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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 2

General comment:

The scientific relevance of this manuscript is considered high and the approaches to model microbes differently are comprehensive and backed by literature. The general structure of paragraphs is mainly easy to follow, although confusions in the abstract, introduction and method sections can come up, which is addressed more in detail in 'Minor revisions'. The conclusions are rational, although further computational and temporal analysis would support claims. For increasing the comparability to literature and clarifying the motivation some major revisions are necessary before publication.

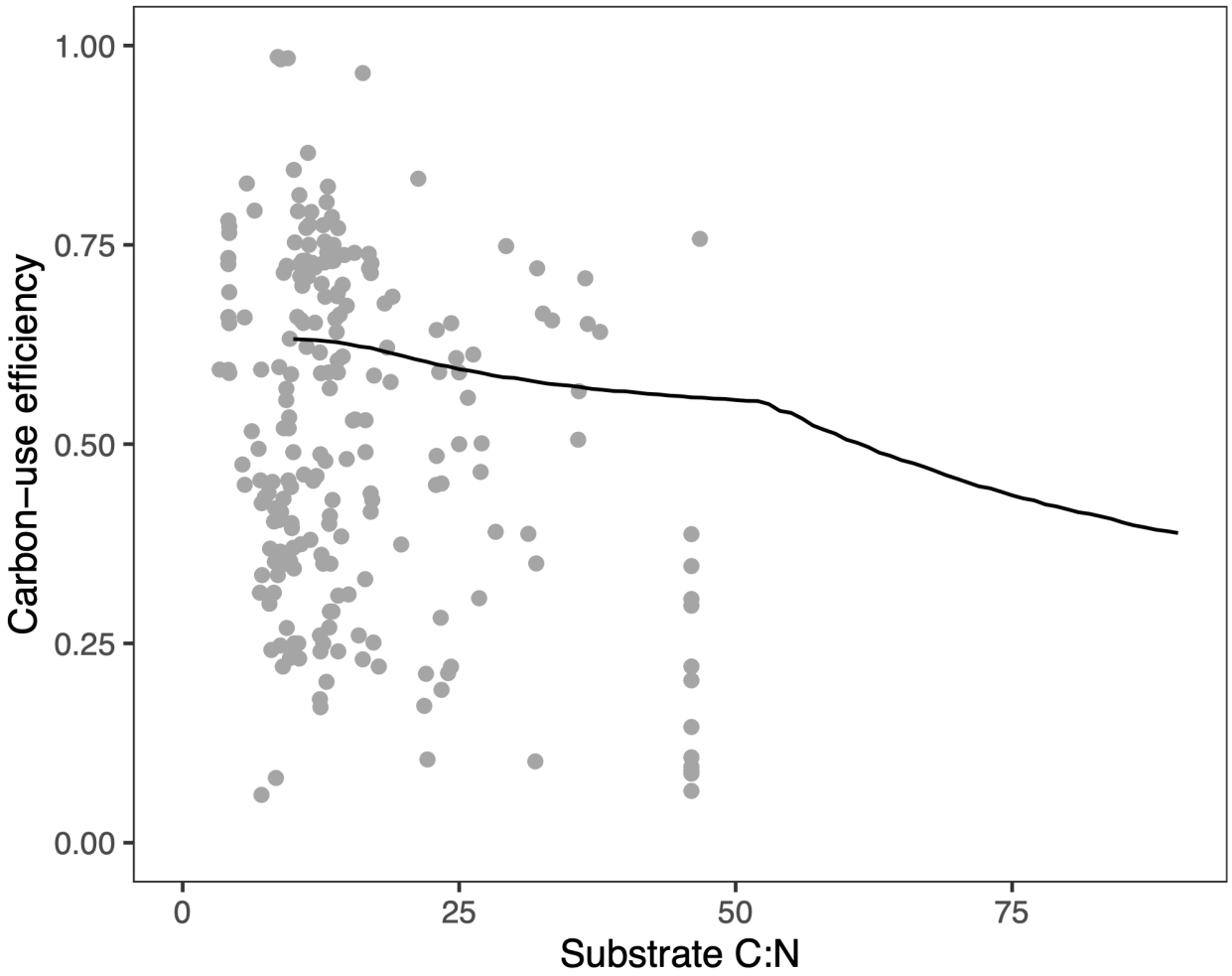
Major revisions:

In line 261 the CUE used in this study is defined. While this is common for most models, the innovative formulations in this study open the question what should be also subtracted in the numerator (e.g. overflow C or enzyme C). A comparison with what is actually measured in CUE lab or field experiments and what models represent as CUE would be very interesting. This is also necessary for the next major revision point.

Indeed, CUE formulations vary depending on the C losses that are accounted for. Geyer et al. (2016) did a thorough examination of different CUE formulations. Many studies subtract the production of extracellular enzymes (and metabolites) when calculating microbial growth efficiencies (Manzoni et al., 2012). Geyer et al. (2016) also include definitions that even subtract microbial death. Although the accounted C losses will depend on the method, our study focuses on consequences for soil C fate. Therefore, we only subtract CO2 production from C uptake. In the main text we justify this choice by noting that CO2 production implies an effective C loss from the system, while extracellular enzymes can be recycled as growth substrate. In reality, enzymes could even be stabilized forming aggregates or interacting with minerals. In the time scale of our simulations (a couple of years) our calculation of CUE is appropriate for assessing soil C balance. We will emphasize these points in section 2.5.

Compare to actual CUE-C:N data from field/lab studies. At least one plot reproducing real-world data for supporting the necessity of avoiding decreasing CUE at high C:N, as this motivation is repeated throughout the study.

We generated a figure showing 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, CO2 overflow, fixed stoichiometry, and no nutrient allocation (i.e., overflow only scenario). We can include this figure in supplementary materials.



Minor revisions

L35: A large body of literature supports the opposite, i.e. no temporal predictive power exists.

We thank the reviewer for noting this point. Our intention is not to portray microbially-explicit models as the panacea. We recognize that there is still a lot of uncertainty in these predictions. We rather want to stress the direction that soil biogeochemistry research took in the last decades and why overflow respiration is currently present in many models. We will edit this phrase as “Some of these modeling improvements…”.

L63: Flexible biomass stoichiometry needs introduction. Also method section does not describe how that is handled in the model or to what range ‘flexible’ refers.

We will include clarifications in the introduction as well as in section 2.3. In the introduction we will specify “…, biomass stoichiometry can be flexible (variation of biomass C:N with substrate C:N; Allison, 2012; Manzoni et al., 2021)”. In section 2.3, we will include: “In default conditions, biomass C:N:P is kept fixed by regulating excretion (Eq. (A5)-(A8)). This is done by defining minimum elemental quotas (i.e., elemental proportion of biomass). If flexible biomass stoichiometry is allowed, the minimum quota of each element decreases (original quota minus tolerance range). This allows biomass C:N:P to fluctuate within the tolerance range.”

Additionally, in section 2.4 we will mention that: “Biomass C, N, and P quotas were set to 0.92, 0.069, and 0.011 with tolerance ranges of 0.06, 0.056, and 0.01 respectively.”

L70-71: ‘sequential nature’ must be explained. I assume that overflow would only happen when the proposed mechanisms don’t resolve the imbalance.

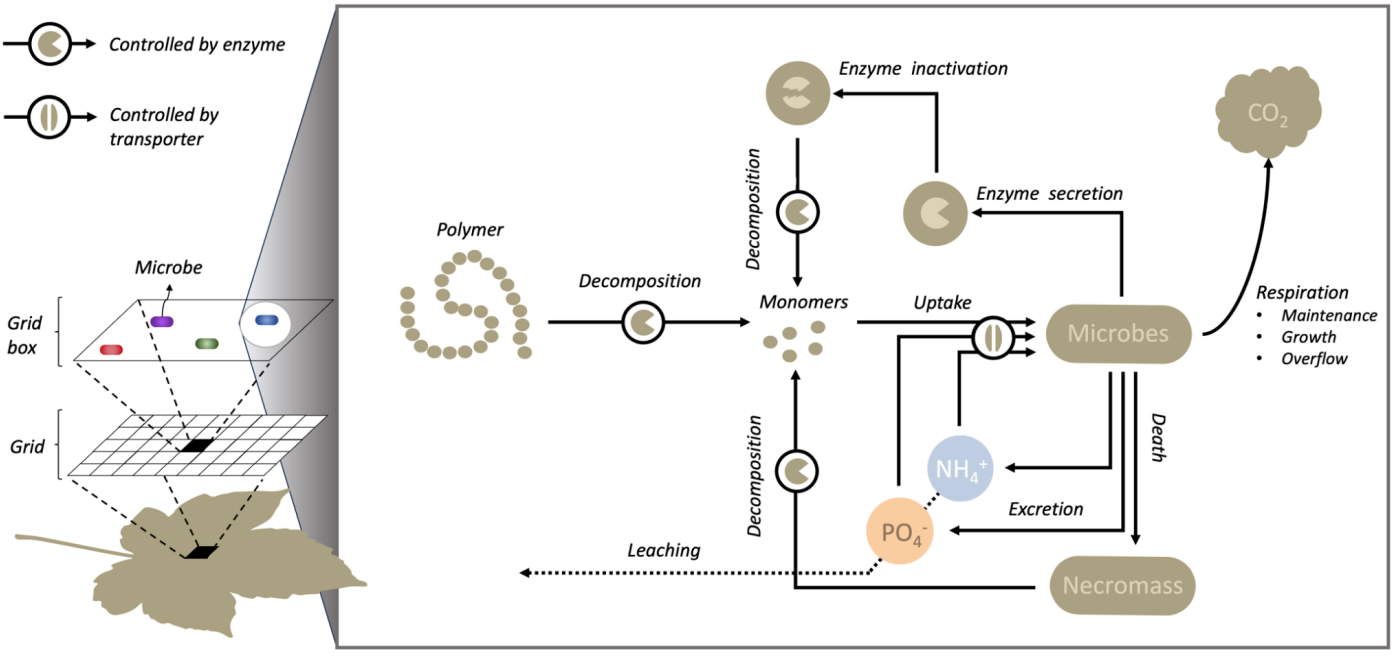
That is correct, we will expand the meaning of this ‘sequential nature’ in the introduction as: “…these mechanisms are sequential in nature, that is, nutrient allocation and flexible biomass stoichiometry happen before excretion.”

L88-90: three mechanisms are proposed here, but Table 2 lists four factors. Maybe put '(i.e. flexible stoichiometry, enzyme and uptake allocation)' after the 'physiological acclimation mechanisms'. Table 2 is a good overview, and the manuscript (including the abstract) would be easier to follow if each section presented the mechanisms in the same order and used consistent wording throughout.

We strongly agree with the reviewer and will revise the wording of our mechanisms throughout the manuscript. We will keep the terminology shown in Table 2.

L127-128: Necromass decomposition being modulated by enzymes is not in the scheme.

We have edited the figure to clarify this issue. Figures 1 and 2 are now combined.



L131: The initialization of the enzymes, biomass, etc. pools is missing.

We will include descriptions of the initial conditions of enzyme, inorganic nutrients, monomers, and biomass pools in section 2.2. These changes will read: “Initial simulation conditions included no enzymes present (i.e., all enzymes have to be produced) and no inorganic nutrients, while the initial monomer pool was assumed to be 1% of the initial litter pool. Grid size was 100 x 100, where the probability of each taxon occupying a given box is 0.01. For additional details on simulation conditions, see section 2.4. The full parameter table is available in supplementary materials.”

L132-139: First stating what the study does and then mentioning what DEMENT would in theory provide (or is lacking) would improve readability. Same for 2.3 & 2.3.3.

We will add topic sentences at the beginning of sections 2.2, 2.3, and 2.3.1 clarifying our goals. These sentences will read:

- Section 2.2: “Before running simulations, microbial taxa must be defined based on traits like extracellular enzymes and transmembrane transporters.”
- Section 2.3: “Our study required the manipulation of mechanisms involved in the reduction of elemental imbalance. These include overflow respiration, nutrient allocation strategies, and flexibility of microbial biomass stoichiometry.”
- Section 2.3.1: “Consequently, we develop the corresponding mechanisms of enzyme and uptake regulation for our further analyses.”

L175: Sigmoid function is described also in Eq. 1.

We will include the citation of Eq. 1 as suggested.

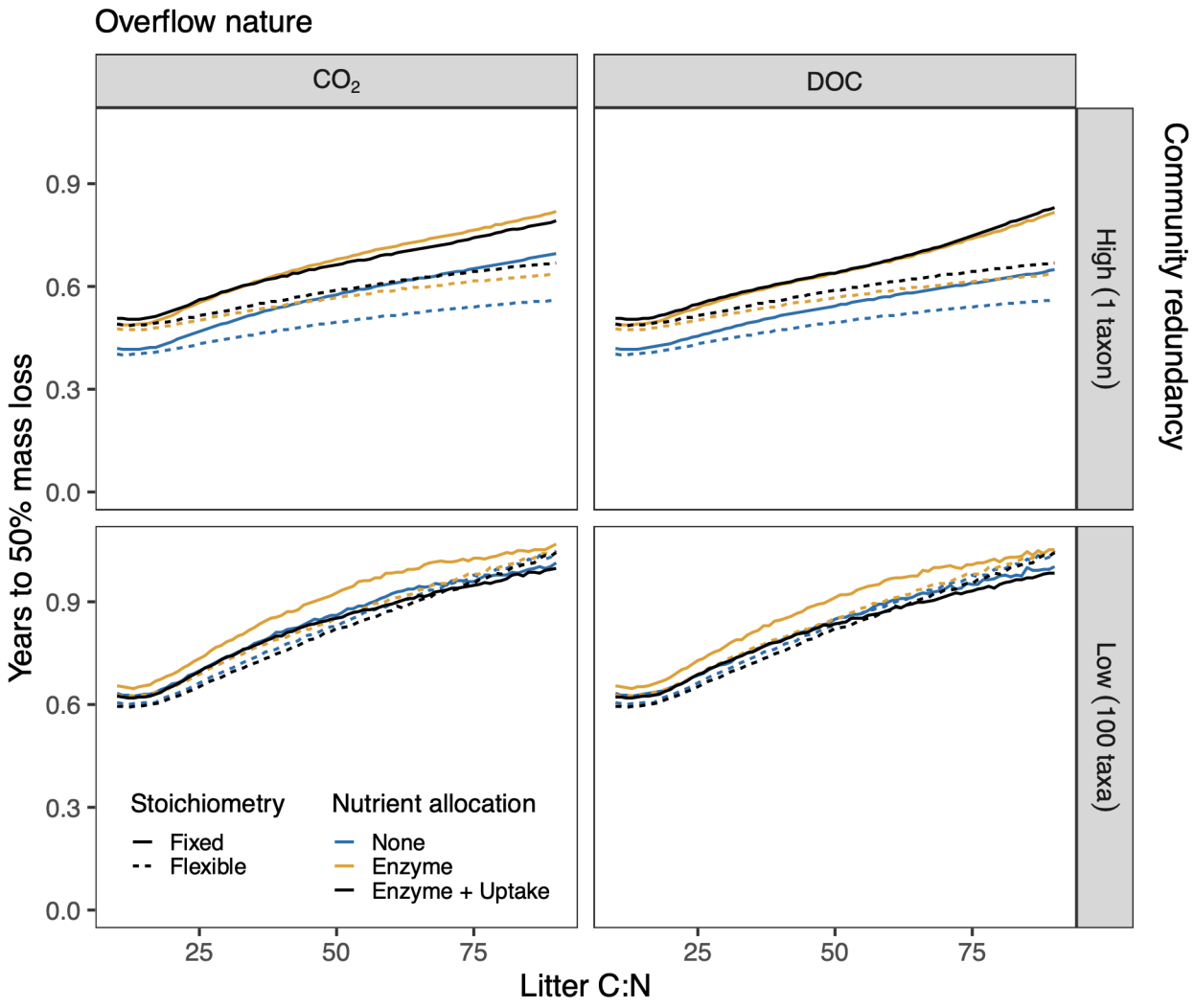
L202-203: Why is constitutive production necessary at all?

The original DEMENT function for enzyme production includes a constitutive (proportion of biomass) and an inducible (proportion of carbon uptake) component. It

was formulated like this for consistency with known enzyme production mechanisms in fungal decomposers, as many carbon-degrading enzymes are produced at low, constitutive levels. As the reviewer cleverly notes, it is not necessary for our purposes to keep this formulation. However, instead of eliminating this component, we opted for implementing our enzyme repression mechanism for both enzyme production components. By doing this, we obtained our desired behavior without substantially altering the model structure.

L213-214: I would find it valuable to see time-to-50% shown across the C:N range and model version.

We have included this figure in supplementary materials and will refer to it in section 2.4.



L214-215: In this case, the pulse actually has no duration.

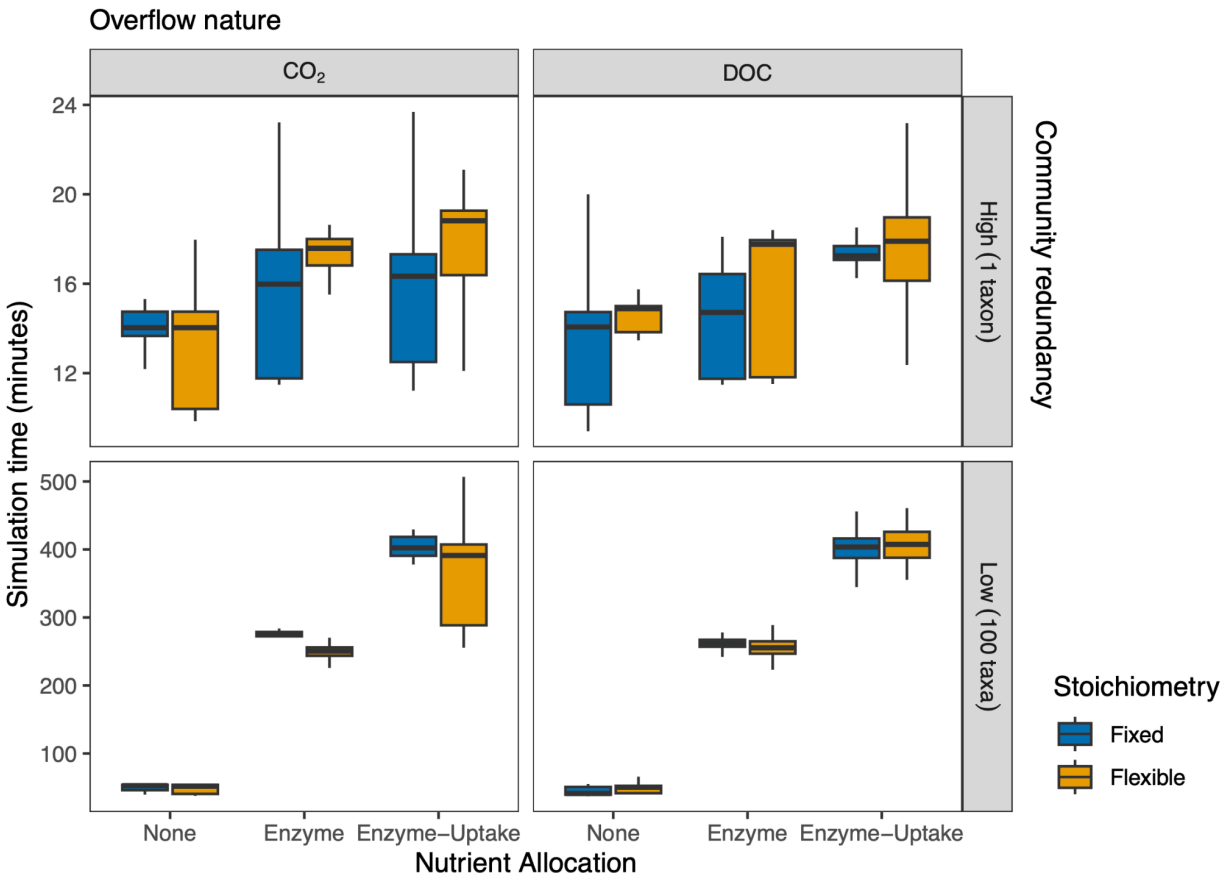
That is correct, but we did run our pulses up to 999 days, so although data beyond 50% mass loss was not used for results, it exists and is available.

L228: The range would be interesting, as bacteria would have rather different values.

We will mention the elemental quotas and ranges used in section 2.4: “Biomass C, N, and P quotas were set to 0.92, 0.069, and 0.011 with tolerance ranges of 0.06, 0.056, and 0.01 respectively.”

L366: Some numbers on the duration of the experiment with the different versions would be interesting (in the supplement).

We generated a figure depicting simulation time for our different scenarios. We will refer to this figure in the discussion section.



L378-379: Assimilation efficiency would also be interesting to compare to different CUE formulations (see major revisions).

Assimilation efficiency is defined as the proportion of C remaining after respiration by uptake (Hagerty et al., 2018). In our simulations, this quantity is equal to 0.84. As we note in the main text, DEMENT has three respiration sources; growth, maintenance,

and overflow. Growth respiration is defined as C uptake times assimilation efficiency. Our definition of C-use efficiency accounts only for C losses as CO2 (i.e., total respiration). Therefore, C-use efficiency will always be lower than assimilation efficiency, since some amount of maintenance respiration is always expected.

L424-425: Could these data be used for comparison with the results of this study? (see major revisions).

The data by Fujita et al. (2014) includes measured C and N mineralization rates but lacks estimates of C-use efficiency. Nevertheless, we generated a figure with empirical C-use efficiency data compiled by Manzoni et al. (2017).

L439-440: Non-linearity in ESMs is also avoided for computational costs.

We thank the reviewer for pointing this out. Although potentially none of our mechanisms could be practical in an ESM context, dissolved organic C overflow is still the simplest to implement. Its relevance could be explored in a “medium-scale” ecosystem model where a potential simplification of this mechanism could be evaluated and subsequently implemented in an ESM. We will add a short phrase in the main text addressing this point.

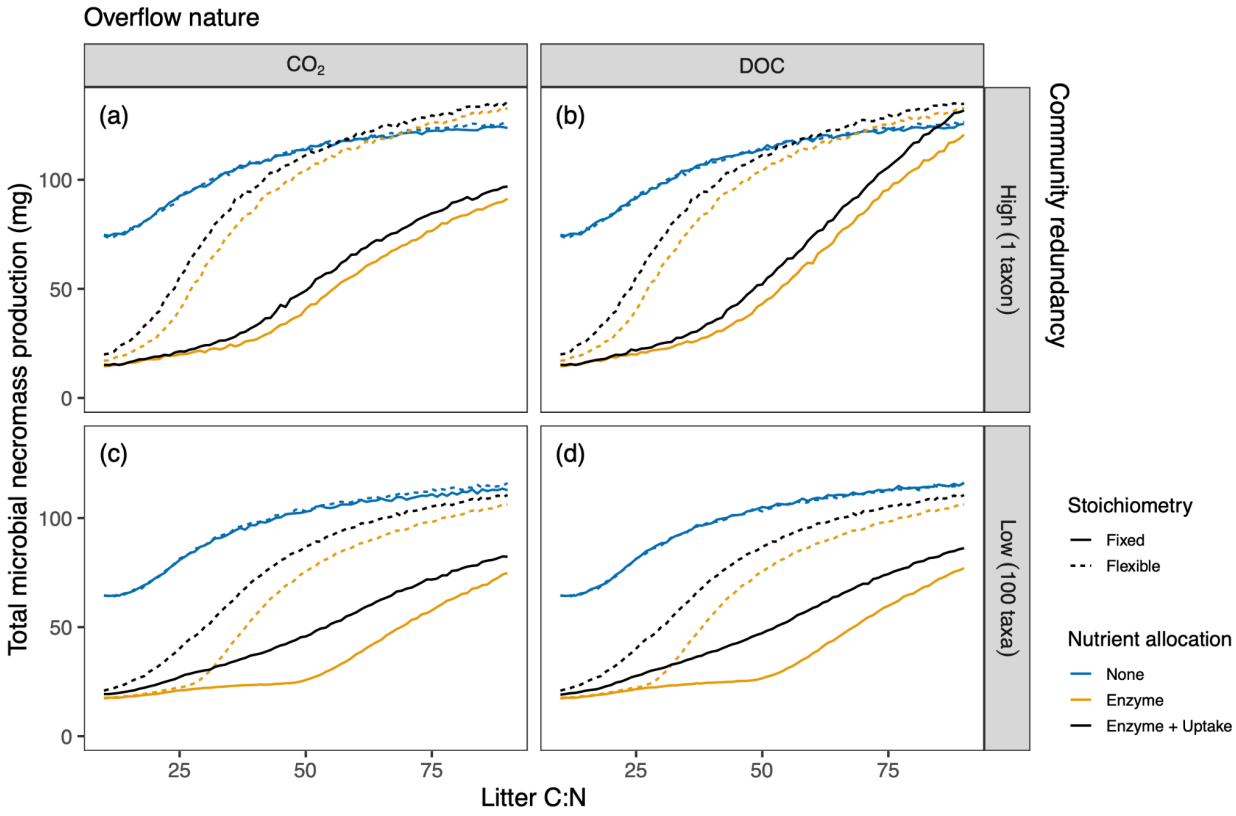
L453-454: A predictive component, or at least a comparison with real-world data, would be needed to support this claim.

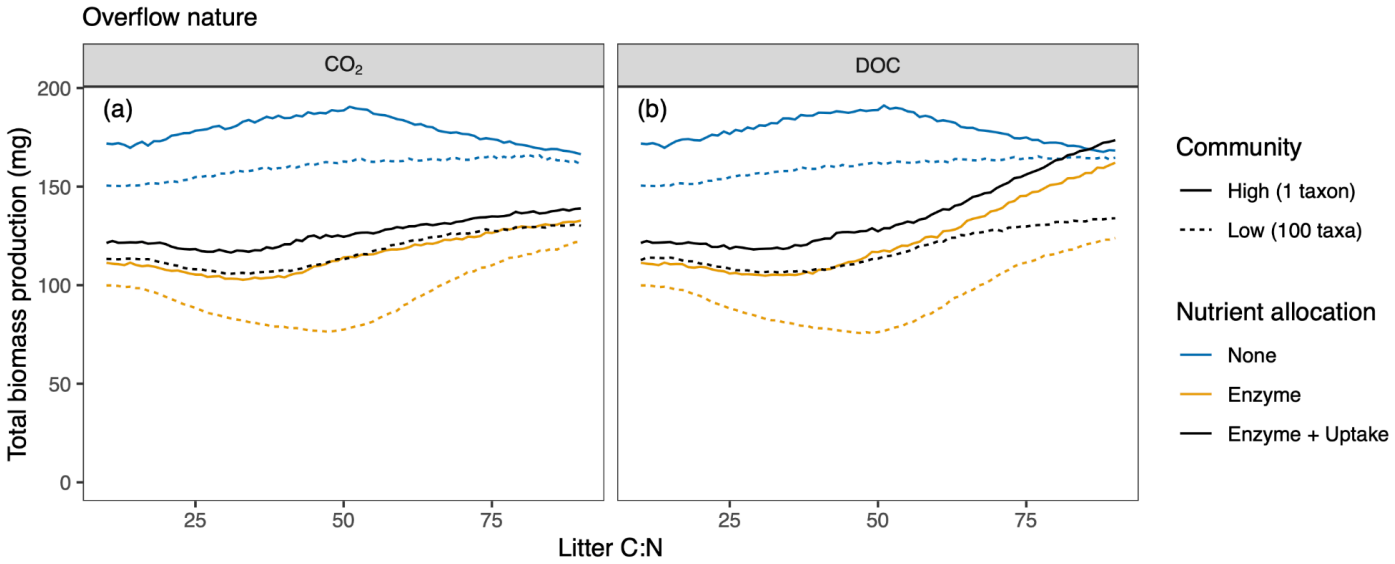
We will modify the phrase, clarifying that we advocate for future studies exploring the consequences of this mechanism. Our intention is only to generate interest in exploring this avenue.

Technical problems:

Figs. S7, S8: units are given in g, but total initial litter C is 344.56 mg cm^{-2}.

Thank you for noticing this detail. We have corrected these figures in the supplementary.





2.3.3 does not follow paragraph numbering correctly.

Thank you, it is now fixed.