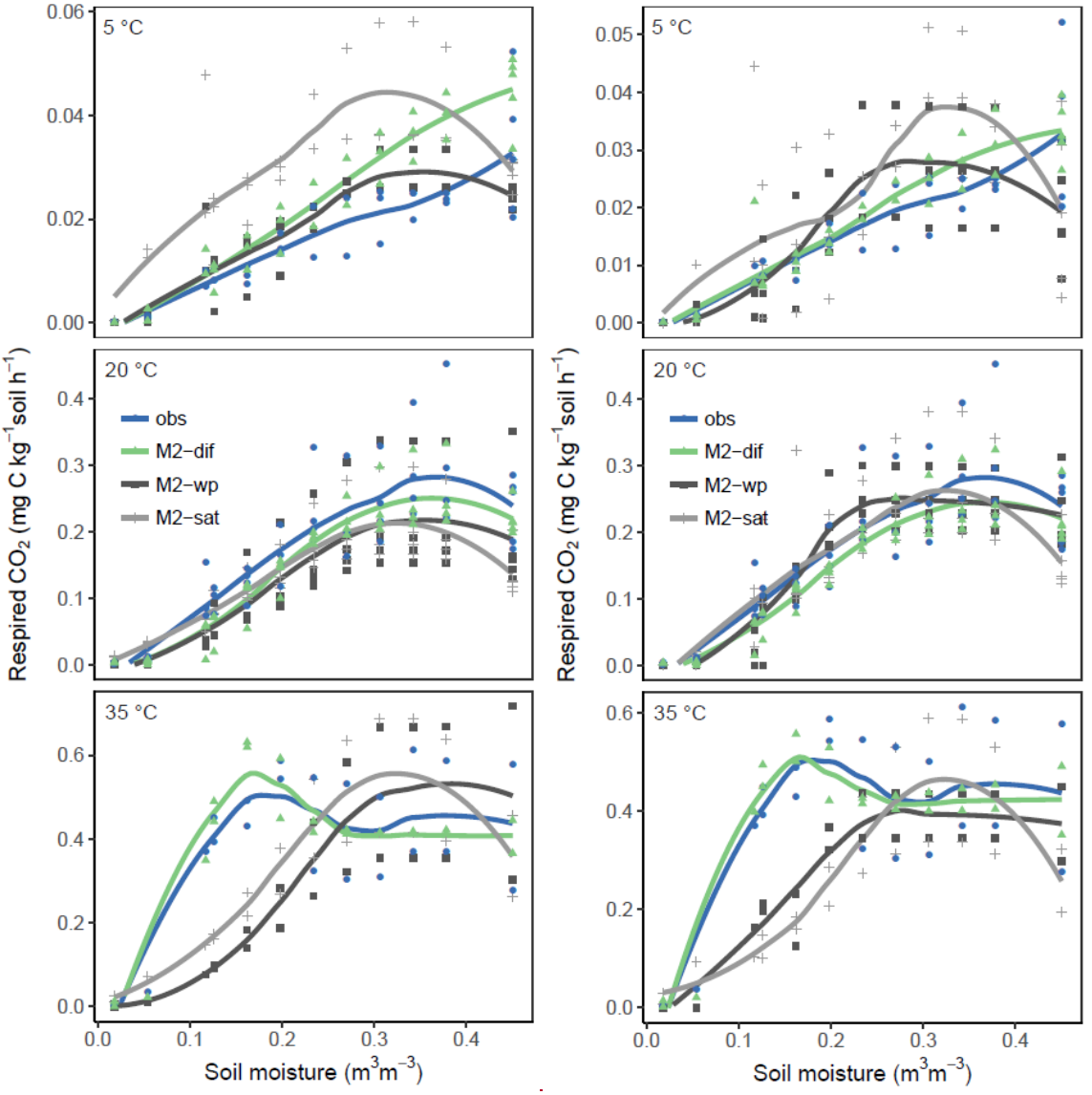
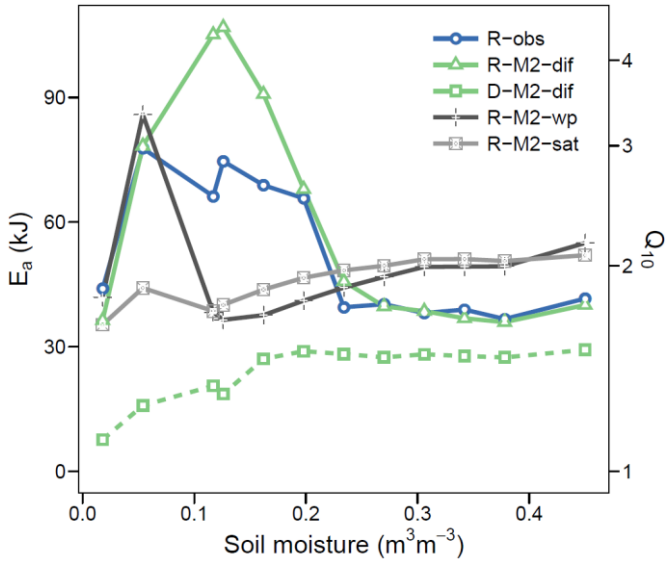


Figure 4: Relationship of soil respiration with volumetric soil moisture. Results shown over three temperatures levels (5, 20, 35 °C) for the observed data (obs) and three model versions (M2-dif, M2-wp and M2-sat). ~~Broad l~~Lines are smooth loess fits depicting the mean relationship.



~~Figure 5: Fitted temperature sensitivities of respiration and decomposition fluxes, showing activation energy (E_a) fitted using the whole temperature range (5-35 °C) and the equivalent Q_{10} derived for the temperature range 15-25 °C. Sensitivities are shown for respiration fluxes of observational data (R_obs) and of three model versions (R_M2_dif, R_M2_wp and R_M2_sat).~~

~~For comparison, the sensitivity of the decomposition flux from model M2-dif is also included (D-M2-dif).~~

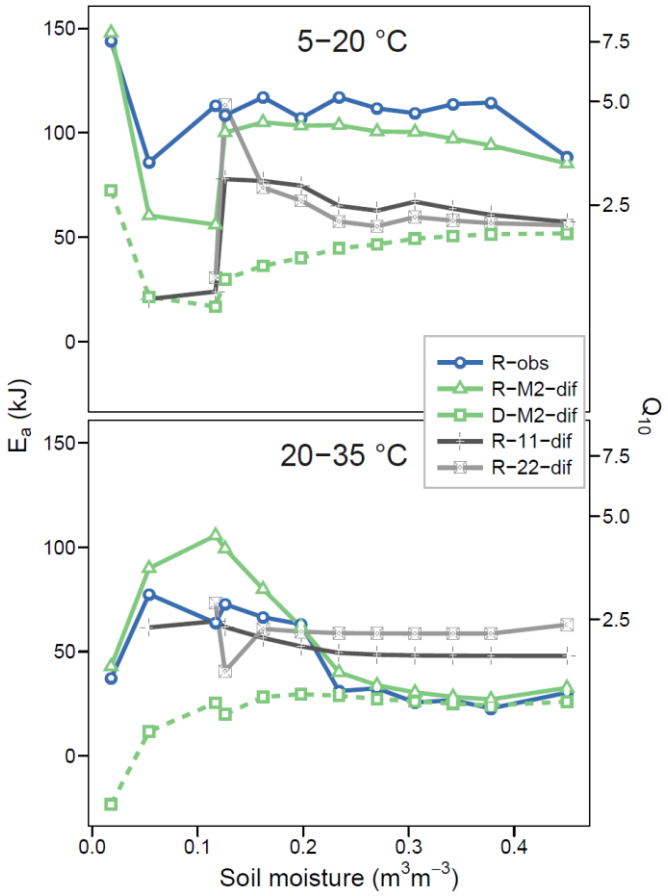


Figure 5: Temperature sensitivities of respiration and decomposition fluxes, showing activation energy (E_a) fitted using two temperature ranges (5-20 and 20-35 °C) and the equivalent Q_{10} derived for a 10 °C range. Plotted are observed respiration data (R-obs) and three models with different reaction kinetics (R-M2-dif, R-11-dif and R-22-dif). The sensitivities of the decomposition flux from model M2-dif is included for comparison (D-M2-dif).

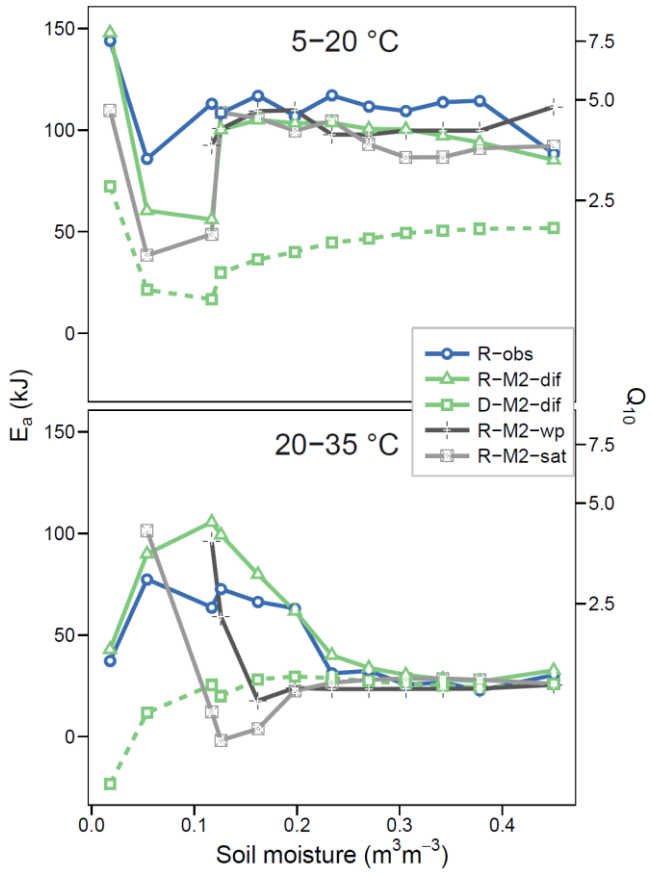


Figure 6: Equivalent to Figure 5 but showing observational data (R-obs) next to models with different moisture functions (R-M2-dif, R-M2-wp and R-M2-sat).

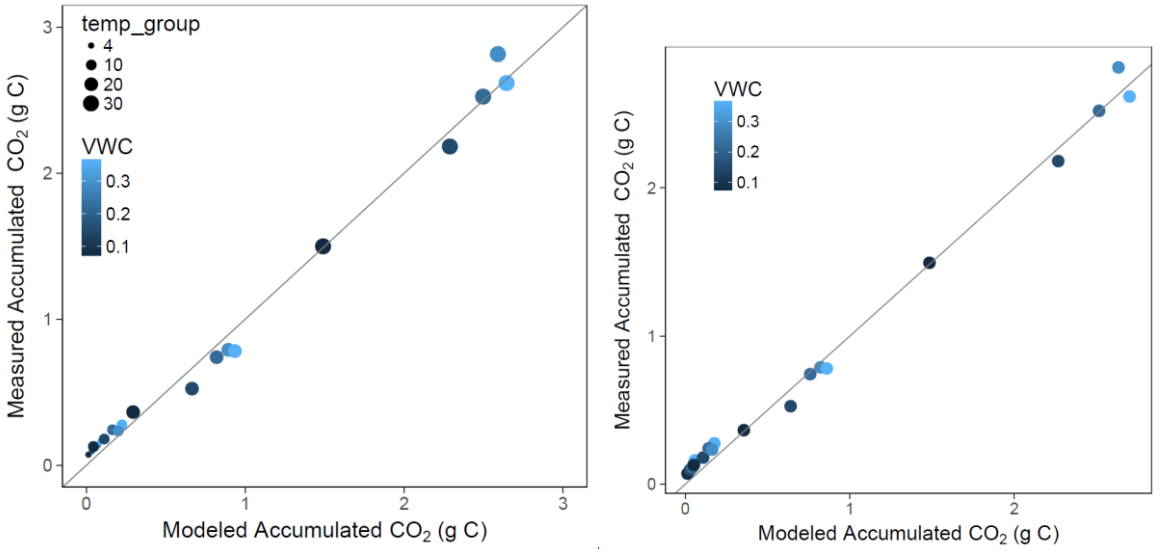


Figure 7: Model vs measured accumulated CO_2 after simulating the experiment from Reay~~Ray~~ et al. (2005). Colour depicts the range of volumetric water content (VWC) ~~size the temperature group~~. The model R^2 is 0.99.

5

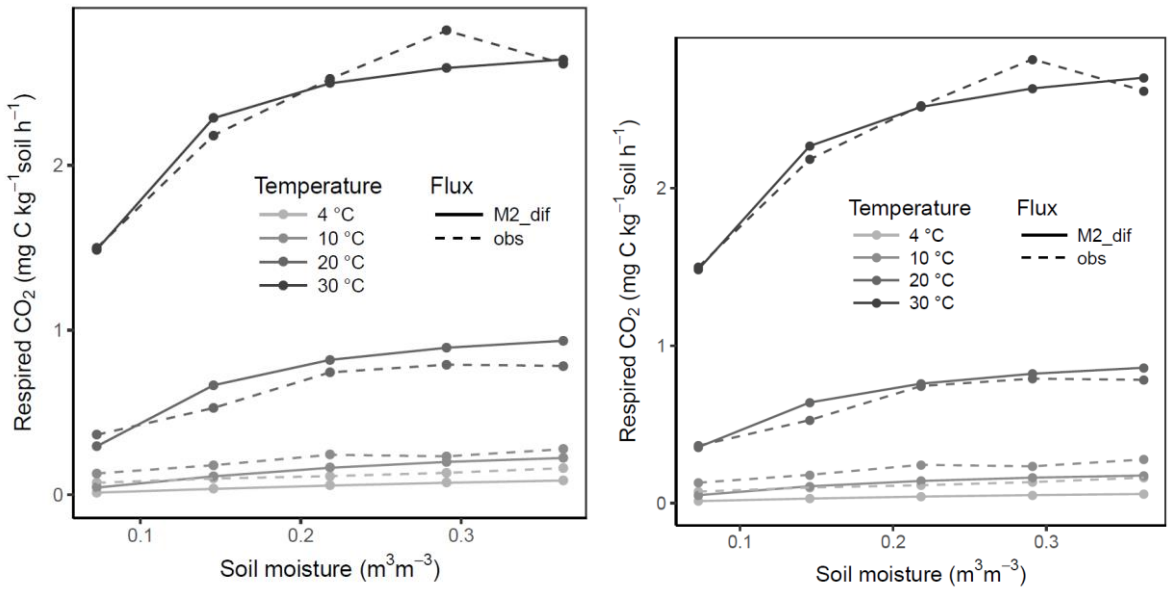


Figure 8: Relationship of soil respiration with volumetric soil moisture shown for model M2-dif and observations from the validation data (obs). Results are shown over four temperatures levels.

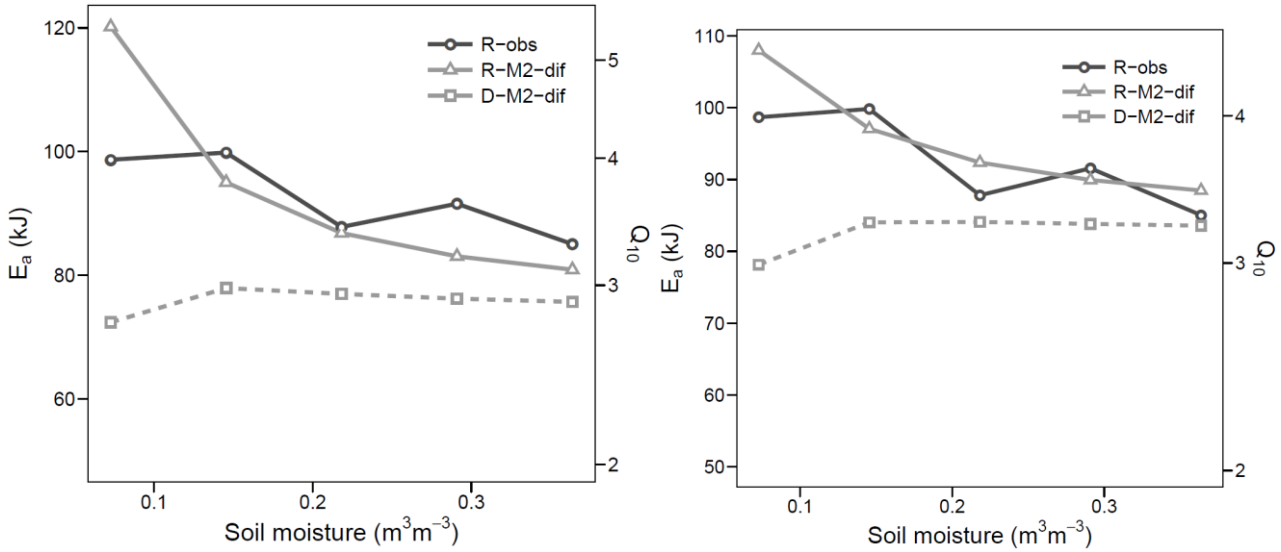


Figure 9: ~~Fitted temperature sensitivities of respiration and decomposition fluxes, showing~~ Apparent **activation energy (E_a)** and equivalent Q_{10} (~~valid~~ for the temperature range 15-25 °C) during the validation step. Values fitted to observed respiration (R-obs) as well as modelled respiration (R-M2-dif) and decomposition (D-M2-dif). ~~, fitted using the whole temperature range (5-35 °C) and the derived equivalent Q_{10} valid for the temperature range 15-25 °C. Sensitivities are shown for respiration fluxes of the validation data (R-obs) and for the respiration and decomposition flux of model M2-dif.~~

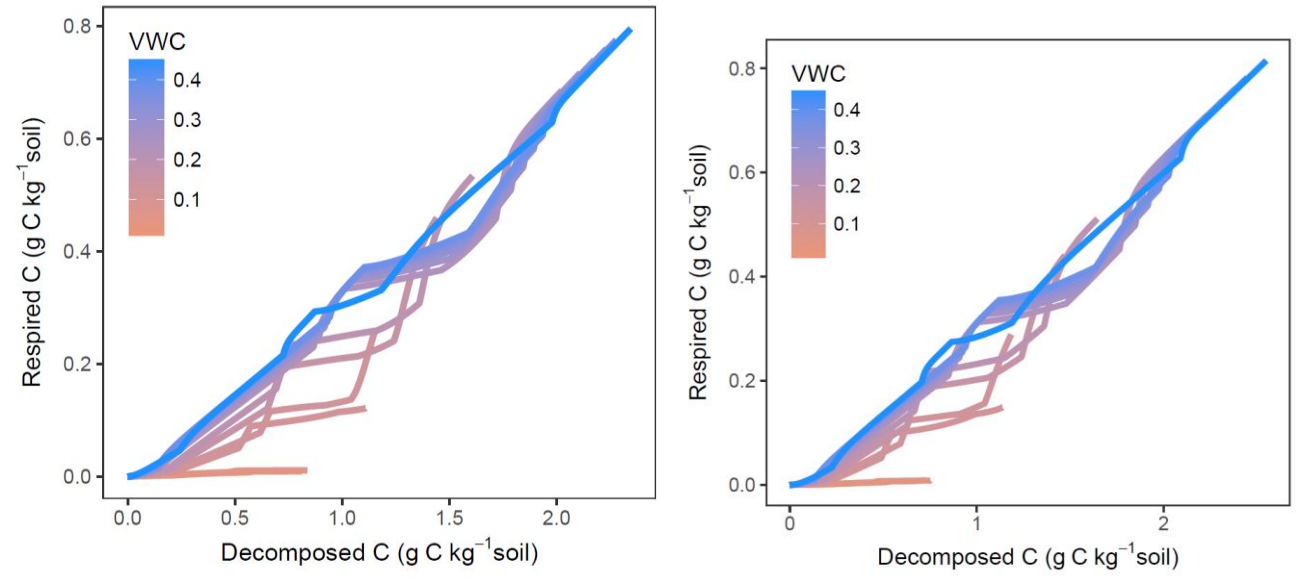


Figure 10: Modelled decomposed vs. respired C shown as accumulated values over the entire simulated incubation, including temperature steps. ~~.~~ Each line is a sample at a different moisture content.

Tables

Table 1: ~~Model p~~Parameters of model M2-diff~~parameters~~, calibrated and non-calibrated, with results of a sensitivity analysis (Sens). ~~Calibrated parameters are rounded to two significant digits. The~~ Sens ~~column values are~~ shows a relative measure of the sensitivity of the model C_P pool to small perturbations in the parameter values. Values are rounded to two significant digits.

Name	~~Value~~Value	Units	Sens
	Calibrated parameters		
g_0	0.98~~2.2~~	h^{-1}	~~4.6~~3.5
~~E_{a_K}~~	~~89~~	~~kJ~~	~~4~~
~~E_{a_r}~~	~~95~~	~~kJ~~	~~3.8~~
E_{a_V}	94~~87~~	kJ	-1.2~~3.6~~
f_D	9.1~~2~~e-5	kg kg^{-1}~~-~~	0.00
f_E	6.8~~1~~e-4	kg kg^{-1}~~-~~	~~-~~0.05~~404~~
f_M	0.08~~71~~ ~~071~~	kg kg^{-1}~~-~~	0.37~~45~~
~~f_{ug}~~	~~0.7~~	~~-~~	~~2.5~~
f_{ge}	0.034~~2~~ ~~5025~~	kg kg^{-1}~~-~~	0.07~~404~~
K_{D_ref}	62~~50~~	kg C m^{-3}	2.7~~4.1~~
n	2.3	-	0.6~~6.4~~
m	1.1~~2~~	-	0.00
r_{ed_ref}	5.6e~~5e-~~ 4	h^{-1}	0.04~~303~~
r_{md_ref}	~~9~~1~~.~~.95e-~~ ~~4~~1.5e~~-3~~	h^{-1}	0.03~~303~~
r_{mr_ref}	1.5~~4.2~~e -5	h^{-1}	0.00~~01001~~
V_{Dm}~~$V_{D\_}$~~ $_{ref}$	0.37~~53~~ ~~5~~	h^{-1}	-0.64~~1.5~~
V_{U_ref}	0.11~~09~~ ~~2~~	h^{-1}	0.5~~35~~
θ_{th}	0.063	m^3m^{-3}	0.00
	~~*Non-calibrated parameters*~~		

~~T_{ref}~~	~~293~~	~~°K~~	~~-~~
	Non-calibrated parameters		
E_{a_m}	10	kJ	~~4.6~~0.61
E_{a_e}	10	kJ	1.7~~2.2~~
f_{ug}	0.7	kg kg^{-1}	1.3
~~θ_{th}~~	~~0.063~~	~~m^{3}m^{-3}~~	~~0~~

Table 2: Different model versions with their weighted and unweighted root mean squared errors (RMSE, in units mg C kg Soil^{-1} h^{-1}) and R^2 after parameter calibration ~~(in units: mgC kgSoil^{-1} h^{-1})~~. F_{PD} = decomposition flux, U_D = dissolved C uptake flux, 1~~st~~ = first order kinetics, 2~~nd~~ = ~~simple~~ second order kinetics, M = Michaelis-Menten kinetics, M_r = reverse Michaelis-Menten kinetics. ~~The remaining combinations of 1st and 2nd order reaction kinetics showed similarly high RMSE and are not shown.~~

Model name	F_{PD}	U_D	Moisture effect	RMSE (weighted)	RMSE (unweighted)	R^2
11-dif	1st	1st	Diffusion	0.284~~.34~~	0.0801~~.08~~	0.81~~-42~~
22-dif	2nd	2nd	Diffusion	0.284~~.45~~	0.0801~~.78~~	0.82~~-44~~
M1-dif	M	1st	Diffusion	0.22121	0.06506	0.87686
M2-dif	M	2nd	Diffusion	0.22424	0.069707	0.87484
MM-dif	M	M	Diffusion	0.245	0.078808	0.84282
M2-sat	M	2nd	Eq. (201):	0.3229	0.10909	0.6571
M2-wp	M	2nd	Eq.	0.2733	0.09311	0.7859
M_r2-dif	M_r	2	Diffusion	0.24	0.070	0.85