Dear Dr. Weintraub,

Thank you for the opportunity to revise our manuscript entitled “Metabolic tradeoffs and heterogeneity in microbial responses to temperature determine the fate of litter carbon in simulations of a warmer world”. We found the reviewer comments very helpful in revising our manuscript. One of the things which became apparent during the review process is that our experimental design and how the different manipulations in the model were achieved was unclear. Therefore, we re-made figure 1 and added additional information to the legend incorporating reviewer comments. Another key point which was brought up is that we had not sufficiently discussed the nuances and shortcomings of the model, so it seemed like we were overstating our results as the be-all and end-all. We have added additional qualifying statements throughout the manuscript (including modifying the title) to make it more apparent that we view our work as the first step of something potentially worthwhile pursuing under other modeling frameworks and validating using empirical fieldwork. We believe the only comment we did not adjust our manuscript in response to is Anonymous reviewer 2’s request to run additional simulations to parse out which features of fungi make it so their CUE temperature response is more influential on model outputs; we feel this is beyond the scope of our current manuscript in part because it would require modifying the model structure itself, and not just parameters. A line by line response (plain text) to individual reviewer comments (italics) can be found below, revised from the original versions posted in the discussions based on responses to additional reviewers and the latest manuscript draft.

Thank you again for the opportunity to revise,

Grace Pold

**Response to Anonymous referee 1:**

Thank you for your comments. Here is a line by line response to your concerns:

*”Especially when the authors refer to Fig.1, which could be a great roadmap to the different scenarios tested, there are almost always mix ups with which letter represents which scenario. The figure itself also looks kind of distorted, at least in the pdf that I got. Lines that should be straight are not and dots are not round.”*

We identified one mix-up in the figure caption and remedied it. We have also remade figure 1 in a different software and added additional text to figure references on lines 95-100 to help guide the reader in its interpretation.

*“In Figure 3 the legend suddenly brings up the categories ’continuous’ and ’discrete’, which have not been mentioned before and are not explained in the figure caption.”*

The language has been remedied to be homogeneous and heterogeneous and so consistent throughout.

*In figure 4, the order of categories is 'decrease', 'increase', and 'none', while in the corresponding section in the manuscript it is different.*

The order has been changed to "increase", "decrease", "none" to keep in line with figure 1 and with the text where figure 4 is referenced.

*"Also in figure 4 there is a graph with an axis title that says 'CUE at 15◦C' and one with just 'CUE' what is the difference?"*

"CUE at 15ºC" refers to the biomass-weighted CUE of the surviving community without 5ºC of warming (ie $C_r + C_e + C_u$). "CUE" refers to the CUE at the temperature where the simulations took place (ie $C_r + C_e + C_u + C_t$*(temperature – 288ºK)). The appropriate equations have been added into "Analysis of outputs" section (line 125).

*"The reference list includes duplicates."*

Thank you. The duplicate Pietikäinen reference has been removed.

*"A lot of the data that is mentioned in the text is not shown in any graphs or tables or wrongly referenced to e.g. lines 204-207."*

We believe there is only one piece of data is not shown in a table or graph ("Intriguingly, neither litter ($r=0.16$, $p=0.23$) nor microbial biomass pool sizes ($r=-0.44$, $P < 0.001$) positively correlated with extracellular enzyme investment"). As it is a post-hoc attempt to explain correlations between litter or microbial biomass carbon pools, it can be adequately described by a simple statistic and so does not need to appear in a table or figure.

Perhaps the reviewer was confused by tables and figure references sometimes appearing after two ideas derived from the same table or figure are stated?

"*1. The title as well as the abstract are quite catchy but I think are overselling what the results of the paper show. Especially since the authors themselves state that their findings partially contradict findings from other modelling studies and empirical evidence is lacking."*

The title has been modified to "Metabolic tradeoffs and heterogeneity in microbial responses to temperature determine the fate of litter carbon in simulations of a warmer world" to clarify that these are simulations. The final sentence of the abstract has been modified to reiterate the contingency of the results on model structure as follows: "Together these results implicate a role for diversity of taxon-level CUE responses in driving the fate of litter C in a warmer world within DEMENT, which should be explored within the framework of additional model structures and validated with empirical studies."

*"The forth hypothesis is not very well introduced before"*

Sorry – this was a little hidden in the sentence "In other instances, CUE may be modeled as fixed within taxa, such that changes in community-level CUE with warming are the result of shifts in the dominant group or groups of organisms present as a function of their dietary preferences and/or C:N ratio (wieder et al. 2013, Sistla et al. 2014)." We appended an additional sentence to spell out this point more clearly: "This may occur, for instance, if large C-rich fungi show less-positive responses to warming than small, N-rich bacteria do, due to higher relative C demand of the former." (line 68)

**Response to Bin Wang:**

Thank you for your thoughtful and helpful review. Here we will highlight the changes we made to the manuscript in response to your comments:

*First, it would be better to provide an explicit equation showing how CUE is calculated in DEMENT: CUE = Ci + (T-15)*Ct, where Ci is the intrinsic CUE, which is calculated as a function of baseline CUE and numbers of enzyme and transporter. This equation, though simple, would first make the writing much easier to organize and follow with regard to what specific changes have been modified in this study. This equation could be listed around line 80 on page 4.*

The suggested modification has been made on line 89.

*In addition, the first sentence in section 2.3 about Running DEMENT could be moved above to the very beginning of section 2.2 to introduce the modifications.*

Good idea. The sentence has been moved up.

*Also, it might be better to include the DEMENT GitHub repository URL.*

Thank you – the link has been fixed. Although given GitHub's status as an ICE contractor, redirecting people to its services doesn't seem particularly ethical.

*The results of Pold et al. clearly indicate the role of CUE variability in regulating the fate of litter C in response to temperature (and likely moisture). Although I overall agree with all of the results and discussions, there is one spot in the very beginning of the results section was particularly not clear to me: in the paragraph around line 130, first, to my understanding references to Figure. 1A and Fig.1B should be reversed.*

Thank you – the references have been swapped.
*As regards the homogeneous scenario, the authors stated "all taxa had an identical temperature sensitivity that was equivalent to the cross-taxon mean (0oC-1). . .". I am not sure what exactly the constant Cr value is across taxon, 0, some value positive or negative, as illustrated in Fig.1 A, Ai, Aii?*

The cross-mean average in this scenario is zero in this instance. We have clarified this in the text (lines 94 and 144).

*Also, throughout the discussion and results, the terms used to describe heterogeneity in the specific parameter Cr and homogeneous community are not easy to follow, and are sometimes confused with descriptions of other microbial models that are not microbial explicit. I recommend being explicit and consistent throughout the ms when using heterogeneity and homogeneity to describe the variation in Cr and across-taxon variability, as well as different models.*[GP1]

Language has been added into the paragraph beginning on line 164 to clarify that some models are inherently homogeneous, while others (like DEMENT) can have heterogeneity introduced to the microbial community.

*A final note is regarding the definition of CUE. CUE in theory is an emergent property. However, CUE in DEMENT and other models, considering a modelling tradeoff of complexity vs. efficiency, is still more like a prescribed parameter. This means current modelling framework largely cannot address causes underpinning CUE variability. Therefore, if Pold et al. could acknowledge the difference between CUE in this study and emergent CUE somewhere in the manuscript, that would make this paper even more informative, though it already is.*

We absolutely agree with this comment and have added text at the end of the paragraph "Comparison to empirical warming studies" to include this (line 320).

**Response to Anonymous Referee 2:**

Thank you for your helpful and insightful comments on our manuscript. Please find the reviewer comments in italics, followed by our responses.

*"Overall, the work is interesting and timely but results and conclusions were sometimes overstated. Simulations are not the same as observations and at best provide possibilities when all underlying assumptions are accepted as adequate to the goals of the project."*

Agreed. We have rephrased some of our results and conclusions to highlight the modeling nature of this study.

*"Abstract: the next-to-last sentence was an important summary of the work, but it was inverted and complex to unpack. Consider rewording and maybe dividing into two sentences."*

Thank you. We modified the sentence to read "Litter C loss was exacerbated by variable and elevated CUE at higher temperature, which effectively lowered costs for extracellular enzyme production"

*"The 2nd paragraph of the introduction was a good synopsis of the state-of-art for estimating CUE and logical argument for a more flexible approach."*

Thank you.

*"The 3rd paragraph of the introduction makes a case for taxonomic variation in extracellular enzyme production with temperature that may affect growth efficiency, but it takes a couple of reads to understand this point. Consider revising to clarify."*

Thank you. We moved up extracellular enzyme production in the first sentence to make it clear that is what this paragraph is really about, and then re-wrote the rest of the paragraph beginning on line 52.

*"Also, the final sentence links rapid growth to more enzyme production, which seems counter-intuitive given that increased costs associated with producing more enzymes reroute resources from growth. This point needs more support."*

We see how it might be counter-intuitive because uptake is missing from this analysis. Fast-growing taxa need to take up more resources to maintain that growth. It doesn't mean they are growing efficiently. Hopefully the revised paragraph starting on line 52 makes this clearer.

*"The rationale for the third of the selected suite of simulations isn't clear. Is there some reason why bacteria and fungi might have opposite responses to temperature? This adds complexity to the work that needs justification."*

We have observed that there is a greater positive range of Ct values for bacterial isolates relative to fungal isolates in our laboratory study (in review); however, we have thus far been unable to determine what biological factors underlie this difference. So in the absence of a known mechanism causing differences in Ct range between bacteria and fungi, we explored the effect of potential differences in Q10 within the constraints of DEMENT.

*"Did the last 5000 of 6000 iterations approximate steady-state?"*

No. We have never managed to get DEMENT to consistently attain a steady state. In some instances a quasi-steady state can be attained for one or two random seeds (starting communities) out of 20. But this comes with the cost of the litter C stabilizing at a concentration so high the leaves may as well be diamonds and/or those same starting communities not attaining a near-steady state once the temperature is increased in the model. So for all practical purposes, we did not find a steady state attainable with the model. This is consistent with earlier work with DEMENT (ex. Allison 2014, Frontiers in Microbiology).

*"How many of the outputs were omitted due to unrealistically high litter accumulation?"*

0 to 5 per scenario. We have added a row to table 2 showing how many runs were excluded from each scenario based on this criteria.

*"Was there any obvious reasons why this happened, such as too little biomass or constrained enzyme production? This might be an interesting result."*

It is almost always due to taxon diversity loss. By chance, taxa capable of producing enzymes to effectively break down one of the substrate classes is lost, and the result is that litter component accumulates at or near the rate of daily litter addition.

*"Line 114: what is "biomass-weighted CUE? "*

'Biomass-weighted CUE' means that the CUE is calculated not as just the average for each taxon, but rather the contribution of each taxon to the final CUE calculation increases proportionate to the fraction of total microbial biomass that taxon contributes. A definition has been added into the revised version on line 125.

*"Why were richness and diversity calculated on different sets of data? they cannot be compared for insights to evenness, for example on line 138."*

Great catch – thank you. This was an unintentional carry over from looking at model stability. The values have been updated in Table 2 and the text.

*"Line 135: I didn't understand the statement. Was the dampening effect a result of extending the microscale model across a macroscale environment? The next line suggests not but refers to the current study instead of the citation."*

Yes – Weider et al. 2015 proposes that microscale models lead to more homogeneous predictions as they are scaled up to the macroscale because any given single parameter will lead to fluctuations, but that the variation in physiology averages out when different phenotypic populations can asynchronously respond to their environment. However, we found that introducing variability in the CUE temperature response – equivalent to sampling a greater physiological parameter space (or pulling from a macroscale-type pool of phenotypes) – increases uncertainty in the response of litter decomposition to simulated warming. We have broken up this sentence to clarify the intended meaning.

*"Line 138: minor issue: MBC is listed as 0.15 in text but 0.16 in table 2."*

Thank you for catching the rounding error. The value has been corrected in the text.

*"Richness and ˇ diversity were not calculated on the same data, so this comparison is questionable."*

The values have been updated to represent calculations made on the same data.

*"Lines 140-144: Although the explanation for these patterns is logical, cause-and-effect relationships are problematic given the complexity of this model, non-linear relationships among variables, and variations in drivers."*

Correct. We have added a sentence reiterating that this is a conjectured driver.

*"Line 162: minor issue is the word in parentheses "positive"? ˇ Figure 1 wasn't easy to understand, especially the dots. Were they output or illustrative of some specific information? Didn't figures 1G and 1H contrast fungi and bacteria? The revisions were better, but still confusing. "*

Each dot represents a taxon. The figure does not represent model output, but rather is intended to show the experimental design for the simulations (ie whether Ct was fixed or allowed to vary in a scenario, and whether the values possible in the simulation depended on the number of enzymes a taxon had or its status as a bacterium or fungus). Additional text has been added to the figure legend stating that each dot represents one of 10 example taxa in the simulations, although 100 taxa were actually used in the model. We have also updated the figure as there was a mistake in the representation of fungal and bacterial values in the revised figure for scenarios E-H.

*"Lines 182-4: The responses of kinetic parameters to temperature aren't consistent among the available studies, and most studies report only apparent activities. So, this statement is more speculative than it seems."*

This is an excellent point. We have modified the language (lines 200-203) to clarify that the stated relationships are assumed in the version of DEMENT we used, although they do not always represent reality.

*"Paragraph around line 190: Maybe I missed it but some of this information would help the reader better understand the purpose of some of the selected simulation treatments if provided earlier in the modeling description."*

Additional information has been added into the "Modifications to DEMENT" paragraph (lines 94-100) to link simulations and hypotheses and demonstrate that some scenarios were included to maintain distributions of Ct and enzyme counts which were comparable, so that the effect of Ct could be parsed out from other drivers (lines 105-107).

*"Line 224-5: This sentence seems to conflate competition with CUE and enzyme costs, but this idea seems like it would be limited to differential responses of taxa rather than a generic response of all taxa to temperature. Right? In any case, the results of DEMENT are not necessarily proof of concept for the real world."*

Yes, this is correct that we are linking competition with CUE and enzyme costs, as these are the central drivers of microbial carbon balance (plus transporters - which we did not examine) in DEMENT. We meant this statement only to explain the patterns observed for DEMENT, and absolutely DEMENT is its own world apart from the real one.

*"Line 234: simulations show the results of a possible synergy within a modeling context, only. "*

Agreed. We have added in this clarifying statement.

*"Lines 246-9: Again, why were these scenarios chosen?"*

A sentence has been added in: "All four scenarios were tested in order to isolate the effect of changing taxonomic domain-Ct relationships from simply changing Ct or taxonomic domain independently." (line 271-272)

*"Line 260: An alternative sensitivity analysis targeting individual model characteristics defining differences between fungi and bacteria would have been appropriate, nor would it take thousands of simulations to explore. "*

This is correct. We did not try this, but our experience is that DEMENT becomes very unstable very fast when you tweak anything. Parsing out the drivers of the differences between bacteria and fungi would require not only changing parameters, but also the model itself, as three of the differences between the two groups are hard-coded into the model (motility, nutrient translocation, and how organisms divide). Therefore, we think the suggested sensitivity analysis is outside the scope of the present study.

*"Line 267-8: This would be a good place to remind readers of the differences in fungi and bacteria that might explain this result. "*

The sentence "Nonetheless, differences in C and nutrient translocation abilities, and in cell size, stoichiometry and turnover rates still defined the two groups." was added to line 291

*"Line 290: Fitting between a range of -48 to +178% doesn't seem impressive, particularly given some of the omissions. What about the omitted simulations?"*

The omitted simulations still have a respiration response to temperature within this range, although the absolute values of respiration are ~6% lower on average than the simulations which were not excluded. The language has been changed in the text to state that the simulated changes in respiration are narrow compared to the range of observed respiration responses (line 314).

*"Line 291-3: This is the clearest, simple statement of the study results, and its buried here."*

Thank you. We have now reiterated it in the conclusion.

*"Lines 301-3: Although interesting, this statement only adds another level of confusion. This paragraph isn't needed. "*

The paragraph from L296-305 has been removed.

*"Fig. 2. Were the temperature responses of soil bacteria isolates on the 4 different media indistinguishable? Ditto for the soil community responses."*

Temperature response is, on average, the same for all media types for isolates. But substrate does matter for the temperature response of a given isolate. Soil community responses do differ based on substrate (ex Frey et al. 2013, glucose vs. phenol). Thus together, this indicates the potential for either species sorting or differential stimulation of communities at different temperatures. We have a manuscript in review on this.

*"Fig. 3. How did continuous and discrete compare to heterogeneous or homogeneous? The revisions helped."*

Thank you. We replaced all the continuous and discrete language with heterogeneous and homogeneous, respectively.

Biogeosciences (bg)

# Metabolic tradeoffs and heterogeneity in microbial responses to temperature determine the fate of litter carbon in simulations of a warmer world

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**Abstract.** Climate change has the potential to destabilize the Earth's massive terrestrial carbon (C) stocks, but the degree to which models project this destabilization to occur depends on the kinds and complexities of microbial processes they simulate. Of particular note is carbon use efficiency (CUE), which determines the fraction of C processed by microbes that is anabolized into microbial biomass rather than being lost to the atmosphere as carbon dioxide. The temperature sensitivity of CUE is often modeled as an intrinsically fixed (homogeneous) property of the community, which contrasts with empirical data and has unknown impacts on projected changes to the soil C cycle under global warming. We used the DEMENT model—which simulates taxon-level litter decomposition dynamics—to explore the effects of introducing organism-level heterogeneity into the CUE response to temperature for decomposition of leaf litter under 5°C of warming. We found that allowing CUE temperature response to differ between taxa facilitated increased loss of litter C, unless fungal taxa were specifically restricted to decreasing CUE with temperature. Litter C loss was exacerbated by variable and elevated CUE at higher temperature, which effectively lowered costs for extracellular enzyme production. Together these results implicate a role for diversity of taxon-level CUE responses in driving the fate of litter C in a warmer world within DEMENT, which should be explored within the framework of additional model structures and validated with empirical studies.

## 1 Introduction

Soil heterotrophs are central to the cycling and recycling of the 60 gigatons of organic carbon (C) that plants deposit onto and into the ground each year. How well these inputs are converted into relatively stable soil organic matter depends on temperature, moisture, chemical composition, and soil mineralogy, which interact to influence microbial physiology (Manzoni et al., 2012; Kallenbach et al., 2016; Oldfield et al., 2018). Predictions regarding how soil C stocks will respond to climate change are, in turn, highly sensitive to how carbon use efficiency (CUE)—or the fraction of C taken up by a cell and incorporated into biomass rather than being respired—-changes with temperature (Allison et al., 2010; Wieder et al., 2013; Allison, 2014; Li et al., 2014; Sistla et al., 2014; Tang and Riley, 2015). As such, quantifying microbial decomposer CUE and its responsiveness to environmental change has been subject to intensive study (Devêvre and Horwáth, 2000; Frey et al., 2013; Blagodatskaya et al., 2014; Lee and Schmidt, 2014; Spohn et al., 2016a, b; Öquist et al., 2017; Malik et al., 2018; Geyer et al., 2019; Malik et al., 2019; Zheng et al., 2019).

Soil microbial communities show considerable differences in how their metabolisms respond to elevated temperatures, with their CUE increasing (Öquist et al., 2017; Zheng et al., 2019), decreasing (Devêvre and Horwáth, 2000; Frey et al., 2013; Öquist et al., 2017; Li et al., 2018; Zheng et al., 2019) or remaining unaffected by warming (Dijkstra et al., 2011b; Öquist et al., 2017; Walker et al., 2018; Zheng et al., 2019). However, models of the soil C cycle generally assume either no change (Allison et al., 2010; Li et al., 2014; Wieder et al., 2014) or a fixed and homogeneous decrease in CUE with temperature (Allison et al., 2010; Wieder et al., 2013; Allison, 2014; Li et al., 2014). When CUE is allowed to directly increase with temperature, this temperature response is homogeneous across taxa (Frey et al., 2013; Ye et al., 2019). In other instances, CUE may be modeled as fixed within taxa, such that dynamic changes in community-level CUE with warming are the result of shifts in the dominant group or groups of organisms present as a function of their dietary preferences and/or C: nitrogen (N) ratio (Wieder et al., 2013; Sistla et al., 2014), rather than any inherent differences in the temperature sensitivity of the constituent community members. This may occur, for instance, if large C-rich fungi show less-positive responses to warming than small, N-rich bacteria do (Pietikäinen et al., 2005; DeAngelis et al., 2015). Therefore, models have thus far insufficiently accounted for how the temperature sensitivity of central metabolism may differ between microbes, such that intrinsic differences in efficiency between taxa (“heterogeneity") above and beyond temperature-driven differences in substrate supply may also drive microbial community trajectories.

Heterogeneity in the temperature sensitivity of growth efficiency across taxa could be driven by differences in the rate-limiting step of central metabolic pathways (Dijkstra et al., 2011a), or in how well the proteins responsible for the extracellular processing and uptake of environmental nutrients are able to maintain activity as temperature increases (Allison et al., 2018; Alster et al., 2018). For instance, there is some evidence that bacteria benefit more than fungi from an increase in temperature, as their growth rate has been observed to decrease less rapidly with temperature above its optimum than a fungal community (Pietikäinen et al., 2005). The respiration rate for the two groups could not be isolated in that study, and so direct differential effects of temperature on CUE could not be parsed out. However, the CUE has been observed to differ in how sensitive it is to nutrient limitation for bacteria and fungi, such that differences in the temperature sensitivity of CUE between the

two groups may play out as a consequence of changing nutrient demands (Keiblinger et al., 2010; Sinsabaugh et al., 2016). Therefore, the temperature range over which an an organism can maintain efficient growth is one important dimension of its niche (Cavicchioli, 2016) and may differ between taxa.

The temperature response of CUE may also differ between taxa because of varying investments in extracellular enzyme production. Extracellular enzyme production can impose substantial metabolic costs on the cell (Allison, 2014; Malik et al., 2019), as C which could otherwise be allocated to growing the cell must instead be spent producing amino acids and ATP to synthesize the enzymes (Kaleta et al., 2013; Kafri et al., 2016). Thus if taxa need to produce more extracellular enzymes in order to support rapid growth at higher temperature, then their CUE could decrease with temperature. However, some taxa may have mechanisms to adjust central metabolism so growth remains efficient at elevated temperatures, as hinted by a weak positive correlation between the Q10 of CUE and cellulolytic potential in soil communities (Zheng et al., 2019). Nonetheless, the most probable relationship between CUE temperature response and extracellular investment is currently unknown.

We explored whether interactions between the temperature sensitivity of intracellular (i.e. CUE) and extracellular (i.e. litter decomposing enzyme) metabolic processes of cells can explain why CUE is observed to increase with temperature in some soils, and decrease in others. We used the litter decomposition model DEMENT (Allison, 2012) to evaluate four hypotheses: 1) allowing temperature response of CUE to differ (be heterogeneous) between taxa increases uncertainty in projected litter decomposition dynamics because more diverse phenotypic combinations exist for competitive selection (i.e. species sorting) to act upon; 2) this heterogeneity favors a community with higher CUE, in turn leading to higher microbial biomass and greater litter C loss with warming; 3) forcing the temperature response of CUE to increase with the number of enzymes an organism produces causes greater litter C loss than when the two factors vary independently, because increasing CUE with temperature offsets the increased costs against CUE associated with copious enzyme production; and 4) the magnitude of litter C loss with warming is greater when the CUE of the C-rich fungal functional group increases with temperature than if only the N-rich bacterial functional group does due to higher relative C demand of the former.

## 2 Methods

### 2.1 DEMENT background and model design

DEMENT (Allison, 2012) is a litter decomposition model designed to simulate the loss of leaf C through time. The principal advancement of DEMENT over its predecessors is that it is both microbially- and spatially-explicit. The model is able to simulate inter- and intraspecific microbial interactions, with a primary focus on the tradeoff between the ability to take up and digest substrates, and the metabolic costs of creating and maintaining the machinery required to do so. Because these tradeoffs are both explicit and variable across taxa, DEMENT is an ideal model for evaluating how the physiology and ecology of microbes affects C stocks in a changing world. Furthermore, DEMENT allows for consideration of how heterogeneous responses across taxa (rather than using some homogeneous cross-taxon mean) can facilitate soil C responses to climate change. Full details about the setup and execution of DEMENT are available elsewhere (Allison, 2012, 2014; Allison and Goulden, 2017); here we describe the controls on CUE in the model which are relevant to our study.

Intrinsic CUE—the maximum CUE an organism could attain under ideal temperature and stoichiometry—is calculated for each taxon as a function of the baseline CUE at 15 °C ($C_r$), and the number of enzymes ($N_e$) and uptake transporters ($N_u$) the taxon can produce. In turn, how much CUE is decreased due to enzyme and transporter production depends on the cost per enzyme ($C_e$) and cost per transporter ($C_u$) (Table 1). The C used in enzyme synthesis is considered a loss from the cell, and is therefore not reported as microbial biomass C. The intrinsic CUE of each taxon is adjusted for temperature, decreasing by 0.016°C$^{-1}$ by default (i.e. $C_t$ = -0.016°C$^{-1}$), consistent with a global meta-analysis (Qiao et al., 2019). Therefore, CUE is calculated as $CUE = C_{\mathrm{i}} + (temperature - 288) * C_{\mathrm{t}} + C_{\mathrm{e}} * N_{\mathrm{e}} + C_{\mathrm{u}} * N_{\mathrm{u}}$, where temperature is in Kelvin.

### 2.2 Modifications to DEMENT

DEMENT v0.7.2 was downloaded from GitHib (https://github.com/stevenallison/DEMENT), and modified as follows. $C_r$ was adjusted downwards from its original published value of 0.58 to 0.38 at 15°C; this not only improved model stability (Table S1), but is also consistent with a comparative modeling study completed by Li et al. (2014), several $^{18}O$-$H_2O$ based CUE measurements (Spohn et al., 2016a, b; Geyer et al., 2019), and for the structural components of litter modeled by the soil C model MIMICS (Wieder et al., 2015a). By default, the temperature sensitivity of CUE ($C_t$) is fixed to take on the same value (ie is homogeneous) for all taxa; therefore we modified the model so $C_t$ could vary around the mean in different ways (Figure 1). In the first set of scenarios, $C_t$ varied independent of the taxonomic identity or number of enzymes a taxon produced (Figure 1B and hypotheses 1 and 2). In the second scenario, $C_t$ was limited to either increasing (Figure 1C) or decreasing (Figure 1D) as a function of the number of enzymes a given taxon had (hypothesis 3). In the third, bacteria were constrained to have a positive and fungi a negative $C_t$ (Figure 1E), fungi a positive and bacteria a negative $C_t$ (Figure 1F), both bacteria and fungi to have a positive $C_t$ (Figure 1G), or both have a negative $C_t$ (Figure 1H; hypothesis 4). In all instances, $C_t$ was selected at random from a uniform distribution bounded by +/- 0.022°C$^{-1}$ at the upper and/or lower limits (scenarios B-H, Fig. 1), or assigned a fixed value equivalent to the cross-taxon mean (scenario A, Fig. 1; scenario $A_i$, $A_{ii}$ Fig. S1). These values are within the range of temperature sensitivities observed for both bacterial cultures in the lab and for field communities (Figure 2), as well as values inferred based on modeling CUE against mean annual temperature on a global basis (Sinsabaugh et al., 2017; Ye et al., 2019). It was necessary to force the temperature sensitivity of CUE to take on a zero-centered uniform distribution so that simulation outputs in which extracellular enzyme counts were linked to the $C_t$ could be compared to those scenarios where they were not linked, without changing the distribution of extracellular enzyme counts present in the community.

### 2.3 Running DEMENT

DEMENT was run on the Massachusetts Green High Performance Computing Cluster for 6,000 model days using 59 different independent starting seeds and a 100x100 grid size. “Control" runs were completed at 15°C (equivalent to April to November mean soil temperature for a northern mid-latitude temperate deciduous forest (Boose, 2001)), while “heated" runs were completed at 20°C (Allison, 2014). The first 1000 days of each resultant output file was excluded from the analysis because of rapid shifts in the microbial community during this time. In addition, outputs were filtered to exclude any seeds where the substrate pool was two or more times greater at the end of the model run than the median during the preceding 5000 days, indicating

unrealistic, unconstrained litter accumulation. R version 3.4.0 was used for all runs and analyses (R Core Team, 2016). A full set of parameters and the model used to run all these simulations can be found in the supplementary files “params.txt" and “DEMENTmodel.R", respectively.

### 2.4 Analysis of outputs

The model outputs of interest were litter organic matter (LOM), microbial biomass carbon (MBC), respiration rate, richness and diversity of the surviving community, median number of enzymes per taxon for taxa alive during the 5000-day simulation, fungal:bacterial biomass ratio for surviving taxa, and reference and simulation temperature CUE. Two diversity metrics — richness and Shannon’s H — were calculated using median daily values for the microbial community over the 5000 days of simulation and the vegan package (Oksanen et al., 2017). Biomass-weighted CUE was reported for communities at 15°C (“reference temperature CUE") or at the simulation temperature (“simulation temperature CUE"), calculated using the formula $\sum_{d=1000}^{6000}\sum_{i=1}^{100} C_r + C_e * Ne_i + C_u * Nu_i + C_{ti} * (temperature - 288) * biomass_i / \sum_{i=1}^{ntaxa} biomass_i$, where 1000 and 6000 are the days of the simulation the outputs were examined over; 100 is the number of taxa the model was initiated with; $Ne_i$ is the number of enzyme genes taxon i has; and $Nu_i$ is the number of uptake transporter genes the taxon has; and temperature is the simulation temperature in °K.

In order to determine whether warming and model parameterization affected model outputs, we used mixed effect models with starting seed as a random effect and warming or simulation scenario as fixed effects using lmer in lme4 v 1.1-17 (Bates et al., 2015). Data were visually assessed for normality and homoskedasticity using qqplots and residual plots following log-transformation. Significantly different pairwise differences were subsequently identified using emmeans v.1.3.0 (Lenth et al., 2019), with a stringent Bonferoni-corrected p-value cutoff of $P < 0.0001$. Warming effect sizes are plotted as the natural log ratio of model outputs in heated:control scenarios. Figures were generated using ggplot2 (Wickham, 2009), and asterisks and letters denoting significant effects of experimental factors were added in Inkscape (Inkscape’sContributors, 2003–2019).

## 3 Results and discussion

LOM and MBC content were both generally higher than observed in environmental samples, leading to MBC:LOM ratios at the high end of ranges observed in the field (2-11% vs. 1-5% (Santos et al., 2012; Xu et al., 2013)). LOM and MBC values were within the range previously observed for simulations using DEMENT with daily litter inputs (Allison et al., 2014), but greater than those with just a single litter pulse (Allison, 2012; Allison and Goulden, 2017; Evans et al., 2017), indicating that these high biomass and litter C values can be attributed to these substrate inputs.

### 3.1 Intertaxon variability

To evaluate the effect of intertaxon CUE variablity on LOM stocks, we ran the model at 20°C (“heated") under two scenarios, and then compared the results to runs at 15°C (“control"). In the first “homogeneous" scenario all taxa had an identical $C_t$, equal to $0°C^{-1}$ (Figure 1A). In the second “heterogeneous" scenario, $C_t$ was assigned from a random uniform distribution bounded

by -0.022 and 0.022°C$^{-1}$ (Figure 1B). Therefore, the mean $C_t$ of the heterogeneous scenario was 0°C$^{-1}$, matching that of the homogeneous scenario.

Introducing intertaxon differences in CUE temperature response caused the characteristics of the initial microbial community (starting seed) to have a greater impact on litter decomposition than when all taxa had an identical temperature response (Table 2). This contrasts with the dampening effect proposed to explain instability in small-scale microbially-explicit models compared to their macroscale counterparts (Wieder et al., 2015b). Specifically, Wieder *et. al.* proposed integrating diverse physiology into C-cycling models should allow different microbial subpopulations to follow distinct trajectories which average out to a more consistent community-level mean and greater certainty in model projections. However, we found that the additive effect of increased physiological diversity was to increase, rather than decrease, uncertainty in the present simulations. The median-standardized interquartile ranges of both MBC (0.25 vs. 0.16) and LOM (0.28 vs. 0.14) increased with the introduction of a variable $C_t$. Through species sorting, this heterogeneously-responding microbial community became less diverse than both the homogeneous and control communities (Table 2). The communities characterized by heterogeneous $C_t$ maintained a higher median microbial biomass—driving two and a half times more LOM loss—than the communities characterized by a homogeneous $C_t$ (Figure 3). Intriguingly, neither litter (r=0.16, $P = 0.23$) nor microbial biomass pool sizes (r=-0.44, $P < 0.001$) positively correlated with extracellular enzyme investment; thus, a (non-significant) 28% increase in the median enzyme count is unlikely to have driven the increased decomposition under the heterogeneous scenario. Instead, increased decomposition and increased biomass are likely the consequence of elevated CUE under warming conditions. Nonetheless, the non-linearity of the model limits the degree to which causal relationships can be drawn between changes in litter C and the microbial parameters.

The homogeneous community scenario tested here is akin to the fixed "no adaptation of CUE" scenario reported in a number of other studies (Allison et al., 2010; Li et al., 2014; Sistla et al., 2014), because the cross-taxon mean used is zero temperature response. However, the effect of inter-taxon differences in $C_t$ ("heterogeneous") is less studied. Our results of reduced LOM loss in the absence of acclimation are consistent with two previous studies, but contrast with others. In an ecosystem-level model parameterized for an arctic tundra system, Sistla *et al.* (2014) found that greater soil organic matter (SOM) loss occurred with warming when the microbial community was able to dynamically acclimate its CN ratio (and in turn efficiency), than when the CUE was effectively fixed. Likewise, Allison (2014) found greater potential for increased LOM accumulation under warming when there was greater absolute variation in CUE across taxa (i.e. $C_r$ from 0.18 to 0.58 rather than 0.38 to 0.58) (Allison, 2014), although $C_t$ was always consistent across taxa in these simulations. On the other hand, a comparison of models where taxon-level differences in CUE or $C_t$ are not possible (i.e. $C_t$ is intrinsically homogeneous at the community level) showed that soil organic matter loss increases when organisms do not adapt (Li et al., 2014). Similarly, Wieder *et al.* found that greater SOM loss occurred if the CUE was directly insensitive to temperature than when $C_t$ was negative (Wieder et al., 2014). These microbially-explicit decomposition models vary in if and how they link CUE to microbial traits, and so our findings support the concept that nuances in how different components of CUE respond to warming is an important control on the fate of litter C (Hagerty et al., 2018).

### 3.2 Confirming the role of $C_t$ as an additional niche dimension

We allowed for CUE to increase with temperature for a subset of taxa in a way that most previous modeling efforts have not, and so it is possible that our results deviate from those of prior studies not because of variation in $C_t$, but rather because our simulations explore novel (positive $C_t$) parameter space. To facilitate comparison with previous decomposition modeling studies, we ran DEMENT simulations to test the effect of $C_t$ being homogeneous vs. heterogeneous when CUE was either always positive (homogeneous $C_t$ = 0.011 $^oC^{-1}$ (Figure S1A$_i$); heterogeneous $C_t$ = 0 to 0.022 $^oC^{-1}$ (Figure S1G)) or always negative (homogeneous $C_t$ = -0.011 $^oC^{-1}$ (Figure S1A$_{ii}$); heterogeneous $C_t$ = -0.022 to 0 $^oC^{-1}$ (Figure S1H)). In contrast to when $C_t$ was allowed to vary over the whole spectrum of values, introducing heterogeneity in CUE did not increase inter-run uncertainty in LOM or MBC pools (Table S2). We also found that less LOM accumulated when CUE showed a variable decrease with warming than a fixed one (Figure S2), which could be attributed to a reduction in MBC. By contrast, the homogeneous zero-centered and homogeneous positive $C_t$ scenarios, and the heterogeneous zero-centered and heterogeneous positive $C_t$, behaved more similarly to one-another in that warming decreased LOM while increasing MBC and CUE to a greater degree in the heterogeneous than homogeneous scenarios (Table S2). This finding reinforces the idea that if warming favors decomposer taxa capable of maintaining efficient growth, then soil C loss will be accelerated. Nonetheless, the strongly selected-for positive CUE response is rarely observed in complex soil communities. This indicates that additional tradeoffs with CUE temperature response are likely at play when CUE is either unaffected or decreases with temperature, but that these tradeoffs are missing in the formulation of DEMENT used in this scenario. One such tradeoff possible to explore within the framework of DEMENT is the allocation of resources to extracellular enzyme activity.

### 3.3 Linkages between CUE temperature response and extracellular enzyme allocation

Microbes depend upon extracellular enzymes to break down substrates in the environment into digestible pieces, and enzyme activities can be, like CUE, responsive to temperature (Wallenstein et al., 2010; German et al., 2012; Allison et al., 2018). Soil extracellular enzymes often are active *in-situ* at temperatures much below their apparent activity optima (German et al., 2012; Pold et al., 2017; Alster et al., 2018). Therefore, warming is assumed to enable them to process substrates at a higher rate in DEMENT (Allison, 2012), increasing the supply of growth substrates to microbes. However, the affinity of enzymes for their substrates may also decrease as temperature increases (German et al., 2012; Allison et al., 2018), as is assumed in DEMENT. If this is the case, then unless enzyme $V_{max}$ increases faster with temperature than $K_m$, additional resources must be diverted from growth to enzyme production to maintain microbial growth substrate supply rate. Therefore, taxa *may* differentially-allocate resources to enzymes and so demonstrate a relationship between the temperature sensitivity of CUE and the number of enzymes they produce.

We evaluated whether litter decomposition changed its trajectory when the organisms with the greatest genomic potential to break the litter down (i.e. enzyme counts) also showed the most- or least-positive growth efficiency response to warming. In the "increase" scenario, we simulated a positive relationship between temperature sensitivity of CUE and extracellular enzymes, where $C_t$ increased linearly from -0.022$^oC^{-1}$ for organisms with no extracellular enzyme production potential to 0.022$^oC^{-1}$ for

those organisms capable of producing the model maximum of 40 enzymes (Figure 1C). In the “decrease" scenario, we simulated a negative relationship between temperature sensitivity of CUE and extracellular enzymes, where the opposite relationship was imposed with $C_t$ decreasing with enzyme counts (Figure 1D). These scenarios were then compared to the “heterogeneous" scenario (aka “no relation", as described above), where $C_t$ varied across the same range, but independently of the number of enzymes an organism could produce. Therefore, the starting distribution of $C_t$ and enzymes per taxon was identical across scenarios, and only their relationship with one-another changed.

More taxa survived to the end of the simulation when warming was applied under the “increase" scenario than either the “decrease" or “no relation" scenario (median of 13 versus 7 and 8, respectively, median absolute deviation = 2.97 in all cases). Under the ”increase" scenario, taxa had 70% more enzymes each than the “no relation" scenario, and more than three times as much as the “decrease" scenario (Table 2). This relationship caused the CUE of surviving taxa to be 20-37% lower at 15$^{o}$C for the “increase" scenario compared to the others, but this deficit was diminished at 20$^{o}$C. As a result, the “increase" scenario led to higher respiration and a greater LOM loss under warming than under the ”decrease" scenario, despite an overall smaller microbial biomass pool (Figure 4).

How the relationship between $C_t$ and extracellular enzymes drives favorable trait combinations in DEMENT can also be observed in Figure 5. Surviving taxa retained a median enzyme count of at least 30 and a realized CUE temperature response of no less than 0.0158$^{o}$C$^{-1}$ under the “increasing" scenario ($\rho$=0.64, $P < 0.001$), but there was no relationship between realized CUE temperature response and enzyme production under either the “decrease" or “no relation" scenarios. The selection for community capable of maintaining high CUE at high temperatures was much weaker when it was associated with reduced enzyme production. When there was no relationship between $C_t$ and extracellular enzyme production costs, however, communities were able to attain a high realized CUE temperature response over a much wider range of median enzyme costs.

These findings indicate that response traits—which determine how an organism reacts to changes in temperature (e.g. CUE temperature response)—and effect traits—which determine how an organism alters its environment (e.g. litter decomposition potential)—interact to determine the fate of organic C within DEMENT. However, contrary to our hypothesis, adjusting DEMENT to allow for this tradeoff did not substantially alter how community-level CUE responds to temperature. The observation that LOM is reduced further when enzyme production is effectively cheaper contrasts with earlier work with DEMENT (Allison, 2014) showing smaller litter C pools under both ambient and elevated temperature when enzymes and transporters were cheaper to produce. However, our results are consistent in that microbial biomass was lower when enzyme costs are high, and that the microbial community was able to maintain a higher CUE under warming no matter the enzyme costs. The mechanisms underlying these phenomenological similarities differ, however, due to differences in how $C_t$ was parameterized in the two sets of model simulations. Specifically, although microbes were able to attain high CUE at elevated temperatures in our simulations by balancing the benefits of elevated CUE at higher temperatures with the costs of enzyme production against CUE, CUE always decreased with temperature in earlier work with DEMENT (Allison, 2014). Furthermore, enzyme production costs varied both with and independently of enzyme counts in previous DEMENT simulations (Allison, 2014).

Within the framework of DEMENT, increased CUE is likely needed to offset the costs of extracellular enzyme production that allow taxa to remain competitive at elevated temperatures, However, there is a paucity of empirical evidence regarding

the hypothesized correlations between temperature sensitivity of CUE and enzyme investment in soil systems. By examining correlations between the number of enzymes an organism can produce and its CUE temperature response at the end of the DEMENT model run, we see that there is likely to be either no or a positive correlation between the two variables, rather than a negative one (Figure 6). Limited data from bacterial isolates grown in the lab also support this, whereby CUE temperature response is either positively correlated or uncorrelated with the number of enzymes produced (Pold et al., in prep). Furthermore, although the mechanisms underlying the isolate response remain unclear, it is consistent with the scenario of $C_t$ and enzyme counts being positively correlated. Specifically, we found isolates with lower CUE at 15°C (more extracellular enzymes in DEMENT) were more likely to have a positive CUE temperature response than those with a higher CUE (fewer enzymes in DEMENT). Together, these insights support a synergism between CUE temperature response and enzyme production, rather than a tradeoff within the model. Because our DEMENT simulations indicate that selection for organisms characterized by high, positive CUE temperature responses with warming can alter both the directionality and extent of projected C loss, we propose it is important for other models to explore how possible increases—rather than just decreases—in $C_t$ affect terrestrial C projections.

### 3.4 Ecological relevance of microbial metabolic diversity—bacteria vs. fungi

Across scenarios, fungi generally dominated the microbial biomass C pool (Table 2), as is typical for litter decomposition (Chapman et al. (2013), and references therein). This pattern occurred despite generally lower biomass-weighted CUE for surviving fungal taxa, and preferential loss of fungal taxa across most scenarios (Table 2). The lower CUE for surviving fungi was not driven by higher enzyme costs than for bacteria, as median biomass-weighed enzyme costs were not statistically different (pairwise t-test $P > 0.4$) and differed by less than one enzyme for the two groups. To test whether modeled differences in fungal vs. bacterial cell sizes and stoichiometry were driving this pattern, we tested how forcing differences in $C_t$ in the two groups would impact the decomposition rate.

DEMENT was run with CUE simulated to respond to temperature: 1) negatively for all fungi and positively for all bacteria (F-B+; Figure 1E); 2) negatively for all bacteria and positively for all fungi (F+B-; Figure 1F); 3) positively for all bacterial and fungal taxa (F+B+; Figure 1G) or 4) negatively for all bacterial and fungal taxa (F-B-; Figure 1F). Minimum and maximum $C_t$ were set to -0.022 °C and 0.022 °C, respectively. All four scenarios were tested in order to isolate the effect of changing taxonomic domain-$C_t$ relationships from simply changing $C_t$ or taxonomic domain independently.

Litter C accumulated at higher rates with warming when fungal $C_t$ was negative, regardless of the bacterial $C_t$ (Figure 7, blue, orange). This is consistent with the observation that the CUE of surviving fungi was lower at the simulation temperature in seven out of the eleven scenarios. Because fungi have higher CNP ratios than bacteria (and thus higher C demands per unit biomass), we predicted that if fungi have a negative CUE temperature response, they would be weaker competitors at higher temperature than bacterial taxa, reducing their C demand and mitigating the warming effect on SOC stocks. While this contrasts with the premise that fungi should have a higher CUE (Six et al., 2006) due to their higher CN ratio (Zak et al., 1996), it is consistent with a growing body of literature indicating that substrate quality—rather than the F:B ratio correlated with it—is the underlying driver of differences in CUE between soils (Frey et al., 2013; Thiet et al., 2006; Malik et al., 2018;

Soares and Rousk, 2019). Despite the low nutrient content of the daily inputs to the model (92:0.26:0.02 C:N:P), microbes did not show evidence for nutrient limitation as biomass CN and CP ratios were lower than are typical for soil communities (Xu et al., 2013) (4.1 and 36.7 vs. 7.6 and 42.4, respectively). Thus C was limiting, which could have further disfavored the highly C-demanding fungi when their $C_t$ was negative. The lower CUE (and increased sensitivity to warming) for fungi compared to bacteria under a given scenario was also not driven by increased metabolic costs for enzyme production in fungi, as median biomass-weighted enzyme counts were statistically indistinguishable from those in bacteria.

The litter C pool decreased when fungal CUE increased with temperature (Figure 7), correlating with a smaller microbial biomass pool when bacterial CUE also decreased with temperature. By contrast, the LOM and MBC responses to warming were similar when only the $C_t$ of bacteria was changed, indicating that it is the fungal warming response which really drives changes in litter decomposition in DEMENT. This result is interesting because no *a priori* differences in decomposition or uptake potential were imposed on the two groups, and fungal and bacterial richness was initially equivalent. Nonetheless, differences in C and nutrient translocation abilities, and in cell size, stoichiometry and turnover rates still defined the two groups. Warming decreased the enzyme costs when fungal $C_t$ was positive but bacterial $C_t$ was negative, and decreased them under the opposing scenario, as evidenced by an increase in $C_r$ in the former and decrease in the latter. Nonetheless, as long as both bacterial and fungal CUE did not both decrease their CUE with temperature, community level CUE remained higher at 20$^{o}$C than it was at 15$^{o}$C.

Empirical evidence for high-level differences in the temperature sensitivity of CUE in bacteria and fungi is currently mixed, but indicate CUE temperature response for fungi is unlikely to be more positive than that for bacteria. Zheng et al. (2019) did not find a correlation between the lipid-based fungal:bacterial ratio and $Q_{10}$ of CUE over a range of soils. However, we (Pold et al., in prep) and our colleagues (Eric Morrison, personal comment) have found that fungi tend to show a stronger negative CUE response with warming than do bacteria when examining them in isolation in the lab. This is consistent with the observation that fungal CUE decreases more strongly with warming than bacterial CUE does when $C_t$ is restricted to negative values (Figure 7). It is also consistent with the premise that bacterial growth benefits more from elevated temperature than does fungal growth in some soils (Pietikäinen et al., 2005). Greater empirical insight into the taxonomic drivers of the temperature sensitivity of CUE will assist with constraining the parameterization and projections of microbially-explicit decomposition models such as DEMENT.

### 3.5 Comparison to empirical warming studies

Litter decomposition is typically observed to accelerate under warming (Lu et al., 2013). However, both the chemical composition of the litter and the identity of the living plant community at the site of decomposition are important for the magnitude of this response (Cornelissen et al., 2007; Ward et al., 2015). Consistent with these empirical studies—but inconsistent with a previous publication using DEMENT (Allison, 2014)—we found that litter decomposition was accelerated by warming in seven of ten scenarios. The range of losses and gains of litter C we observed with warming (-62% (scenario C) to +42% (scenario $A_i$)) approximates the -65% to +36% change observed in field experiments (Lu et al., 2013), with the upper limit only being exceeded when $C_t$ is constrained to negative values. Likewise, values for simulated litter respiration response (-5 to +6%) were

within a narrow range compared to those observed for soil respiration in the field (-48 to +178%), and responses for microbial biomass C were also within the observed range (-25 to +58% vs -47 to +86%) (Lu et al., 2013). Our modeled responses to warming thus suggest that one possible explanation for differences in terrestrial C pool responses to warming may be diverse temperature sensitivities of underlying decomposer communities. Nonetheless, a number of additional factors must be taken into consideration when interpreting our results in the context of global climate change, including soil mineral-mediated modulation of substrate supply (Schimel and Schaeffer, 2012; Coward et al., 2018), plant-microbe feedbacks (Melillo et al., 2011; Sistla et al., 2014; Suseela and Tharayil, 2018), and temporal variation in temperature. Furthermore, we modeled CUE as a somewhat inflexible property of individual taxa, rather than as the emergent property of allocation to different, biochemically-important physiological processes that it is Hagerty et al. (2018). These are important features to pursue in future iterations of the model should address.

## 4 Conclusions

Our results indicate that accounting for heterogeneous temperature response increases uncertainty regarding future litter C stocks, but only when $C_t$ does not differ from zero on average. However, by combining simulations, empirical studies, and literature searches, we can conclude that microbes with high enzyme costs are likely to have larger increases in intrinsic CUE with temperature; that taxa can sort on a CUE temperature response axis; and that fungi are more likely to increase CUE with warming than bacteria. The simulations meeting each or all of these criteria lead to loss of litter C under warming, and so DEMENT favors the prediction that litter will to become a net atmospheric C source in a warmer world. Nonetheless, our results also indicate that soil C stocks may change heterogeneously with warming because of diversity in how decomposers' CUE responds to temperature. We encourage models functioning on larger scales to explore the effect of including heterogeneity in the temperature response of CUE in order to determine the robustness of our conclusions to other model structures. However, ultimately increased integration of the growing body of literature on the temperature sensitivity of CUE must be explored for root causes of heterogeneity in temperature sensitivity of CUE in taxa under *in situ* conditions.

*Code and data availability.* The data and modified version of DEMENT used to generate it are available at OSF (https://osf.io/cwep9/?view_only=de0e6ba7b7da493f96531f398ca62c2c) under DOI 10.17605/OSF.IO/CWEP9

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## References


Allison, S.: A trait-based approach for modelling microbial litter decomposition, Ecol. Lett., 15, 1058–1070, 2012.

Allison, S. D.: Modeling adaptation of carbon use efficiency in microbial communities, Front Microbiol, 5, 571, 2014.

Allison, S. D. and Goulden, M. L.: Consequences of drought tolerance traits for microbial decomposition in the DEMENT model, Soil Biol Biochem, 107, 104–113, 2017.

Allison, S. D., Wallenstein, M. D., and Bradford, M. A.: Soil-carbon response to warming dependent on microbial physiology, Nat Geosci, 3, 336, 2010.

Allison, S. D., Lu, L., Kent, A. G., and Martiny, A. C.: Extracellular enzyme production and cheating in Pseudomonas fluorescens depend on diffusion rates, Front Microbiol, 5, 2014.

Allison, S. D., Romero-Olivares, A. L., Lu, Y., Taylor, J. W., and Treseder, K. K.: Temperature sensitivities of extracellular enzyme Vmax and Km across thermal environments, Global Change Biol, 24, 2884–2897, 2018.

Alster, C. J., Weller, Z. D., and von Fischer, J. C.: A Meta-Analysis of Temperature Sensitivity as a Microbial Trait, Global Change Biol, 2018.

Bates, D., Mächler, M., Bolker, B., and Walker, S.: Fitting Linear Mixed-Effects Models Using lme4, J Stat Softw, 67, 1–48, https://doi.org/10.18637/jss.v067.i01, 2015.

Blagodatskaya, E., Blagodatsky, S., Anderson, T.-H., and Kuzyakov, Y.: Microbial growth and carbon use efficiency in the rhizosphere and root-free soil, PloS one, 9, e93 282, 2014.

Boose, E.: Fisher Meteorological Station at Harvard Forest since 2001. Harvard Forest Data Archive: HF001, doi = doi:10.6073/pasta/04076dfd30b286c6c29301b6345a63f5, howpublished= http://harvardforest.fas.harvard.edu:8080/exist/apps/datasets/showData.html?id=hf001, 2001.

Cavicchioli, R.: On the concept of a psychrophile, ISME J, 10, 793, 2016.

Chapman, S. K., Newman, G. S., Hart, S. C., Schweitzer, J. A., and Koch, G. W.: Leaf litter mixtures alter microbial community development: mechanisms for non-additive effects in litter decomposition, PLoS One, 8, e62 671, 2013.

Cornelissen, J. H., Van Bodegom, P. M., Aerts, R., Callaghan, T. V., Van Logtestijn, R. S., Alatalo, J., Stuart Chapin, F., Gerdol, R., Gudmundsson, J., Gwynn-Jones, D., et al.: Global negative vegetation feedback to climate warming responses of leaf litter decomposition rates in cold biomes, Ecol. Lett., 10, 619–627, 2007.

Coward, E. K., Ohno, T., and Sparks, D. L.: Direct Evidence for Temporal Molecular Fractionation of Dissolved Organic Matter at the Iron Oxyhydroxide Interface, Environmental Sci Technol, 53, 642–650, 2018.

DeAngelis, K. M., Pold, G., Topçuoğlu, B. D., van Diepen, L. T., Varney, R. M., Blanchard, J. L., Melillo, J., and Frey, S. D.: Long-term forest soil warming alters microbial communities in temperate forest soils, Front Microbiol, 6, 104, 2015.

Devêvre, O. C. and Horwáth, W. R.: Decomposition of rice straw and microbial carbon use efficiency under different soil temperatures and moistures, Soil Biol Biochem, 32, 1773–1785, 2000.

Dijkstra, P., Dalder, J. J., Selmants, P. C., Hart, S. C., Koch, G. W., Schwartz, E., and Hungate, B. A.: Modeling soil metabolic processes using isotopologue pairs of position-specific 13 C-labeled glucose and pyruvate, Soil Biol Biochem, 43, 1848–1857, 2011a.

Dijkstra, P., Thomas, S. C., Heinrich, P. L., Koch, G. W., Schwartz, E., and Hungate, B. A.: Effect of temperature on metabolic activity of intact microbial communities: evidence for altered metabolic pathway activity but not for increased maintenance respiration and reduced carbon use efficiency, Soil Biol Biochem, 43, 2023–2031, 2011b.

Evans, S., Martiny, J. B., and Allison, S. D.: Effects of dispersal and selection on stochastic assembly in microbial communities, ISME J, 11, 176, 2017.

Frey, S. D., Lee, J., Melillo, J. M., and Six, J.: The temperature response of soil microbial efficiency and its feedback to climate, Nat Clim Change, 3, 395, 2013.

German, D. P., Marcelo, K. R., Stone, M. M., and Allison, S. D.: The Michaelis–Menten kinetics of soil extracellular enzymes in response to temperature: a cross-latitudinal study, Global Change Biol, 18, 1468–1479, 2012.

Geyer, K. M., Dijkstra, P., Sinsabaugh, R., and Frey, S. D.: Clarifying the interpretation of carbon use efficiency in soil through methods comparison, Soil Biol Biochem, 128, 79–88, 2019.

Hagerty, S. B., Allison, S. D., and Schimel, J. P.: Evaluating soil microbial carbon use efficiency explicitly as a function of cellular processes: implications for measurements and models, Biogeochemistry, 140, 269–283, 2018.

Inkscape'sContributors: Inkscape, https://inkscape.org, 2003–2019.

Kafri, M., Metzl-Raz, E., Jona, G., and Barkai, N.: The cost of protein production, Cell reports, 14, 22–31, 2016.

Kaleta, C., Schäuble, S., Rinas, U., and Schuster, S.: Metabolic costs of amino acid and protein production in Escherichia coli, Biotechnol J, 8, 1105–1114, 2013.

Kallenbach, C. M., Frey, S. D., and Grandy, A. S.: Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls, Nat Commun, 7, 13 630, 2016.

Keiblinger, K. M., Hall, E. K., Wanek, W., Szukics, U., Hämmerle, I., Ellersdorfer, G., Böck, S., Strauss, J., Sterflinger, K., Richter, A., et al.: The effect of resource quantity and resource stoichiometry on microbial carbon-use-efficiency, FEMS Microbiol Ecol, 73, 430–440, 2010.

Lee, Z. M. and Schmidt, T. M.: Bacterial growth efficiency varies in soils under different land management practices, Soil Biol Biochem, 69, 282–290, 2014.

Lenth, R., Henrik, S., Jonathon, L., Paul, B., and Herve, M.: emmeans, Iowa State, https://cran.r-project.org/web/packages/emmeans/index.html, 2019.

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

Li, J., Wang, G., Mayes, M. A., Allison, S. D., Frey, S. D., Shi, Z., Hu, X.-M., Luo, Y., and Melillo, J. M.: Reduced carbon use efficiency and increased microbial turnover with soil warming, Global Change Biol, 2018.

Lu, M., Zhou, X., Yang, Q., Li, H., Luo, Y., Fang, C., Chen, J., Yang, X., and Li, B.: Responses of ecosystem carbon cycle to experimental warming: a meta-analysis, Ecology, 94, 726–738, 2013.

Malik, A. A., Puissant, J., Buckeridge, K. M., Goodall, T., Jehmlich, N., Chowdhury, S., Gweon, H. S., Peyton, J. M., Mason, K. E., van Agtmaal, M., et al.: Land use driven change in soil pH affects microbial carbon cycling processes, Nat Commun, 9, 3591, 2018.

Malik, A. A., Puissant, J., Goodall, T., Allison, S. D., and Griffiths, R. I.: Soil microbial communities with greater investment in resource acquisition have lower growth yield, Soil Biol Biochem, 2019.

Manzoni, S., Taylor, P., Richter, A., Porporato, A., and Ågren, G. I.: Environmental and stoichiometric controls on microbial carbon-use efficiency in soils, New Phytol, 196, 79–91, 2012.

Melillo, J. M., Butler, S., Johnson, J., Mohan, J., Steudler, P., Lux, H., Burrows, E., Bowles, F., Smith, R., Scott, L., Vario, C., Hill, T., Burton, A., Zhou, Y.-M., and Tang, J.: Soil warming, carbon–nitrogen interactions, and forest carbon budgets, P Natl Acad Sci USA, 108, 9508–9512, 2011.

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E., and Wagner, H.: vegan: Community Ecology Package, https://CRAN.R-project.org/package=vegan, r package version 2.4-2, 2017.

Oldfield, E. E., Crowther, T. W., and Bradford, M. A.: Substrate identity and amount overwhelm temperature effects on soil carbon formation, Soil Biol Biochem, 124, 218–226, 2018.

Öquist, M. G., Erhagen, B., Haei, M., Sparrman, T., Ilstedt, U., Schleucher, J., and Nilsson, M. B.: The effect of temperature and substrate quality on the carbon use efficiency of saprotrophic decomposition, Plant Soil, 414, 113–125, 2017.

Pietikäinen, J., Pettersson, M., and Bååth, E.: Comparison of temperature effects on soil respiration and bacterial and fungal growth rates, FEMS Microbiol Ecol, 52, 49–58, 2005.

Pold, G., Grandy, A. S., Melillo, J. M., and DeAngelis, K. M.: Changes in substrate availability drive carbon cycle response to chronic warming, Soil Biol Biochem, 110, 68–78, 2017.

Pold, G., Domeignoz-Horta, L. A., Sistla, S. A., Morisson, E., Frey, S. D., and DeAngelis, K.: Carbon use efficiency and its sensitivity to temperature co-vary in soil bacteria, in prep.

Qiao, Y., Wang, J., Liang, G., Du, Z., Zhou, J., Zhu, C., Huang, K., Zhou, X., Luo, Y., Yan, L., et al.: Global variation of soil microbial carbon-use efficiency in relation to growth temperature and substrate supply, Sci Rep, 9, 5621, 2019.

R Core Team: R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria, https://www.R-project.org/, 2016.

Santos, V. B., Araújo, A. S., Leite, L. F., Nunes, L. A., and Melo, W. J.: Soil microbial biomass and organic matter fractions during transition from conventional to organic farming systems, Geoderma, 170, 227–231, 2012.

Schimel, J. and Schaeffer, S. M.: Microbial control over carbon cycling in soil, Front Microbiol, 3, 348, 2012.

Sinsabaugh, R. L., Turner, B. L., Talbot, J. M., Waring, B. G., Powers, J. S., Kuske, C. R., Moorhead, D. L., and Follstad Shah, J. J.: Stoichiometry of microbial carbon use efficiency in soils, Ecol Monogr, 86, 172–189, 2016.

Sinsabaugh, R. L., Moorhead, D. L., Xu, X., and Litvak, M. E.: Plant, microbial and ecosystem carbon use efficiencies interact to stabilize microbial growth as a fraction of gross primary production, New Phytol, 214, 1518–1526, 2017.

Sistla, S. A., Rastetter, E. B., and Schimel, J. P.: Responses of a tundra system to warming using SCAMPS: a stoichiometrically coupled, acclimating microbe–plant–soil model, Ecol Monogr, 84, 151–170, 2014.

Six, J., Frey, S., Thiet, R., and Batten, K.: Bacterial and fungal contributions to carbon sequestration in agroecosystems, Soil Sci Soc Am J, 70, 555–569, 2006.

Soares, M. and Rousk, J.: Microbial growth and carbon use efficiency in soil: Links to fungal-bacterial dominance, SOC-quality and stoichiometry, Soil Biol Biochem, 2019.

Spohn, M., Klaus, K., Wanek, W., and Richter, A.: Microbial carbon use efficiency and biomass turnover times depending on soil depth–Implications for carbon cycling, Soil Biol Biochem, 96, 74–81, 2016a.

Spohn, M., Pötsch, E. M., Eichorst, S. A., Woebken, D., Wanek, W., and Richter, A.: Soil microbial carbon use efficiency and biomass turnover in a long-term fertilization experiment in a temperate grassland, Soil Biol Biochem, 97, 168–175, 2016b.

Suseela, V. and Tharayil, N.: Decoupling the direct and indirect effects of climate on plant litter decomposition: Accounting for stress-induced modifications in plant chemistry, Global Change Biol, 24, 1428–1451, 2018.

Tang, J. and Riley, W. J.: Weaker soil carbon-climate feedbacks resulting from microbial and abiotic interactions, Nat Clim Change, 5, 56–60, 2015.

Thiet, R. K., Frey, S. D., and Six, J.: Do growth yield efficiencies differ between soil microbial communities differing in fungal: bacterial ratios? Reality check and methodological issues, Soil Biol Biochem, 38, 837–844, 2006.

Walker, T. W., Kaiser, C., Strasser, F., Herbold, C. W., Leblans, N. I., Woebken, D., Janssens, I. A., Sigurdsson, B. D., and Richter, A.: Microbial temperature sensitivity and biomass change explain soil carbon loss with warming, Nat Clim Change, 8, 885, 2018.

Wallenstein, M., Allison, S. D., Ernakovich, J., Steinweg, J. M., and Sinsabaugh, R.: Controls on the temperature sensitivity of soil enzymes: a key driver of in situ enzyme activity rates, in: Soil enzymology, pp. 245–258, Springer, 2010.

Ward, S. E., Orwin, K. H., Ostle, N. J., Briones, M. J., Thomson, B. C., Griffiths, R. I., Oakley, S., Quirk, H., and Bardgett, R. D.: Vegetation exerts a greater control on litter decomposition than climate warming in peatlands, Ecology, 96, 113–123, 2015.

Wickham, H.: ggplot2: Elegant Graphics for Data Analysis, Springer-Verlag New York, http://ggplot2.org, 2009.

Wieder, W., Grandy, A., Kallenbach, C., and Bonan, G.: Integrating microbial physiology and physio-chemical principles in soils with the MIcrobial-MIneral Carbon Stabilization (MIMICS) model, Biogeosciences, 11, 3899–3917, 2014.

Wieder, W., Grandy, A., Kallenbach, C., Taylor, P., and Bonan, G.: Representing life in the Earth system with soil microbial functional traits in the MIMICS model, Geoscientific Model Development, 8, 1789–1808, 2015a.

Wieder, W. R., Bonan, G. B., and Allison, S. D.: Global soil carbon projections are improved by modelling microbial processes, Nat Clim Change, 3, 909, 2013.

Wieder, W. R., Allison, S. D., Davidson, E. A., Georgiou, K., Hararuk, O., He, Y., Hopkins, F., Luo, Y., Smith, M. J., Sulman, B., et al.: Explicitly representing soil microbial processes in Earth system models, Global Biogeochemical Cycles, 29, 1782–1800, 2015b.

Xu, X., Thornton, P. E., and Post, W. M.: A global analysis of soil microbial biomass carbon, nitrogen and phosphorus in terrestrial ecosystems, Global Ecol Biogeogr, 22, 737–749, 2013.

Ye, J., Bradford, M. A., Dacal, M., Maestre, F. T., and García-Palacios, P.: Increasing microbial carbon use efficiency with warming predicts soil heterotrophic respiration globally, Global Change Biol, https://doi.org/doi: 10.1111/gcb.14738, 2019.

Zak, D. R., Ringelberg, D. B., Pregitzer, K. S., Randlett, D. L., White, D. C., and Curtis, P. S.: Soil microbial communities beneath Populus grandidentata grown under elevated atmospheric CO2, Ecol Appl, 6, 257–262, 1996.

Zheng, Q., Hu, Y., Zhang, S., Noll, L., Böckle, T., Richter, A., and Wanek, W.: Growth explains microbial carbon use efficiency across soils differing in land use and geology, Soil Biol Biochem, 128, 45–55, 2019.

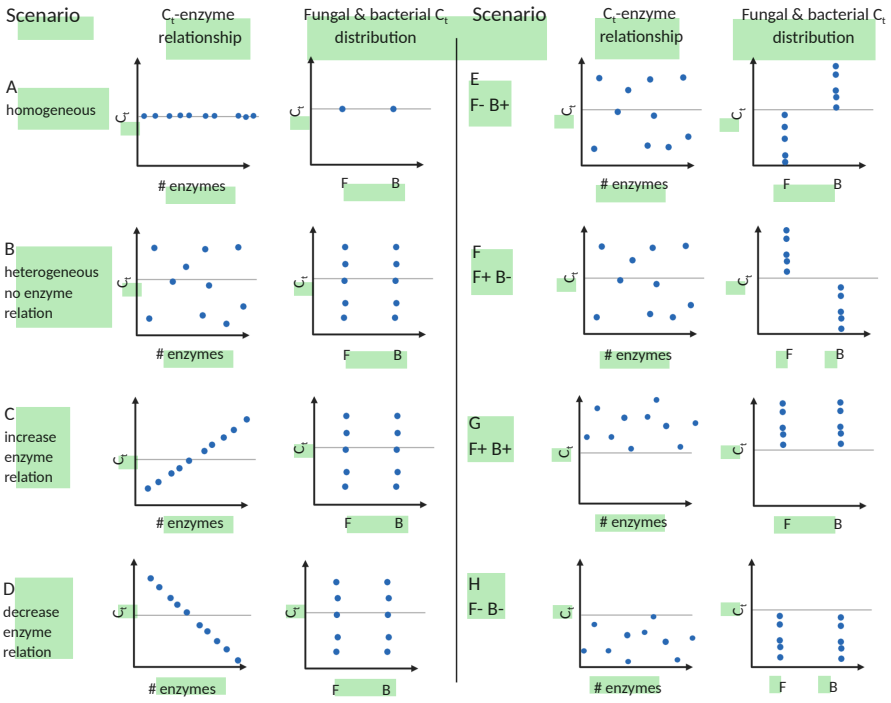


**Figure 1.** Schematic of experimental design used in this study, where CUE temperature response ($C_t$) varies as a function of the number of enzymes and/or taxonomic affiliation of organisms. Graphs show the effects of having homogeneous (A) or heterogeneous (B) $C_t$ across taxa; the effect of forcing a positive ("increase";C) or negative ("decrease";D) correlation between the number of enzymes and $C_t$; bacteria showing a positive $C_t$ and fungi a negative $C_t$ (E); fungi showing an positive $C_t$ and bacteria a negative $C_t$ (F); or fungi and bacteria both having positive (G) or negative (H) $C_t$. Each point represents the combination of traits one of ten taxa in the model might have, although 100 taxa were actually used in the simulations. Horizontal dashed lines indicate a $C_t$ of zero, and clusters of points above and below this line denote when CUE tends to increase or decrease with increasing temperature. The letters F and B in the x-axis of individual graphs denote sensitivities for fungi and bacteria, respectively. The trait values for different mock taxa may not be visible if the values for the traits on the two axes are identical. Figure prepared in BioRender

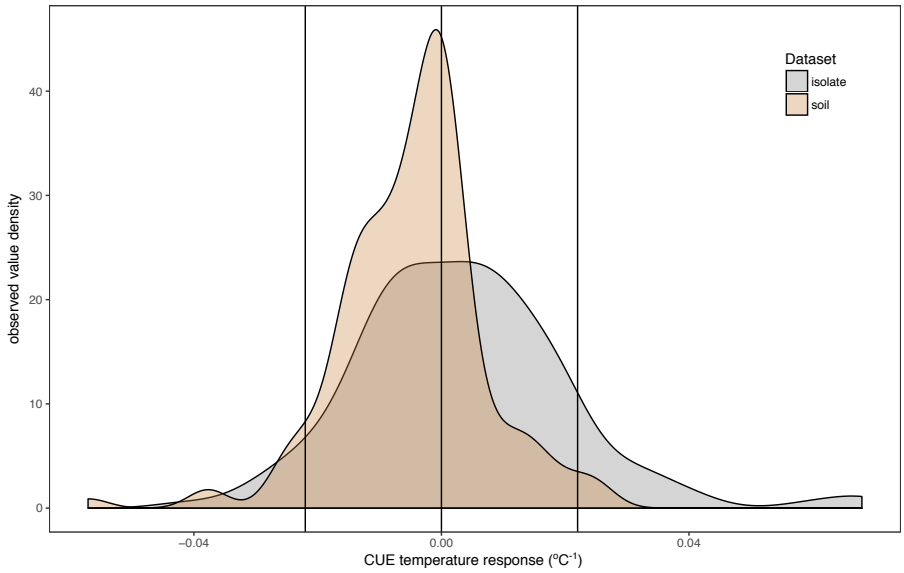


**Figure 2.** Density plot of observed CUE temperature response for 23 soil bacterial isolates grown between 15 and 25°C on four different liquid media types in the lab (n=160 datapoints, grey), and for soil microbial communities grown with various different substrates and temperatures based on a literature search (n=141 datapoints, brown). Vertical lines are placed at 0 (no change in CUE with temperature) as well as the +/-0.022 $^{o}C^{-1}$ upper and lower limits used in the present study. Contributing datapoints are primarily derived from Qiao et al. (2019) and can be found in the "Ct_literatureValues.txt" file in OSF.

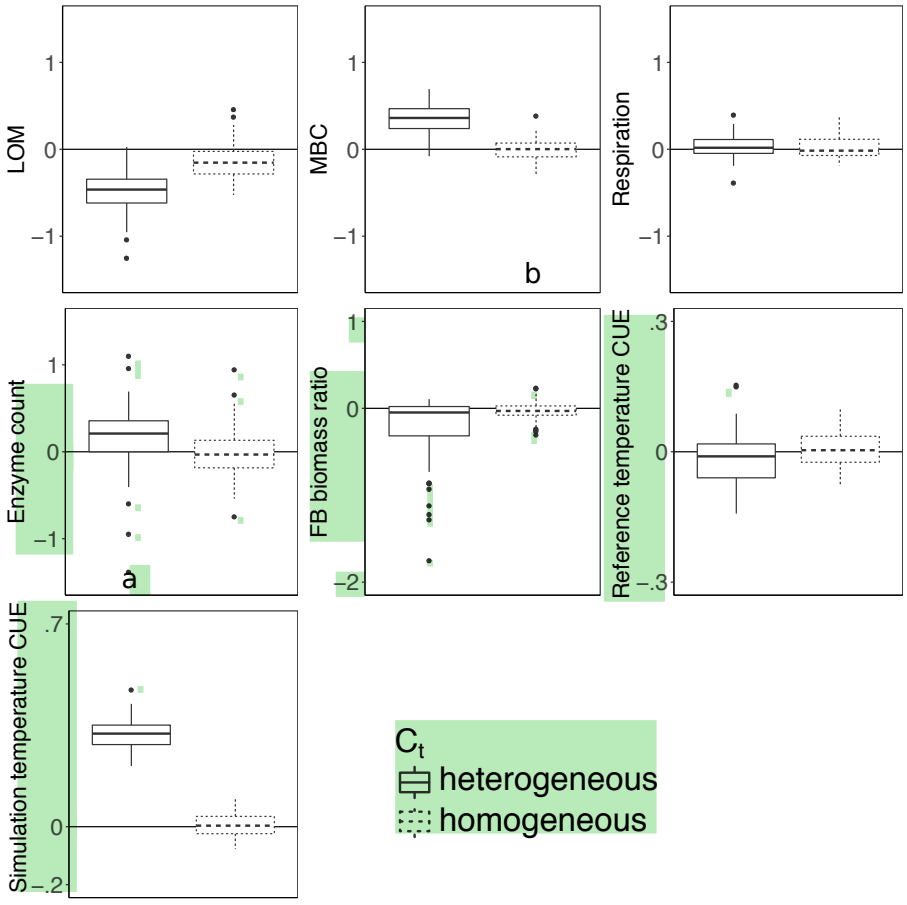


**Figure 3.** Effect of warming 5°C on C stocks and flows in simulations, reported as the natural log of the ratio of values in heated compared to control conditions. CUE temperature response either varied between taxa ("heterogeneous") or took on the fixed cross-sample mean temperature response of zero. Values above the zero line indicate warming increased the value, and values below indicate a decrease with warming. Boxplots denote 1st to 3rd quartiles with the median. Asterisks denote significant warming effect based on a paired Wilcoxon test at Bonferoni-corrected $P < 0.0001$. Letters denote warmed scenarios which are significantly different from one-another by the same criteria. "Reference temperature CUE" denotes the ratio of CUE for the surviving community at 15°C in warmed to the ratio of CUE for the surviving community at 15°control scenarios, while "Simulation temperature CUE" denotes the ratio of CUE in heated scenarios run at 20°C to control scenarios run at 15°C.

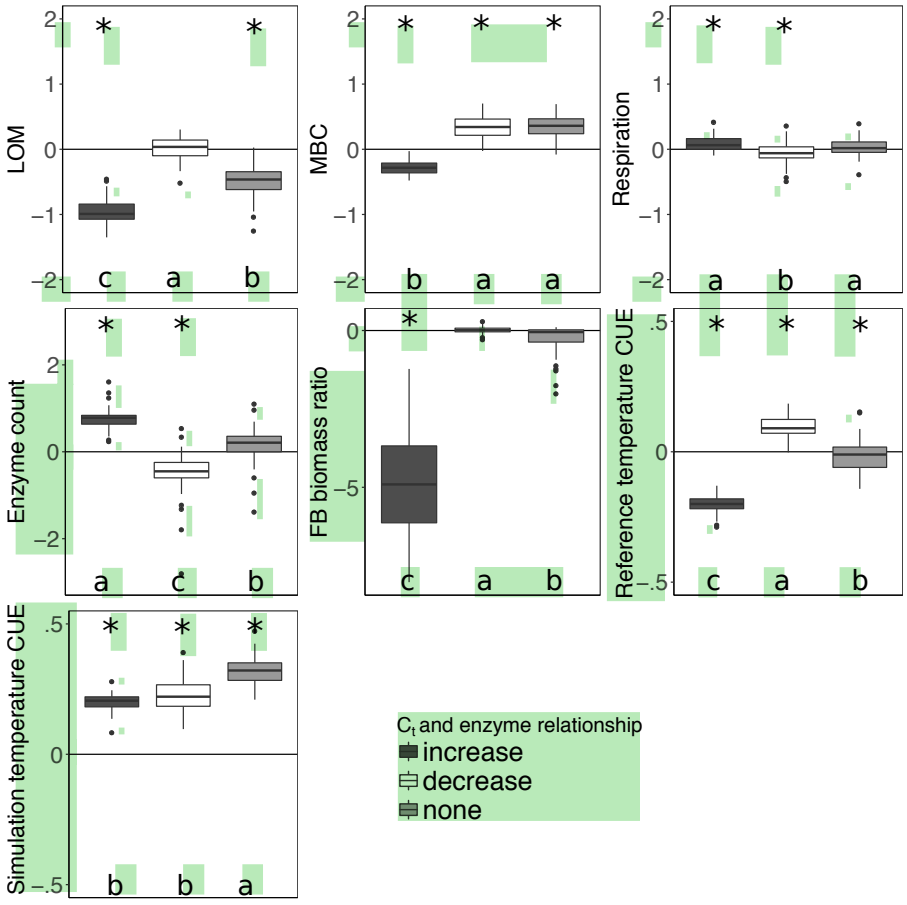


**Figure 4.** Effect of warming 5°C on C stocks and flows in simulations, reported as the natural log of the ratio of values in heated compared to control conditions. CUE temperature response was forced to increase, decrease, or remain independent of the number of enzymes a taxon could produce. Values above the zero line indicate warming increased the value, and values below indicate a decrease with warming. Boxplots denote 1st to 3rd quartiles with the median. Asterisks denote significant warming effect at $P < 0.0001$ after correcting for multiple comparisons using the Bonferoni method. Letters denote warmed scenarios which are significantly different from one-another by the same criteria. "Reference temperature CUE" denotes the ratio of CUE for the surviving community at 15°C in warmed to the ratio of CUE for the surviving community at 15°control scenarios, while "Simulation temperature CUE" denotes the ratio of CUE in heated scenarios run at 20°C to control scenarios run at 15°C.

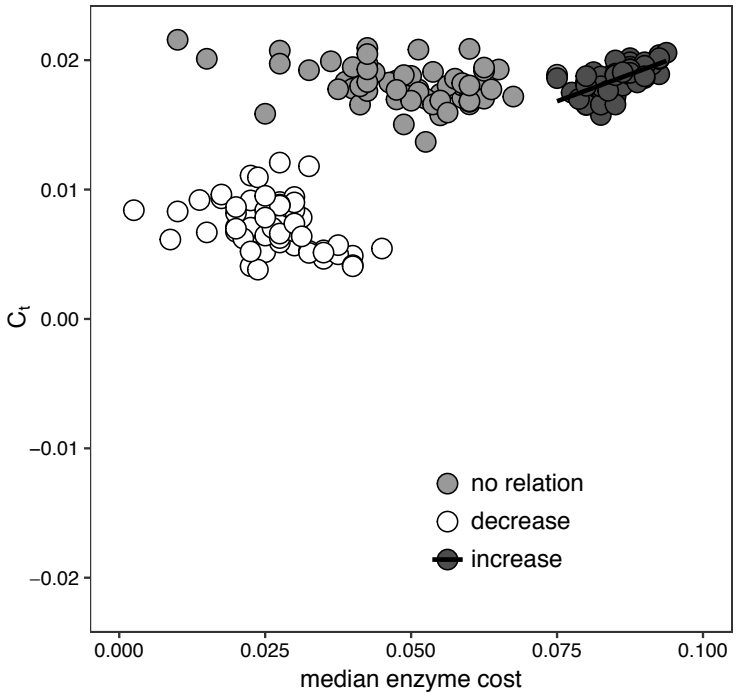


**Figure 5.** Relationship between the enzyme cost and temperature sensitivity of surviving taxa when the relationship between $C_t$ and enzyme count is set to be positive (increase), negative (decrease), or non-existent. Each point represents the value for the median across all surviving taxa for one of 59 starting communities.

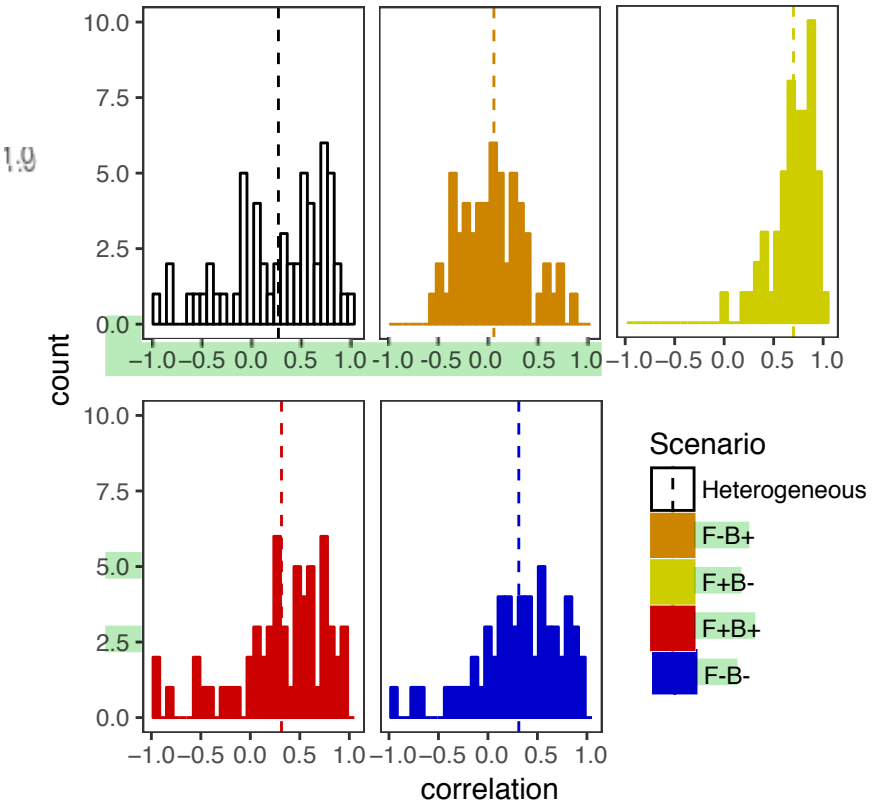


**Figure 6.** Frequency histograms of Pearson correlation coefficients between the number of enzymes taxa which survive to the end of the 6000 day run and their $C_t$. Each panel corresponds to a different scenario shown elsewhere in the manuscript, with the “counts" in each bar corresponding to a single starting seed (community) under that scenario. Vertical lines denote the mean correlation coefficient for the scenario.

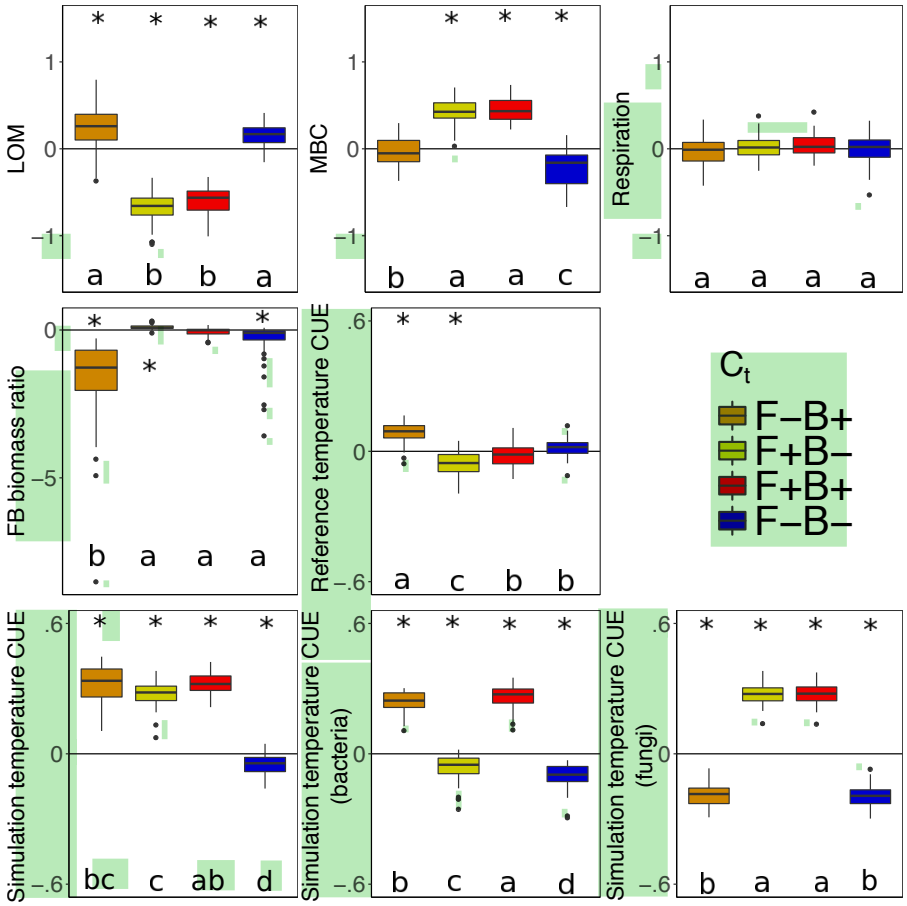


**Figure 7.** Effect of 5°C of warming on components of the litter C cycle when bacteria and fungi show dissimilar (F-B+ (orange), F+B- (yellow)) or similar (F+B+ (red), F-B- (blue)) CUE responses to temperature. Values above the zero line indicate warming increased the value (log ratio positive), and values below indicate a decrease with warming. Boxplots denote 1st to 3rd quartiles with the median. Asterisks denote significant warming effect at $P < 0.0001$ after correcting for multiple comparisons using the Bonferoni method. Letters denote warmed scenarios which are significantly different from one-another by the same criteria. "Reference temperature CUE" denotes the ratio of CUE for the surviving community at 15°C in warmed to the ratio of CUE for the surviving community at 15°control scenarios, while "Simulation temperature CUE" denotes the ratio of CUE in heated scenarios run at 20°C to control scenarios run at 15°C. Values for "simulation temperature CUE" are also shown for bacteria and fungi separately.

**Table 1.** CUE-related model parameters mentioned in this paper. The complete set of parameters can be found in the params.txt file in the supplement.

| Parameter | Value | Units | Description | Reference |
|---|---|---|---|---|
| $C_r$ | 0.38 | dimensionless | CUE at 15$^o$C for a taxon with no transporters or enzymes | this paper |
| $C_e$ | -0.0025 | enzyme$^{-1}$ | change in CUE per extracellular enzyme gene | Allison, 2014 |
| $C_u$ | -0.0071 | transporter$^{-1}$ | change in CUE per transporter gene | Allison, 2014 |
| $C_t$ | -0.022 to 0.022 | $^o$C$^{-1}$ | change in CUE per degree change in temperature from 15$^o$C | this paper |

**Table 2.** Median (or median-standardized interquartile range (IQRm)) output values for various DEMENT model runs, marked according to warming effect (+/-) and model structure effects (letters) determined using Bonferoni-corrected post-hoc tests following linear mixed effect models. Symbols: "+" warming increased value; "-" warming decreased value). Letters: differences between warmed scenarios. All heated scenarios were compared to values in the "control" column to determine the warming effect. Only values within boxes defined by vertical lines were evaluated for significance differences of warming scenario, as different seeds needed to be excluded for failing to constrain litter accumulation in the two boxes. NA indicates that fungi died out completely in many instances (i.e. median fungal biomass of zero), so the parameter output could not be determined. The number in brackets denotes the median excluding the scenarios where all the fungi died out. "Excluded runs" denotes the number of runs removed from analysis due to unconstrained litter C accumulation

| | NON-HEATED | CUE heterogeneity | | CUE-Enzyme relationship | | Fungi and bacteria differ | | | |
|---|---|---|---|---|---|---|---|---|---|
| Fig.1 scenario | NA | A | B | C | D | E | F | G | H |
| $C_t$ ($^oC^{-1}$) | NA | 0 | -0.022 to 0.022 | -0.022 to 0.022 | -0.022 to 0.022 | -0.022 to 0 (F)<br>0 to 0.022 (B) | -0.022 to 0 (B)<br>0 to 0.022 (F) | 0 to 0.022 | -0.022 to 0 |
| enz_cost/ $C_t$ relationship | NA | ind | ind | pos | neg | ind | ind | ind | ind |
| LOM IQRm | 0.2 | 0.14 | 0.28 | 0.33 | 0.15 | 0.39 | 0.29 | 0.23 | 0.3 |
| MBC IQRm | 0.16 | 0.16 | 0.25 | 0.02 | 0.24 | 0.14 | 0.16 | 0.15 | 0.19 |
| Taxon richness | 22 | 23a | 13b- | 22a+ | 14b- | 19a- | 11b- | 19a | 13b- |
| Enzyme count | 16 | 16b | 20.5b | 34.5a+ | 10.8c- | 17.5a | 20.5a+ | 19a | 18a |
| Shannon's H | 1.81 | 2.01a | 1.58b- | 2.11a+ | 1.31b- | 1.93a | 1.21c- | 1.61a | 1.64a- |
| MBC (mg $cm^{-3}$) | 20.9 | 21.0b | 30.5a+ | 15.6c- | 29.3a+ | 20.3b | 33.0a+ | 32.3a+ | 17.2c- |
| LOM (mg $cm^{-3}$) | 583.2 | 496.0b- | 361.5c- | 220.1d- | 603.2a | 746.0a+ | 300b- | 334.6b- | 706.5a+ |
| CUE at 15°C | 0.23 | 0.23b | 0.23b | 0.19c- | 0.25a+ | 0.25a+ | 0.22c- | 0.23b | 0.23b |
| CUE at 20°C | NA | 0.23c | 0.32a+ | 0.28b+ | 0.29b+ | 0.32bc+ | 0.31c+ | 0.32ab+ | 0.22d- |
| FB biomass ratio | 0.86 | 0.84b | 0.81b- | 0.00c- | 0.88a | 0.12b- | 0.96a+ | 0.85a | 0.73a- |
| Respiration (mg $cm^{-3}$ $day^{-1}$) | 0.93 | 0.94b | 0.96ab | 0.99a+ | 0.88c- | 0.91a | 0.94a | 0.96a | 0.94a |
| FB richness ratio | 0.45 | 0.48a | 0.44a | 0.00b- | 0.50a | 0.14c- | 0.67a+ | 0.44b | 0.40b |
| CUE bacteria | 0.27 | 0.27b | 0.26c- | 0.18d- | 0.36a+ | 0.35b+ | 0.26c- | 0.36a+ | 0.25d- |
| CUE fungus | 0.22 | 0.23c | 0.31a+ | NA(0.28)b+ | 0.28b+ | NA(0.18)b- | 0.30a+ | 0.29a+ | 0.18b- |
| Excluded runs | 2 | 1 | 3 | 4 | 0 | 0 | 0 | 1 | 5 |