Reviewer #2 (Citation: https://doi.org/10.5194/egusphere-2026-1571-RC2)

Summary

In this modeling paper, the authors simulated how the priming effect would vary depending on microbial growth traits, specifically differences between copiotrophs and oligotrophs. In the main text, the authors present four model simulations: two single functional group simulations (either copiotrophic or oligotrophic degraders) and two binary functional group simulations (a copiotrophic degrader paired with an oligotrophic non-degrader, or vice versa). The authors use a technique called cybernetic modelling: in their model, complex OM can be degraded to labile OM via the production of exoenzymes, which can then be consumed by microbes, stimulating microbial growth and further exoenzyme production in a feedback loop. The modelling technique allows for microbes to incorporate trade-offs in growth vs. enzyme production over finite time horizons (e.g. allocating energy to enzyme production to favor future growth outcomes, or vice versa). Overwhelmingly, the authors found cumulative positive priming in their model results, with interesting variations across different functional group simulations, with negative priming only occasionally observed when oligotrophic degraders are present. However, temporal modelling reveals that positive priming initially dominates while negative priming starts to become more prevalent as time goes on (complex OM is depleted), suggest the potential for priming effects to invert over time in real ecosystems.

General comments.

This was a very interesting and thorough modelling paper which prompted me to think about microbial regulation of the priming effect. Especially interesting was the authors' finding that magnitude/direction of priming effects may be partially explained by the presence/absence of non-degrading microbes in a system, which is something experimentalists do not generally measure. Surely these results will prompt some interesting experiments and future modelling work.

We thank the reviewer for their kind words and constructive comments, which have significantly improved our manuscript and research. We address the specific comments individually below and highlight the corresponding changes in the manuscript in red.

As I am not a modeler myself, many of my comments are rooted in exploring the biological realism of the model, and I encourage the authors to be explicit in what the model is simulating biologically where they can. The manuscript would also benefit from some more research into what has been found empirically, and discussion of if/why this varies from the model outcomes. Some general points that I think could be clarified/reviewed in the manuscript:

1. Define the explicit difference between copiotrophs and oligotrophs in the model (and if/how this varies from classical definition). Consider using more explicit terminology than "microbial growth traits"

   We adopt the classical microbial ecology definition of copiotrophs and oligotrophs as fast-growing microbes adapted to resource-rich conditions and slow-growing microbes adapted to resource-poor conditions, respectively (Koch, 2001). In our model, this distinction is represented by assigning copiotrophs higher maximum labile OM uptake rates and saturation constants, and oligotrophs lower values (Norris et al., 2021). This captures the contrasting responses of the two microbial traits across all observable levels of labile OM in our simulations (Supplementary Figure S1A).

   However, we acknowledge that this representation differs from classical ecological definitions in several ways: (1) we represent microbial groups as discrete categories, whereas microbial traits likely exist along a continuum (Couso et al., 2023; Stone et al., 2023); (2) trophic strategy is treated as an intrinsic and fixed trait independent of environmental conditions and nutrient

stoichiometry unlike in nature (Couso et al., 2023); and (3) degraders and non-degraders are represented as distinct groups, whereas many microbes in nature can produce low baseline levels of degrading enzymes or can shift strategies depending on environmental conditions (Chen et al., 2021). Please also refer to comments from Reviewer #1: https://doi.org/10.5194/egusphere-2026-1571-RC1.

We have clarified this in the main text in Section 2.1. We have also adopted the reviewer's suggestion to refer to copiotrophic and oligotrophic traits as "trophic traits/strategies" throughout the manuscript to provide a more explicit description of these microbial characteristics, and to refer to the combination of trophic traits and degradation ability as "microbial functional traits" overall.

2. Review the presence of positive vs. negative priming in the literature and how this compares to the model

   The comparison of positive and negative priming in the literature and our model is addressed in Section 4.1, which has now been expanded to include additional literature findings.

3. Review the proportion of labile vs. complex OM in real ecosystems and in priming experiments and how this compares to the model (I think 10% labile OM is actually quite large)

   In real ecosystem and soil priming experiments, the proportion of dissolved organic carbon or labile organic carbon is indeed typically much lower than 10% of total soil organic carbon (Lu and Xu, 2014; Wu et al., 2023). However, total soil organic carbon does not only represent the fraction accessible to microbial communities, as a substantial portion is not readily available for microbial utilization. Therefore, the relative proportion of labile organic carbon within the microbe-accessible soil organic carbon pool may be considerably higher than the values typically reported based on total soil organic carbon. In our modelling framework, complex OM represents the fully degradable and utilizable OM pool rather than the entire soil organic carbon pool. Therefore, examining relatively higher labile OM fractions in our model compared to values typically reported in natural ecosystems and soil priming experiments remains a reasonable representation of the contrasting availability of labile and complex substrates. Moreover, for a hypothesis-driven modeling study, the exploration across the full parameter space, including extreme conditions, is necessary to identify system behavior beyond the specific ranges captured by natural observations or experimental designs. We acknowledge that this rationale may not be immediately apparent to readers and have therefore clarified this assumption in the main text in Section 2.3.

Specific comments.

As there were no line numbers in the submitted manuscript, I've just grouped my in-line comments by the section. If further review is needed, it would be good if the authors could submit a numbered manuscript.

Abstract:

Less than 10% of what?

The text refers to strong positive priming being triggered by labile OM comprising less than 10% of the total OM in the complex and labile OM mixture. This has now been clarified in the manuscript.

I think the abstract should include some brief discussion of the biological relevance of the findings. The finding that copiotrophs induce positive priming is consistent with the idea that priming effect is a byproduct of microbial nutrient mining, right?

We have now linked the model results to biologically relevant interpretations.

Introduction:

Paragraph 1:

What is debated? Whether priming occurs? There is no evidence provided to suggest that priming does not occur.

We agree that the wording was poorly chosen. Our intention was not to imply that the existence of priming itself is debated, but rather that the occurrence of priming across diverse environments and their ecological implications remain actively discussed. We have revised the text accordingly.

Paragraph 3:

This would be a good paragraph to provide some reasons why a microbe would allocate C/energy to growth vs exoenzyme production. This would be a good place to bring in stoichiometry.

We have now provided additional explanations in this paragraph.

Paragraph 4:

This is an interesting topic. Is there any information known about the proportion of degrading to non-degrading microbes in real ecosystems? This would be a good place to look into that and bring it in.

We agree that it would certainly add value to the manuscript to discuss the relative abundances of degraders and non-degraders in real ecosystems and their implications for priming effects. However, quantitative estimates are largely unavailable in the existing literature. While many studies acknowledge the coexistence of degraders and non-degraders, these functional groups are typically inferred rather than explicitly identified and quantified, making robust estimates of their relative abundances difficult to obtain.

Paragraph 7:

This paragraph should include a statement precisely defining cybernetic modelling. Most biogeochemists/ecologists won't know what that is.

Which enzymes/enzyme classes can microbes produce in this model? I'm sure this is addressed later but some brief discussion in the intro would also be good.

We have provided a more detailed and kind explanation of the model for the benefit of general readers. To further clarify, the cybernetic model does not simulate any specific enzymes, instead, we formulate arbitrary enzyme pools upon which specific microbial metabolic functions depend. The resulting induction and suppression of enzyme synthesis can then dynamically represent microbial regulation of the metabolic functions. In this model, we broadly define two enzyme pools i.e., endoenzymes that mediate labile OM uptake and exoenzymes that regulate the degradation of complex OM.

Methods:

Section 2.1:

In the model, is it possible to vary the "quality" of complex OM such that "energy return from degrading complex OM" can vary? Otherwise how does energy return vary? Is the energy return just different for the different types of organisms (e.g. copiotroph, degrader, etc.) that are modeled?

In this study, the potential energy return from complex OM is fixed by the chosen degradation parameters (k_Z, K_Z, Y_S), so we do not vary complex OM "quality" explicitly. Instead, the realised energy return emerges from microbial regulatory decisions that depend on enzyme synthesis kinetics and trophic strategy. Through cybernetic control, degraders decide how much exoenzyme to produce in order to elevate labile OM to an optimal concentration for growth, and this optimal labile OM level

differs between copiotrophic and oligotrophic strategies. Thus, variation in "energy return" in our simulations arises from regulation and trait-dependent capture of a fixed potential yield, rather than from differences in the intrinsic degradability or chemistry of the complex OM itself. In principle, varying k_Z, K_Z, and Y_S, or accounting for the full distribution of polymeric properties (e.g., DP), and OM chemistry would allow a more explicit representation of OM quality (e.g., more or less depolymerizable substrates, OM bioavailability, specific nutrient limitations), and we are currently exploring this as a natural extension of the current framework. However, in this study, we chose to hold these parameters constant to isolate how microbial trophic strategies and interactions alone shape priming outcomes. We have now briefly explained this in Section 4.6.

Section 2.2:

To help the reader follow equations, consider providing units when defining each term in the equations (e.g. some terms are in unit mass, others are in unit mass per unit time, etc). It could be good to put these units along with the definition of each term in Table S1 for easy access to the interested reader.

The units of all model parameters are now provided in Supplementary Table S1 for clarity and ease of understanding. The definitions of the parameters are provided in the main text at the relevant locations where they are first introduced.

I don't think E_i and e_i are defined anywhere. These are the exoenzyme and endoenzyme concentrations right?

The dynamics of exoenzyme and endoenzyme concentrations are given in Eqs. (4) and (5), but may not have been explicitly defined in the text. We have now included this definition in the revised manuscript, along with other model variables.

Section 2.3:

"Complex OM degradation kinetic parameters were also fixed"… this means that the only thing impacting the energy return from complex OM is microbial growth traits, right? I would make this explicit in Section 2.1 where you talk about energy return.

We have now clarified this in Section 2.1 of the main text. Please also refer to our response to your comments under Section 2.1 above.

"Only exoenzyme and endoenzyme synthesis kinetic parameters are randomized in Monte Carlo simulations"… biomass yield ($Y_{X,i}$) is randomized according to Table S1. This isn't an enzyme synthesis parameter, is it?

We thank you for pointing out the accidental omission of biomass yield in this sentence. The sentence has now been revised accordingly.

The parameters that distinguish between copiotrophs and oligotrophs in the model are $k_{S,i}$ and $K_{S,i}$. From the supplementary table, copiotrophs have a higher rate of maximum OM uptake (about doubled) and a much higher saturation constant (one order of magnitude). My conclusion is that copiotrophs are defined in the model by their ability to uptake more labile OM than oligotrophs. I think a sentence like the previous explicitly explaining what differentiates copiotrophs and oligotrophs (biologically, not mathematically) in this model is important. (Also, a sentence about how the fixed numbers for the two differentiating parameters were chosen.) The differentiation between copiotrophs and oligotrophs in the model does not match precisely with biological definitions of copiotrophy and oligotrophy, which is OK, but it needs to be defined.

The parameters $k_{s,i}$ and $K_{s,i}$ are chosen arbitrarily to ensure that the contrasting responses of the two microbial trophic strategies are distinctly observable across all levels of labile OM in our simulations, as shown in Supplementary Figure S1A. These details have also been included in the main text. Please

also refer to our response to your General Comments, specifically Comment #1, where we have clearly defined the similarities and differences between our model definition and the microbial ecology of copiotrophs and oligotrophs.

Moreover, at the request of Reviewer #1, we have also performed a sensitivity analysis to examine whether qualitative trends in the model outputs are affected due to the variability in parameters $k_{S,i}$ and $K_{S,i}$. We briefly discuss this sensitivity analysis results in Section 4.3, with results presented in the Supplementary Fig. S4. Please also refer to comments from Reviewer #1: https://doi.org/10.5194/egusphere-2026-1571-RC1.

Section 2.4:

This section lost me, and I don't have the expertise to comment on it. A number of terms ($\mathbf{A}$, $\mathbf{B}_{ui}$, $\mathbf{p}_{ui}$) are not defined explicitly, but maybe we are getting outside the realm of precise physical definitions here and it's not as important.

We have now explicitly defined these optimal control theory variables in the text. However, we do not elaborate on their mathematical details here, as they extend beyond the physical microbial definitions into control theory. Readers interested in the mathematical formulation are referred to the original source to avoid distracting from the main focus of this study, which is the role of microbial regulation and functional traits in shaping priming responses.

Results:

Section 3.1:

There are no instances of negative priming in the CDG model. Is it mathematically impossible to have negative priming based on the input parameters in the CDG model? If so, why, and is there a biological basis for this? Perhaps there will be more discussion of this later…

The model setting reflecting the trophic strategy of CDG does not completely prevent negative priming, as instances of negative priming are clearly observed in CDG-ODG and CDG-CND microbial group combinations in Supplementary Figs. S2A and S2B. Therefore, we infer that CDG does not inherently or absolutely prevent negative priming, but rather the occurrence of negative priming is context-dependent on the specific microbial interactions and environmental conditions. For example, CDG preferentially responds to resource-rich conditions, leading to a strong positive feedback loop and thus pronounced positive priming. However, in CDG-CND systems, both groups compete for labile OM equally, resulting in less efficient triggering of positive priming and an increased likelihood of negative priming. Conversely, in CDG-ODG systems, the community consists of degraders that can thrive across a wide range of labile OM conditions (with low levels favoring ODG and high levels favoring CDG), forming a strongly degradative community even in the absence of exogenous labile OM. In this case, the addition of labile OM provides only minor enhancement of positive priming and may increase the likelihood of negative priming. Moreover, interactions between the two degraders can also potentially lead to rapid accumulation of labile OM in the system, thereby reducing the subsequent degradation of complex OM. We have now provided additional information on the context-dependent triggering of negative priming in Section 4.3, with additional discussions in the Supplementary Text.

"Owing to the inherent growth traits of copiotrophs and oligotrophs, the population level of CDG is lower at pure complex OM while the population level of ODG is higher." This sentence should be rephrased to be more explicit. For example (not sure if this interpretation is totally correct), "Because copiotrophs have higher labile OM uptake rates than complex OM decomposition rates relative to oligotrophs, oligotrophs are better able to sustain population levels under pure complex OM, resulting in greater biomass under pure OM in ODG than CDG."

We have now rephrased the sentence to provide a clearer and more mechanistic explanation. We cannot determine whether labile OM uptake rates are higher than complex OM degradation rates in copiotrophs, as this dynamically changes due to metabolic regulation in response to changing environmental conditions over time. Therefore, our interpretation is based solely on labile OM uptake kinetics, where oligotrophs exhibit higher uptake rates than copiotrophs under low labile OM conditions, such as those encountered in systems containing only complex OM. We have reflected this clarification in the main text.

Section 3.3:

I would like to see some discussion of the actual timing in this section. The time is in hours. After how many hours did you start to observe a positive priming effect? After how many hours did the positive priming effect go away?

We have now explicitly reported the observations of peak positive and negative priming with respect to time in Section 3.3 and have also discussed the potential inferences for the observations in Section 4.2.

Discussion:

Section 4.1:

This less than 10% number needs a bit more qualifying. 10% labile OM seems very large to me. Imagine if I suddenly added 10% of the carbon pre-existing in a soil sample as labile glucose. I would see a huge priming effect. In nature, labile C (e.g. as root exudates) is not added to systems at such high rates. In artificial substrate addition experiments, I think it will also be rare to see rates of substrate addition this high.

As in our earlier response to your General Comments, specifically Comment #3, we agree that a labile OM fraction of 10% of the total soil OM pool would represent an impractically high C influx in most natural ecosystems or in soil priming experiments. The way we structured the model implies that all complex OM present in the system is potentially degradable and can be fully consumed given a sufficiently extended simulation time. This is unlike the total soil OM that does not only represent the fraction accessible to microbial communities, but also a substantial portion which is not readily available for microbial utilization. Therefore, the fraction of labile OM within the bioavailable portion of soil OM pool may be considerably higher than the values typically reported for natural ecosystem or soil priming experiments. We have now accordingly highlighted the distinction between our model setting and the typical conditions observed in nature and priming experiments in Sections 2.3 and 4.5, as well as slightly modified the representation of a similar threshold in the literature in Section 4.5.

The finding of overwhelmingly positive priming effects should be discussed within the context of the existing empirical literature. I think positive priming effects are probably found more often than negative priming effects, but not overwhelmingly so as in the model. What does this mean? Are we measuring the priming effect inaccurately? Is the model predicting priming inaccurately? Or are there certain systems where negative priming may be more likely, where this model does not apply as well?

We have now added additional discussion of positive versus negative priming in our model in comparison with what is reported in the literature and have also included potential reasons for the sporadic occurrence of negative priming in our model. This could be attributed to the model not accounting for variation in OM chemistry, which are known to significantly influence the direction of priming between positive and negative (Chen et al., 2014; Fu et al., 2022), and could potentially lead to more frequent occurrences of negative priming, albeit still being rarer compared to positive priming (Bastida et al., 2019; Xu et al., 2024).

Section 4.2:

Interesting discussion of the temporal dynamics. This suggests to me that soils which are receiving constant labile C inputs (i.e. anywhere with plants) will be less likely to exhibit a positive priming effect than soils which are, say, newly colonized by plants. Or a seasonal aspect – perhaps a greater priming effect in the beginning of the growing season than the end?

We appreciate this insightful interpretation based on our dynamic model results. Our results are indeed partially consistent with the idea that systems receiving a newly increased input of labile C may exhibit stronger positive priming than systems under continuous labile C supply, as the temporal dynamics in our simulations show that positive priming tends to dominate early after labile OM addition and then weaken, or even transition toward negative priming, at later stages as the system adjusts and complex OM becomes depleted. In that sense, newly colonized soils or soils experiencing the onset of seasonal plant activity could be more likely to display pronounced positive priming than soils under sustained exudation. However, we note that this interpretation should be viewed as a hypothesis generated by the model rather than a direct prediction, because our current framework does not explicitly represent continuous labile OM input but instead simulates a single pulse addition at the beginning of the simulation, and the interpretation above stems the observation of sustained dynamic instantaneous relative priming effects. We have now added a brief discussion in Section 4.2 to highlight that this interpretation may have ecological implications, while noting that it remains a model-based hypothesis that would benefit from future exploration of how temporal patterns of labile C delivery shape priming under more realistic field conditions.

Section 4.3:

"Oligotrophs can deplete labile OM to concentrations well below the threshold required for copiotrophs to persist…" I'm not sure I follow this argument, as it applies to this system. Oligotrophs have lower rates of labile OM uptake – wouldn't that leave more OM left over for the copiotrophic degraders than if it was a copiotrophic non-degrader instead of an oligotrophic non-degrader?

Section 4.4:

It seems like the argument employed in this section (that CDG dominates OND biomass because OND do not consume enough OM to capitalize on priming by CDG) is at odds with the argument employed in the previous section (that OND deplete OM to low enough concentrations that CDG struggle to persist).

We appreciate the reviewer's careful reading and identification of inconsistencies across Sections 4.3 and 4.4, and agree that our original explanations were not sufficiently precise or consistent. In particular, the statement that oligotrophs can deplete labile OM to concentrations below the threshold required for copiotrophs to persist was inaccurate in the context of our model and microbial ecological definition of trophic strategies, and it created an apparent contradiction with the later discussion of CDG biomass dominance, as OND do not consume sufficient labile OM to capitalize on priming driven by CDG. Our intended interpretation is narrower and simpler: the presence of OND alongside CDG only marginally reduces labile OM levels (unlike the stronger effect of CND in the ODG-CND system), while CDG retain greater access to released labile OM. Consequently, the addition of labile OM represents a larger perturbation in the CDG-OND system than in the CDG-only system, thereby producing a stronger positive priming response in the former. We have now revised the discussion accordingly in Section 4.3, and the revised explanation is now consistent with the biomass patterns of CDG and OND reported in Section 4.4.

Section 4.5:

See my earlier comments on the wording of "small amount" re. 10%. It might be interesting to have a section focusing on just what happens between 0 and 10% labile OM, or even 0 and 5%.

As in our response to General Comment #3 and your comment on Section 4.1, we have now accordingly highlighted the distinction between our model setting and the typical conditions observed in nature and priming experiments (i.e., the 10% labile OM fraction in our model is not equivalent to the labile C input percentage in nature and soil priming experiments) in Sections 2.3 and 4.5. As for what happens between 0 and 10% labile OM fraction, priming is already observed at much lower labile OM fractions and generally increases approximately linearly with increasing labile OM up to the threshold. Beyond this threshold, additional labile OM produces little further enhancement of priming as the response becomes nonlinear and approaches saturation. We have now briefly mentioned this in Section 4.5.

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