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Revisiting overflow metabolism and its impact on soil carbon cycling

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Dear editor and reviewers,

Thank you for considering our manuscript for publication and for your thoughtful comments and suggestions. They helped improve the clarity and robustness of the manuscript.

In the attached file we address the reviewer's comments, which have been reprinted in blue. Our responses are in normal font.

Reviewer 1

General comments:

The authors propose three potential mechanisms to mitigate overflow respiration in soil C models that may force a decrease in microbial carbon-use efficiency. They design numerical experiments to test these three mechanisms using DEMENT. The manuscript is well structured and written. However, this is a purely numerical study with no comparisons to either observations or other numerical models. In addition, sensitivity analyses to critical numerical parameters are missing, which may affect the results presented. I recommend major revision before it can be considered for publication. See my detailed comments below.

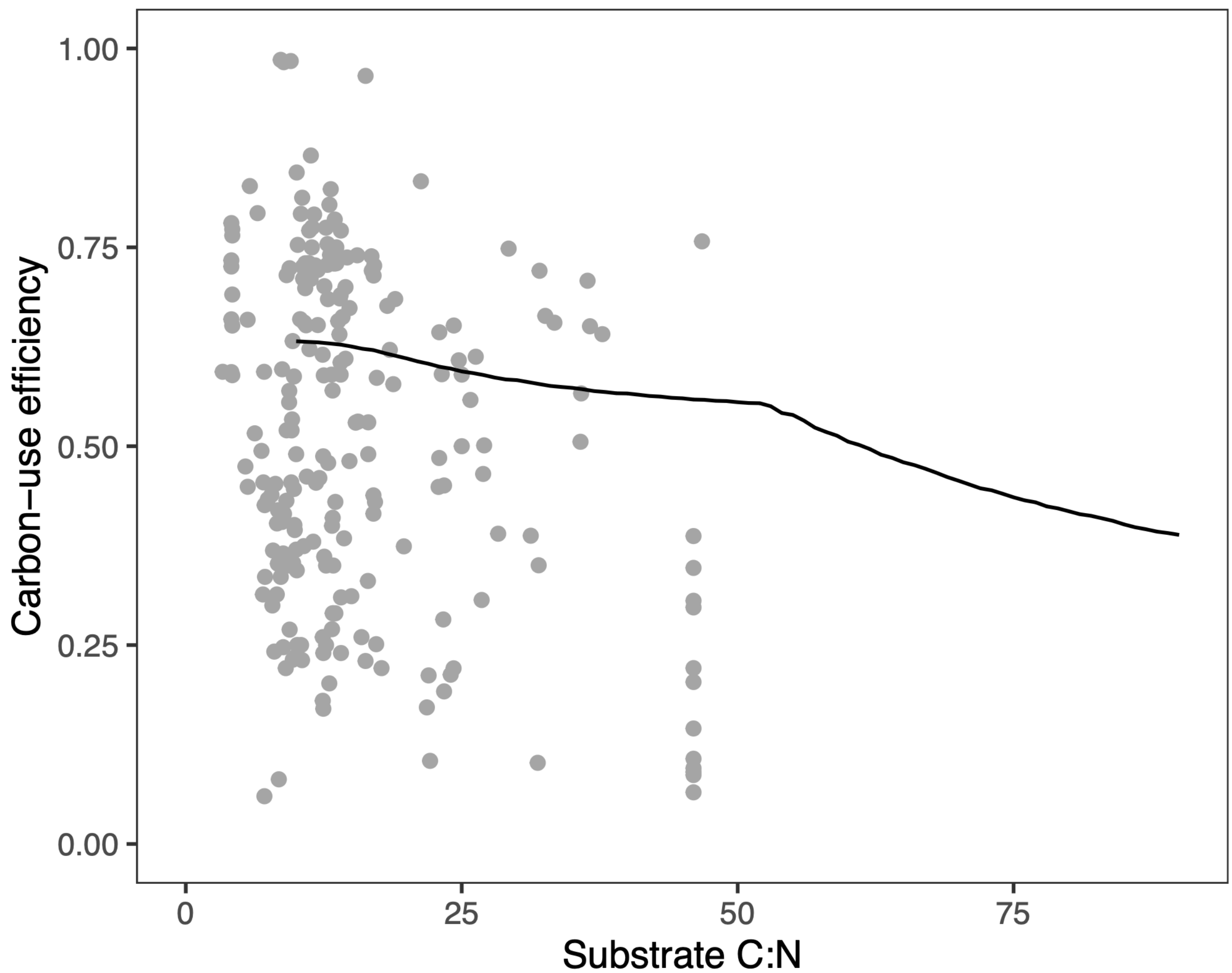
Major comments:

1. This is a purely numerical study with no comparisons to either observations or other numerical models. I would suggest including these comparisons, as they would significantly strengthen the manuscript.

We understand the reviewer's concern about the lack of empirical data. On this note we can say that Manzoni et al. (2021) demonstrates that using bulk measurements of CO2 or litter decomposition it is not possible to distinguish which mechanism microbes are using. Our results reinforce this idea. For this reason, we focus on showing how different model assumptions impact CUE and respiration patterns, instead of trying to determine which mechanism actually occurs in nature. Experiments tracking carbon in soil through different compartments are necessary to clarify this uncertainty.

Our findings are relevant for long term carbon dynamics (decades or centuries). DEMENT is an ideal tool to test short-term detailed microbial dynamics, but it is not adequate to assess long-term biogeochemistry. This has to be performed with the appropriate model, which is beyond the scope of this study

To have a more grounded perspective, we have included a figure containing empirical C-use efficiency data compiled by Manzoni et al. (2017) across substrate C:N. We excluded data from aquatic systems and terrestrial animals, including only data from soil microbes (N = 225). From the available data it is evident that there is a severe lack of measurements for substrate C:N > 50, which is not appropriate to assess overflow respiration. Manzoni et al. (2017) address this issue by including C-use efficiency estimates from a model that uses N mineralization dynamics. We also excluded these estimates since they are not truly empirical. The line corresponds to our DEMENT simulations with 1 taxon, CO_2 overflow, fixed stoichiometry, and no nutrient allocation (i.e., overflow only scenario). We can include this figure in supplementary materials.



2. The proposed three mechanisms works well to mitigate overflow respiration in DEMENT, while it is uncertain for other soil carbon models. How do the authors comment on this uncertainty?

We thank the reviewer for bringing this up. The decrease of carbon-use efficiency for high substrate C:N caused by overflow respiration was described early on by Schimel and Weintraub (2003). The mitigation of this decrease by alternative mechanisms is reported by previous studies (Kaiser et al., 2014; Wutzler et al.. 2017; Manzoni et al., 2021)

Mitigating carbon overflow (and therefore, its effect in the CUE - substrate C:N relationship) is a matter of mass balance. As long as there is an excess of carbon relative to microbial needs (and if overflow is implemented as a mechanism in the model) overflow will happen.

Alternative mechanisms mitigate overflow because they solve the imbalance, that is, they avoid an excess of carbon before excretion can happen. If elemental imbalance is reduced, but not solved, carbon overflow still occurs, but to a lesser extent. This is exactly what happens with our implemented mechanism of enzyme and uptake allocation.

Two things set us apart from previous studies. First, the inclusion of DOC overflow, which, to our knowledge, has not been assessed before; and second, the possibility of implementing all mechanisms in a single simulation. We will reinforce comparisons with previous studies in the discussion section.

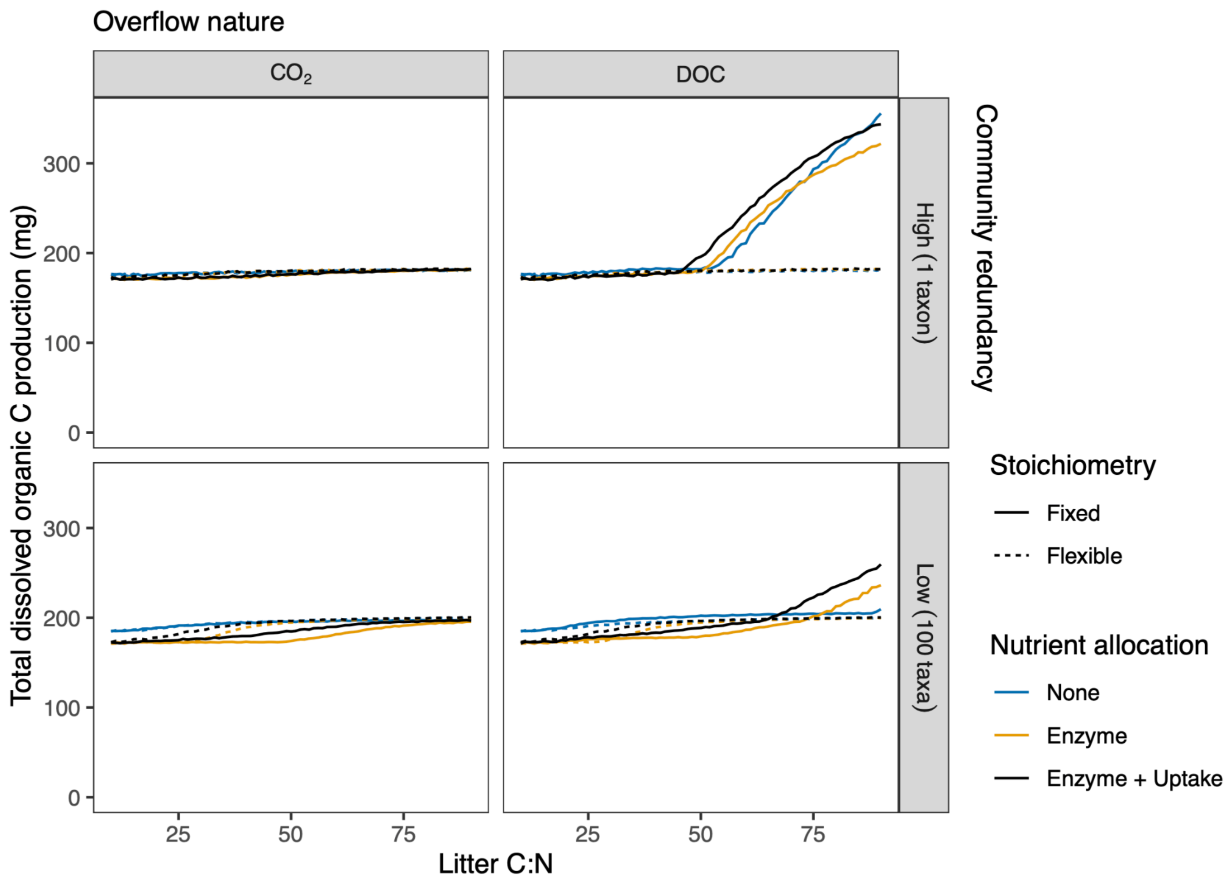
3. I would suggest conducting sensitivity analyses to critical numerical variables (e.g., Vmax and Km), so that we can know when to involve these three mechanisms to mitigate overflow respiration.

To address this concern, we can generate additional simulation scenarios manipulating Vmax and Km parameters for both enzyme and transporters. Perturbations would be performed one at a time by incrementing or decreasing the focal parameter by 10% while keeping the other parameters fixed. This adds to a basal design of 8 simulations that can be replicated in 3 points of the litter C:N gradient (minimum, maximum, and threshold points), yielding 24 simulations. These simulations could be repeated for our two community contexts and two biomass stoichiometry scenarios, summing up a total of 96 simulations. We would compare CUE computed from these perturbed simulations to the CUE from our original simulations.

4. Lines 113-115.The authors conduct numerical experiments with constant environmental variables. However, it remains unclear whether the proposed three mechanisms work with changing environmental variables. The authors should also test their mechanisms in real cases. In addition, the authors focus on CUE responses to the three mechanisms. It is also important to look at other metrics (e.g., DOC), especially when redirecting C overflow to DOC.

As we note in the manuscript, we excluded moisture and temperature forcings from the simulations to facilitate a better causal attribution to our findings. In this version of DEMENT, moisture and temperature are rate modifiers in processes like depolymerization, uptake, and mortality. Temperature also has a negative impact on assimilation efficiency. It is true that changing environmental conditions may obscure the relationships we found. However, it is imperative to describe first the isolated effects of elemental imbalance. Testing these relationships in different environmental conditions goes beyond the aims of our study.

Since overflow respiration is a mechanism directly affecting total respiration and carbon-use efficiency, we focus on those variables due to their relevance in soil carbon balance and climate change studies. We can include a supplementary figure of DOC dynamics for reference, which is shown below, but the patterns are similar to the ones seen in Fig. S4 showing total C uptake.



Minor comments:

1. Line 13. “reasoning” to “reason”.

We will change “reasoning” to “argument” to clarify the statement.

2. I suggest the authors shortening the abstract.

We have reduced the length of the abstract from 332 to 292 words.

3. Line 36. “enabled” to “enable”.

We will modify the wording from “enabled us to identify” to “led to the identification of”. The statement should be in past tense since we are referring to historical events in the literature.

4. Lines 40-41. References are needed to support this statement.

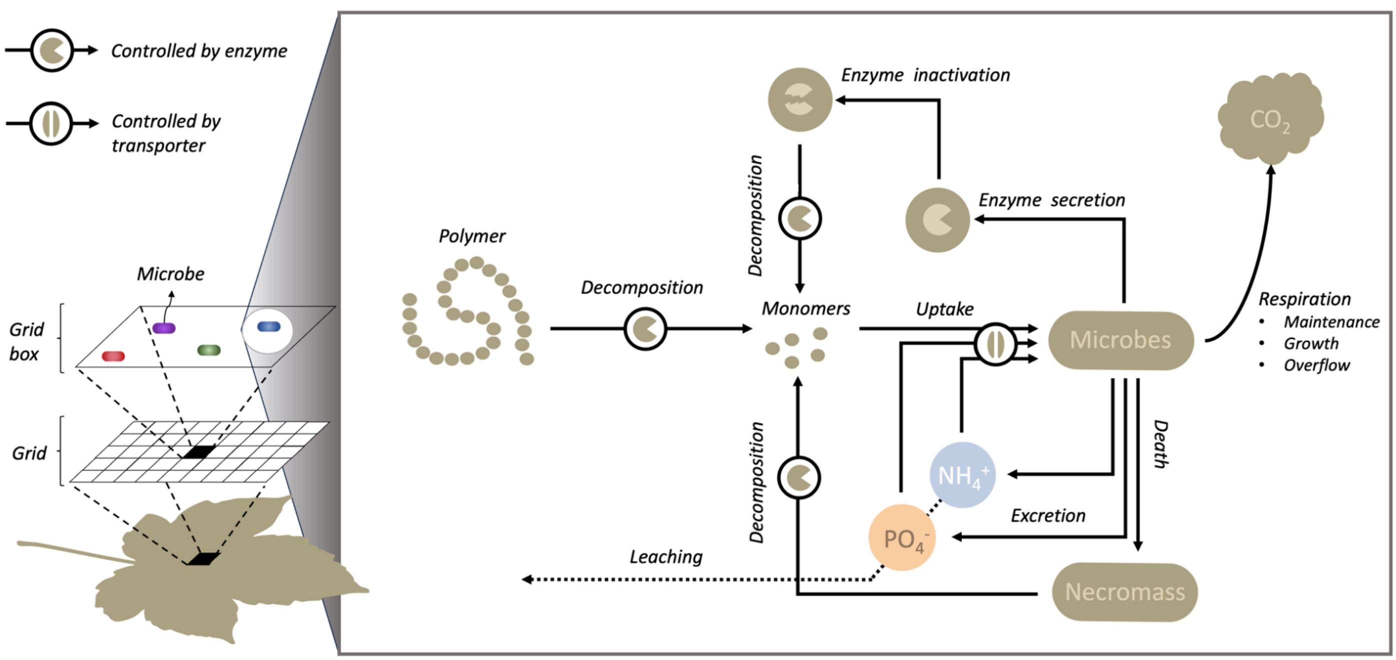
We will add references as suggested. Cleveland and Liptzin (2007) report average soil microbial biomass C:N, as well as plant litter C:N across terrestrial ecosystems. Manzoni et al. (2012) and Sinsabaught et al. (2013) describe the role of elemental imbalance as a driver of carbon-use efficiency.

5. Line 86. "for C:N litter" is unclear.

We will modify the wording from "...the reduction of carbon-use efficiency for C:N litter..." to "the reduction of decomposer carbon-use efficiency feeding on high C:N litter...:

6. Suggest combining Figures 1 and 2 into a single figure.

We have combined Figures 1 and 2 as suggested.



7. Line 175. Suggest moving Figure 3 to the supplementary material as it provides limited information.

We would advocate to keep Figure 3 in the main text since enzyme allocation mechanisms in models are rare and the one presented in this study is different from previous ones. Moreover, it is intuitive to understand how it works with the figure, while equations 1 and 2 are not.

8. Line 343. “decomposers feed” to “feeding decomposers”.

We will change the verb from “feed” to “grow” to clarify the statement

9. Line 351. “maintenance” to “maintenance respiration”.

Will be edited as suggested