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Peer Review File

Microbial dormancy under freeze–thaw cycling regulates alpine soil responses to warming

Corresponding Author: Professor Gangsheng Wang

This manuscript has been previously reviewed at another journal. This document only contains information relating to versions considered at Communications Earth & Environment.

This file contains all editorial decision letters in order by version, followed by all author rebuttals in order by version.

Attachments originally included by the reviewers as part of their assessment can be found at the end of this file.

Version 0:

Decision Letter:

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Dear Professor Wang,

Your manuscript titled "Microbial dormancy under freeze–thaw cycling regulates alpine soil responses to warming" has now been seen by 2 reviewers, whose comments are appended below. You will see that they find your work of some potential interest. However, they have raised substantial concerns that must be addressed. In light of these comments, extensive revisions will be required before we can further consider the manuscript for publication. We would, however, be interested in considering a revised version that fully addresses these serious concerns.

In the revision, please follow the editorial thresholds:
* Provide a compelling analysis of how microbial dormancy regulates alpine soil responses to warming during freeze-thaw cycling by using an advanced process-based model, including clear explanations of the new model advancements and fully considering uncertainties in data and the model, as raised by reviewer #1
* Clearly describe how the model is developed and simulate.

We hope you will find the reviewers' comments useful as you decide how to proceed. If additional work allows you to either incorporate or refute these criticisms, we will be happy to look at a substantially revised manuscript. If you choose to take up this option, please either highlight all changes in the manuscript text file, or provide a list of the changes to the manuscript with your responses to the reviewers.

When resubmitting, please provide a point-by-point response to the reviewers' comments. Please submit your responses as a separate file, distinct from your cover letter where you can add responses to the Editors' comments that you do not want to be made available to the reviewers. Word files are preferred. We recommend that any figures, tables or graphs that are included in the response to reviewers are also included in the main article or Supplementary Information.

Please bear in mind that we will be reluctant to approach the reviewers again in the absence of substantial revisions.

If the revision process takes significantly longer than three months, we will be happy to reconsider your paper at a later date, as long as nothing similar has been accepted for publication at Communications Earth & Environment or published elsewhere in the meantime.

We are committed to providing a fair and constructive peer-review process. Please do not hesitate to contact us if you wish to discuss the revision in more detail.

Please use the following link to submit your revised manuscript, point-by-point response to the reviewers' comments with a list of your changes to the manuscript text (which should be in a separate document to any cover letter), a tracked-changes version of the manuscript (as a PDF file) and any completed checklist:

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Please do not hesitate to contact us if you have any questions or would like to discuss the required revisions further. Thank you for the opportunity to review your work.

Best regards,

Guopeng Liang,PhD
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REVIEWER COMMENTS:

Reviewer #1 (Remarks to the Author):

In this study, the authors developed a freeze–thaw-enabled microbial-ecological model and integrate it with multi-year whole-soil warming experiments in an alpine meadow on the Qinghai-Tibetan Plateau where freeze–thaw cycles frequently happen to investigate warming effects on soil carbon dynamics. The authors found that their model could reproduce the observed soil carbon dynamics, and they found the minimal enzyme responses and seasonal microbial activity shifts via dormancy regulation. Also, they reported persistent declines under warming of carbon use efficiency. Their long-term projections (50 years) suggested soil carbon loss due to enhanced microbial biomass and oxidase activity. Overall, this manuscript is well-written, sufficient in data collection, and well-organized in results display. However, some issues can be found as below.

Major concerns

1. The authors claimed that this study features to investigate warming effects on soil carbon dynamics from genes to ecosystems. But it is unclear: 1) What genetic data were used in this study? 2) How were genetic data integrated with the model? 3) And from genes to ecosystems, scaling up is an important concept to be considered, how did the authors address that issue? The authors may want to clarify.
2. GPP data were used to force the model. Based on the model framework in figure 1, litter from aboveground and belowground of vegetation serve as the source of carbon input as litter. GPP was replaced as the forcing data may call this study into some questions: 1) GPP is not equal to biomass, did the authors do any work on investigating the empirical associations between productivity and biomass and then litter production? 2) Warming may change the litter quality by altering vegetation community composition, nutrient uptake, etc., did the authors examine the litter quality responses to warming? Since that will affect POM and MOM production based on the model framework. 3) Warming can also change carbon allocation of plants, that will affect carbon inputs into the ecosystem and further the fate of carbon components. Did

the authors investigate warming effects on plant allocation in the community? And how did you account for that? The authors may want to explain and make it more specific in methods description.
3. The authors acknowledged that the model used in this study is available in GitHub at https://github.com/wanggangsheng/MEND. However, the latest update was found 3 years ago, no model contents about freeze-thaw cycle were found. So, it is not like the model versions used in this study, the authors may want to update the GitHub repository or the source of model codes available.
4. The microbial responses and their regulating role were reported as a key role in soil carbon dynamics to warming with freeze-thaw cycles, but DOC was not found in the analysis or discussion, although the increases in MBC or substrate depletion were mentioned important in this study for their long-term response patterns. In addition, during model calibration and validation phases, MBC was overestimated under warming in 2019, but it was largely underestimated under both control and warming in 2021. Why is that? And inorganic nutrients were overall underestimated. Why? Are they related to physical fragmentation and release of soluble carbon and nutrients that not captured by the model? Please explain.
5. The authors conducted long-term simulation of 50 years to project soil and microbial responses to warming using the new model they developed. However, it is unclear: 1) What are the forcing data? And what years are used for analysis? Any considerations of vegetation and microbial communities shifts under warming in the long term? 2) MBC increased under warming, why is that? What is the response of DOC to warming? Is the increase of MBC due to parameterization? Because MBC under warming was largely overestimated compared with control in 2019. Additionally, the authors came to the conclusion that “Long-term projections reconceptualize soil carbon loss (–2.2%) as arising primarily from enhanced microbial biomass and oxidase activity, reframing carbon decline as a consequence of microbial physiological adaptation rather than substrate depletion alone.” How come the carbon decline as a consequence of microbial physiological adaptation rather than substrate depletion alone? I do not think the results in this study support this conclusion. As I mentioned earlier, it is unclear whether vegetation and microbial shifts were considered in long-term project, and how microbial physiological adaptation was considered in the model was unclear. And DOC was not included in the analysis, how did the authors claim that substrate depletion was also responsible for soil carbon response to warming in the long term?

Minor points
L. 320-444: What statistical analysis approached were used in this study? And what variables were used to force the model? And what are their durations?

Reviewer #2 (Remarks to the Author):

This manuscript presents a timely study that examines how freeze–thaw cycles and microbial dormancy influence soil C processes in alpine systems. Extending MEND model to MEND-FT by incorporating Freeze-Thaw (FT) influenced microbial dormancy and testing it against field warming experiments on the Qinghai–Tibet Plateau and found the new model performed better on simulating microbial physiology and showed increased warming effects in non-growing season adds great value in understanding how microbial processes regulate alpine soils to warming. The paper is clearly written, and results show are noteworthy. My major concern is that the authors did not clearly explain the linkages between the added new freeze–thaw–dormancy processes in the model and the enhanced performance on CUE, enzyme activity, and Ninorg, etc. I recommend the manuscript to be published after addressing the following major and minor comments I have:

Major: The manuscript would benefit from a clearer mechanistic explanation linking the newly implemented freeze–thaw–dormancy processes to the model’s improved performance in simulating microbial CUE and enzyme production. It is not clear why MEND-FT predicts a lower CUE than MEND-old (Fig. 4a), or why MEND-FT captures the observed decrease in enzyme activity in August 2021 while generally predicting higher enzyme activity under warming elsewhere. Throughout the manuscript, the authors emphasize that model outputs align with observations, but they do not explain the underlying mechanisms driving this agreement. As this is a modeling study, I encourage the authors to present key intermediate variables or fluxes to illustrate how the new processes translate into these outcomes and thereby clarify the causal chain within the model.

Line 22: This work doesn’t seem to involve any genes data, or am I missing something here?

Line 51: “substrate availability for decomposition” is not essentially physical soil process, but a biological one.

Line 74: Does the approach/framework in this manuscript incorporates genes data?

Line 115-131, How are all these objects weighted in the multi-object weighted calibration, I could not find the values of the different weights wi in the Supplementary Info. Have the authors tested alternative weighting schemes to evaluate sensitivity? The uncertainties or rationale for these weights should be discussed, at least in the Supplementary Information.

Fig. 3, For MBC and Ninorg, why is the results in 2020 not shown here and why only Augst? Is that the only data found in that existing literature? The authors should state the data availability and span in the “Study site and data compilation” section in the Methods without readers having to going back to the literature to check.

Fig. 4, why is only one month of one year (August 2021) was included in here? Is it because you only had microbial data in that particular month? The authors referred all the microbial data they compared to into one sentence from line 437 to 439, they should be clearer about which months and years are the data available there.

Fig. 5a showed that the rMBA on the rise most of the time during the experimental years even in control scenarios, which left me wondering if the model is not spun up to steady state. Could the authors provide any info on the model spin-up and steady state in the setup?

In Fig. 5b, rMBA seems to decrease with warming in the short-term experiment, yet in the long-term warming projection (Fig. 5e) it slightly increases. The authors should discuss why the short- and long-term responses differ.

Also again why in Fig.5c-d, Why are only one thaw month and one freeze month shown in Fig. 5c–d? Even if patterns across different years' thaw-freeze months are similar, the authors should consider aggregating all thaw/freeze months or moving other months' results to Supplementary Figures. Also, please clarify in the caption whether the dots represent daily modeled values.

Line 204-210, it's unclear and underexplored here, why adding the freeze-thaw dormancy leads to less enzyme production. Here the authors explained as "This improvement is due to the model's representation of enzyme production depending on microbial biomass and substrate availability", but I believe both MEND_old and MEND_FT has this representation. The difference seems to stem from MEND-FT simulating less microbial biomass under warming (Fig. 5b), but the mechanism behind this remains unexplained. Is it due to reduced CUE with warming in MEND-FT? And if so, why does this occur in MEND-FT but not in MEND-old?

Moreover, the decline in enzyme production appears mainly in the growing season, while oxidative enzyme activity increases during thaw and frozen months (Fig. 5). The authors should clarify why enzyme responses differ across seasons and, if possible, show intermediate variables or model outputs to illustrate these contrasting behaviors.

Line 227-228, It is not clear how the model produces enhanced N mineralization after adding the FT cycle. Both microbial biomass and rMBA decrease with MEND-FT, so how can a smaller active biomass lead to greater mineralization? The authors should examine additional model outputs to clarify this mechanism and, if possible, include supplementary figures showing the relevant processes or fluxes that drive this result.

Line 239-244, again it is unclear why the new model achieved high CUE here compared to the MEND_old.

Line 278-279, Finally the authors explained here the possible reasons for CUE declined, but since this is a modeling study, the authors should be able to track all the changes to show exactly what occurred to decrease the microbial CUE.

Line 287-289 Does the MEND-FT model include a dynamic, C-targeting enzyme allocation scheme? What mechanisms allow it to capture the relatively stronger increase in oxidative versus hydrolytic enzymes under warming, while the MEND-old can't?

Line 305-307 Did the authors also conduct long-term projections using MEND-old? If so, did MEND-old predict similar trends in microbial biomass and enzyme activities? Presenting both would clarify whether the freeze–thaw mechanism truly alters long-term outcomes. At least a summary in Supplementary Information would be valuable.

Line 330-363: The use of ALTscalar suggests that the model represents soil as a single layer, with freeze–thaw effects scaled by the thawed depth fraction. Could this simplification introduce uncertainties compared to a vertically resolved soil model? Would a multi-layer implementation, with microbial dormancy driven directly by depth-dependent temperature and moisture, produce similar outcomes?

Fig. S3a There is no error bar here?

Table S7 This table clarifies a bit why many figures show data for only one or two months, but it is never cited in the main text or figure captions. Please reference it explicitly. It also highlights large differences in data frequency between microbial and SOC observations, which may affect the weighting in the multi-objective calibration, which the authors should discuss. Plus, the authors should discuss the uncertainties from relying on such limited microbial data for model calibration and validation.

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Decision Letter:

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Dear Professor Wang,

Your manuscript titled "Microbial dormancy under freeze–thaw cycling regulates alpine soil responses to warming" has now been seen by our reviewers, whose comments appear below. In light of their advice we are delighted to say that we are happy, in principle, to publish a suitably revised version in Communications Earth & Environment.

We therefore invite you to revise your paper one last time to address the remaining concerns of our reviewers. At the same time we ask that you edit your manuscript to comply with our format requirements and to maximise the accessibility and therefore the impact of your work.

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We hope to hear from you within two weeks; please let us know if you need more time.

Best regards,

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Mengjie Wang
Associate Editor, Communications Earth & Environment
Consulting Editor, Communications Sustainability

REVIEWERS' COMMENTS:

Reviewer #2 (Remarks to the Author):

This manuscript presents a timely and valuable study examining how freeze–thaw cycles and microbial dormancy regulate soil carbon processes in alpine ecosystems. The authors have undertaken a very careful and thorough revision in response to my previous comments and provided clear, point-by-point responses to all my questions. I appreciate the substantial effort that went into this revision. In particular, the clarification of model assumptions and calibration processes, the much-improved discussion of microbial physiological responses simulated by the old and the new model, and the expanded explanations of the freeze–thaw–dormancy mechanisms have strengthened both the transparency and interpretability of the work.

Overall, I find that the authors have addressed my previous concerns, and the manuscript now presents a clear mechanistic framework linking freeze–thaw dynamics, microbial dormancy, and soil carbon responses to warming. The integration of experimental observations with the MEND-FT model provides a meaningful contribution to understanding cold-region carbon cycling and its sensitivity to climate change. I am pleased to see the manuscript strengthened through revision and only have two very minor comments for the authors to consider before publication:

Minor comments:
1. Figures S7 and S9 appear to have visible outline artifacts around the panels. Please check the export/rendering settings to ensure consistent formatting.
2. In the Discussion (Lines 370–376), the text suggests that reductions in freeze months are less pronounced than those in thaw months. Based on the figure, both appear to show small net reductions, and the relative difference is not visually clear. Please clarify or quantify this comparison.

With these minor issues addressed, I recommend the manuscript for publication.

Microbial dormancy under freeze–thaw cycling regulates alpine soil responses to warming

(COMMSENV-25-4161)

Response to Comments from Reviewers

We have addressed the reviewers' comments and questions point-by-point. The original reviewer's comments are *italicized*, and our Response to the reviewer's comments follow. The numbers of lines and pages in the text are referred to the revised version.

A. Response to Reviewer #1:

A1. *In this study, the authors developed a freeze–thaw-enabled microbial-ecological model and integrate it with multi-year whole-soil warming experiments in an alpine meadow on the Qinghai-Tibetan Plateau where freeze–thaw cycles frequently happen to investigate warming effects on soil carbon dynamics. The authors found that their model could reproduce the observed soil carbon dynamics, and they found the minimal enzyme responses and seasonal microbial activity shifts via dormancy regulation. Also, they reported persistent declines under warming of carbon use efficiency. Their long-term projections (50 years) suggested soil carbon loss due to enhanced microbial biomass and oxidase activity. Overall, this manuscript is well-written, sufficient in data collection, and well-organized in results display.*

Response: Thank you for the complimentary comments!

A2. *The authors claimed that this study features to investigate warming effects on soil carbon dynamics from genes to ecosystems. But it is unclear: 1) What genetic data were used in this study? 2) How were genetic data integrated with the model? 3) And from genes to ecosystems, scaling up is an important concept to be considered, how did the authors address that issue? The authors may want to clarify.*

Response: We appreciate the reviewer's insightful questions regarding the role of genetic information, model integration, and cross-scale interpretation. We clarify these points below:

(1) Genetic data used in this study

We acknowledge that no direct genetic (e.g., metagenomic) data were used in this study. This limitation reflects the lack of available molecular observations for the long-term field warming experiment analyzed here (Zhang et al., 2023; Chen et al., 2024). Instead, the model was calibrated and validated using multiple microbial and ecosystem-level observations, including enzyme activities, microbial carbon use efficiency (CUE), microbial biomass carbon (MBC), microbial C:N ratio, soil organic carbon (SOC), soil respiration, and inorganic N pools. These measurements provide direct constraints on microbial functional expression and ecosystem-scale carbon–nitrogen dynamics, even in the absence of explicit genetic

measurements.

(2) Integration of genetic data within the MEND framework

While genetic data are not used here, the MEND (Microbial-ENzyme Decomposition) modeling framework is designed to integrate such information when available. Prior studies have integrated microarray-based gene abundances into MEND by linking gene signals to modeled enzyme pools or activities, thereby using genetic data as quantitative indicators of microbial functional potential (Gao et al., 2020; Guo et al., 2020; Wang et al., 2022; Tao et al., 2024). This capability underpins the framework's extensibility, even though it was not activated in the current application.

In these prior applications, gene are not used individually, but are first aggregated into functional guilds corresponding to key biogeochemical processes, such as lignin- and cellulose-degrading enzymes for SOC decomposition; nitrogenases for N fixation; ammonia oxidases for nitrification; and nitrate, nitrite, nitric oxide, and nitrous oxide reductases for stepwise denitrification (Wang et al., 2022).

Importantly, genetic information is typically incorporated using response ratios (i.e., log-proportional changes in gene or guild-level abundances between treatments, such as warmed vs. control soils or elevated vs. ambient resource availability), rather than absolute gene abundances (Gao et al., 2020; Wang et al., 2022; Tao et al., 2024). These response ratios are used as quantitative indicators of treatment-induced shifts in microbial functional potential and are mapped onto corresponding enzyme-mediated process rates (e.g., SOC decomposition, nitrification, denitrification). This strategy reduces sensitivity to sequencing depth and site-specific background differences, while emphasizing treatment-induced functional shifts.

(3) Scaling from genes to ecosystems

We agree with the reviewer that scaling is a critical issue. In this manuscript, the phrase "from genes to ecosystems" refers to the scalable design of the modeling framework rather than the specific data streams used in this paper. Conceptually, MEND addresses scaling in three steps: (i) Process-level representation. The model explicitly represents microbial physiology and functional processes (e.g., enzyme production and activity, microbial dormancy and resuscitation,) at the process level. (ii) Propagation to ecosystem fluxes. These microbial-enzymatic dynamics are propagated to soil C-N pools and fluxes at the ecosystem scale through mass-balance and kinetic formulations. (iii) Optional molecular constraints. When available, genetic data can directly constrain enzyme-mediated processes, thereby strengthening mechanistic realism across scales.

(4) Manuscript revisions for clarification

To avoid misunderstanding, we have revised the manuscript to clarify this distinction between framework capability and data availability, as detailed below.

Abstract (Line 19–23, Page 2): "Here, we develop a freeze-thaw-enabled microbial ecological model (MEND-FT) and integrate it with multi-year whole-soil warming experiments in an alpine meadow on the Qinghai-Tibetan Plateau to investigate warming effects on soil carbon dynamics from microbial physiology to ecosystem processes."

Introduction (Line 72–78, Page 4): "Given the strong linkages between warming-induced microbial processes and carbon dynamics, a comprehensive approach integrating field observations, laboratory incubations, and process-based modeling is needed. Such a framework enables a mechanistic representation of microbial physiology at the process level, propagates these microbial-enzymatic dynamics to soil C-N pools and fluxes at the ecosystem scale through mass-balance and kinetic formulations, and allows genetic data, where available, to directly constrain enzyme-mediated processes, thereby strengthening mechanistic realism across scales (Gao et al., 2020; Wang et al., 2022; Tao et al., 2024)."

A3. *GPP data were used to force the model. Based on the model framework in figure 1, litter from aboveground and belowground of vegetation serve as the source of carbon input as litter. GPP was replaced as the forcing data may call this study into some questions: 1) GPP is not equal to biomass, did the authors do any work on investigating the empirical associations between productivity and biomass and then litter production? 2) Warming may change the litter quality by altering vegetation community composition, nutrient uptake, etc., did the authors examine the litter quality responses to warming? Since that will affect POM and MOM production based on the model framework. 3) Warming can also change carbon allocation of plants, that will affect carbon inputs into the ecosystem and further the fate of carbon components. Did the authors investigate warming effects on plant allocation in the community? And how did your account for that? The authors may want to explain and make it more specific in methods description.*

Response: Thank you for these detailed questions. We agree that GPP is not equal to biomass and we did not treat it as such. In our implementation, daily GPP data were used as the continuous carbon influx driver to provide the model with temporally resolved inputs, while the conversion from photosynthetic uptake to litter inputs was handled through an explicit transfer parameter rather than assuming GPP directly represents standing biomass.

(1) Empirical linkage among GPP, biomass, and litter production

We did not have plot-level time series of biomass, litterfall, root turnover, or rhizodeposition for the field warming experiment, so we could not derive a site-specific empirical association between productivity and biomass and then litter production. Instead, we followed a common practice in terrestrial ecosystem modeling: derive plant detrital inputs as a

fraction of assimilated carbon (GPP or NPP) and partition them into aboveground and belowground litter inputs, which then feed soil pools.

This design is explicit in several ecosystem models. For example, in TECO, carbon fixed by photosynthesis first enters a non-structural carbon pool, is then allocated to vegetation biomass pools, and litter inputs arise from biomass turnover (Weng and Luo, 2008). Likewise, DALEC represent carbon pools connected by fluxes, where carbon enters via GPP and is propagated through allocation and turnover pathways (Famiglietti et al., 2021). In DayCent, long-term SOC dynamics are driven by plant production and residue returns to soil, together with daily soil biogeochemical processes (Bista et al., 2024).

Given the missing site-level litter input measurements, we adopted a parsimonious prior to translate GPP into litter inputs. Specifically, we parameterized the plant-to-litter transfer using the synthesis constrains of Fan et al. (2023), who estimated that, on average, 48% of GPP is transferred to litterfall, partitioned into ~20.4% aboveground and ~27.7% belowground litterfall. Nonetheless, we acknowledge that a fixed mapping from GPP to litter inputs can overlook seasonal lags and environmental controls, and this limitation is expected to be more pronounced in ecosystems with large long-lived woody biomass pools (Fan et al., 2023).

(2) Warming effects on litter quality and consequences for POM/MOM formation

We agree that warming may influence litter quality and plant carbon allocation through shifts in species composition or nutrient uptake, which could alter the partitioning and quality of litter inputs and hence affect POM and MOM formation (Hong et al., 2021). However, site-level litter chemistry and trait data were not available for this experiment, so we could not explicitly represent warming-driven litter-quality shifts. We now state this limitation clearly and frame it as a priority for future experiment–model integration.

(3) Warming effects on plant carbon allocation

We also agree that warming may shift plant allocation (above vs. belowground), which would alter both the quantity and vertical distribution of carbon inputs to soil (Zhou et al., 2022). Because allocation or turnover observations were not available, we did not prescribe treatment-specific allocation changes. To represent treatment effects, we scaled the daily GPP time series by the observed annual NPP ratio between warming and control plots (Chen et al., 2024), thereby translating treatment-induced productivity differences into varying litter input. We have clarified this assumption and its implications in the Methods and Discussion (see below).

(4) Revisions in the manuscript

We have revised the Methods to clarify the forcing and transfer assumptions:

Methods (Line 602–608, Page 30): “We used daily GPP as a flux driver to represent the

temporal dynamics of photosynthetic carbon uptake. Daily litter carbon input was parameterized as a fraction of daily GPP based on the plant-to-litter transfer efficiency reported by Fan et al. (2023), and then partitioned into litter entry pools using the fractions in the model configuration. To represent treatment effects on carbon input, we scaled the daily GPP time series by the observed annual NPP ratio between warming and control plots (Chen et al., 2024), thereby translating treatment-induced productivity differences into varying litter input."

We also strengthened the Discussion to acknowledge limitations and future needs:

Discussion (Line 417–431, Page 21): "Uncertainties remain in quantifying carbon transfer from photosynthetic production to soil inputs. Using daily GPP as model forcing enables continuous carbon influx, but assuming a relatively constant relationship from GPP to litter input may overlook seasonal lags and environmental controls (Fan et al., 2023). Although evidence suggests that NPP can be close to litterfall under steady state and grasslands often show tighter coupling between productivity and turnover than forests (Fan et al., 2023), warming may still alter carbon transfer pathways by changing plant carbon allocation (Zhou et al., 2022) and litter quality (Hong et al., 2021), which could affect both quantity and biochemical composition of POM and MOM inputs. Moreover, belowground inputs may include rhizodeposition in addition to root turnover, the lack of site-level data on litter chemistry and allocation precludes an explicit representation of these processes. Future work combining long-term measurements of productivity, allocation, and litter decomposition are needed to better constrain these parameters and reduce associated uncertainties."

A4. *The authors acknowledged that the model used in this study is available in GitHub at https://github.com/wanggangsheng/MEND. However, the latest update was found 3 years ago, no model contents about freeze-thaw cycle were found. So, it is not like the model versions used in this study, the authors may want to update the GitHub repository or the source of model codes available.*

Response: Thanks for pointing this out. We have now updated the GitHub repository with the latest version of the MEND-FT model, which includes the freeze-thaw module. The updated code is available at https://github.com/wanggangsheng/MEND-FreezeThaw.git

A5. *The microbial responses and their regulating role were reported as a key role in soil carbon dynamics to warming with freeze-thaw cycles, but DOC was not found in the analysis or discussion, although the increases in MBC or substrate depletion were mentioned important in this study for their long-term response patterns. In addition, during model calibration and validation phases, MBC was overestimated under warming in 2019, but it was largely underestimated under both control and warming in 2021. Why is that? And inorganic nutrients were overall underestimated. Why? Are they related to physical fragmentation and release of*

soluble carbon and nutrients that not captured by the model? Please explain.

Response: We appreciate the reviewer's comment. DOC was not explicitly included in the analysis or discussion because direct DOC observations were not available for the study site. We acknowledge that substrate (e.g., DOC) availability is a key factor of microbial responses to warming, particularly under freeze-thaw conditions. In MEND model, this control is mechanistically represented through dissolved organic matter (DOM) pool, which governs microbial carbon and nitrogen uptake through a Michaelis-Meten formulation. Specifically, DOC control on microbial growth is regulated by relative substrate availability [φ = DOM/(DOM + KsDOM)], where and KsDOM is the half-saturation constant for microbial uptake. To better illustrate how warming and freeze–thaw cycles regulate microbial responses via substrate supply, we have new results showing warming-induced changes in φ (Supplementary Fig. S5, see below).

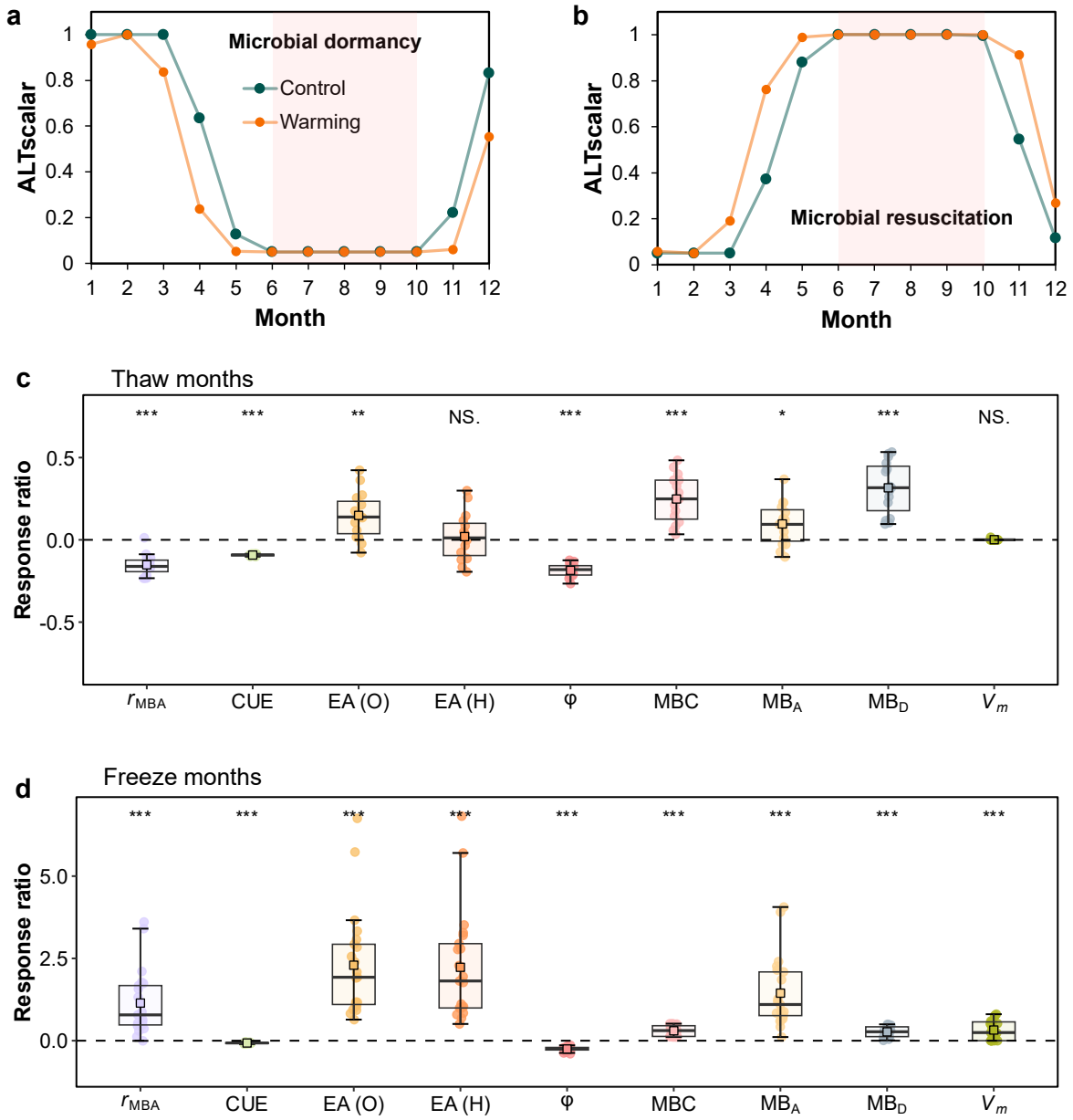

Fig. S5 | Freeze-thaw regulation (a–b) and simulated monthly microbial responses to warming across (c) all thaw-period months (June to October) and (d) freeze-period months (November to May). Variables include active fraction (r_{MBA}), microbial carbon use efficiency (CUE), enzyme activities (EA) of oxidases (O) and hydrolases (H), substrate availability (φ = DOM/(DOM + KsDOM)), microbial biomass carbon (MBC), active microbial biomass (MB_A) and dormant microbial biomass (MB_D), and biomass-specific maintenance rate (V_m). Differences between RR values and 0 were tested using the Wilcoxon test. "***" denotes a significance difference at $p < 0.01$; "NS." means not significant ($p > 0.05$).

We added the following text to describe warming-induced changes in φ:

Results (Line 193–200, Page 10): "In addition to microbial responses, relative substrate availability (φ = DOM/(DOM + KsDOM)) declined under warming during both thaw months (mean RR = −0.18) and freeze months (RR = −0.25) (Supplementary Fig. S5c, d). Despite concurrent changes in microbial activity and biomass pools, the reduction in φ indicates stronger substrate limitation on microbial uptake under warming. This pattern reflects the temperature sensitivity of the microbial uptake half-saturation constant (KsDOM), which increases under warming and thereby lowers effective substrate affinity and reduces φ for a given DOM level."

Regarding the mismatches in MBC and inorganic nutrients (N_{inorg}) during calibration and validation, we added the following explanation in the revised manuscript:

Discussion (Line 293–303, Page 15): "Both MBC and N_{inorg} were primarily used as low-frequency constraints to calibrate key model parameters (Table S7). In contrast, soil respiration constituted the most abundant observational constraint (i.e., 32 data points), far exceeding the only two observations for MBC and two for N_{inorg}. Consequently, parameter estimation was more strongly influenced by respiratory fluxes than by microbial or nutrient state variables. The overestimation of MBC under warming in 2019 and its underestimation under both control and warming treatments in 2021 likely reflect pronounced interannual variability in microbial responses (Liu et al., 2010), which are difficult to constrain when observations are sparse. Although these discrepancies (|PBIAS| = 16–76%), remain within ranges commonly considered acceptable in biogeochemical modeling (Wang et al., 2015; Cameron et al., 2022), they highlight the challenge of reproducing year-specific microbial dynamics using parameters constrained primarily by low-frequency observations."

Discussion (Line 304–313, Page 15–16): "Similarly, N_{inorg} (the sum of nitrate and ammonia) were overall underestimated. This bias likely reflects missing or simplified representations of rapid, pulse-driven nutrient release associated with physical fragmentation of soil aggregates, microbial cell lysis, and enhanced mineralization during freeze–thaw transitions (Song et al., 2017). Such processes can generate short-lived but substantial increases in inorganic N that are difficult to capture without high-frequency nutrient measurements. While the MEND model represents biologically mediated mineralization pathways, it does not explicitly resolve physical disruption processes, which may partly explain the underestimation of inorganic nutrients. Nevertheless, the magnitude of bias (|PBIAS| = 15–72%) remains within the generally acceptable bias threshold of 70% for N modeling (N. Moriasi et al., 2007). "

A6. *The authors conducted long-term simulation of 50 years to project soil and microbial responses to warming using the new model they developed. However, it is unclear: 1) What are the forcing data? And what years are used for analysis? Any considerations of vegetation and microbial communities shift under warming in the long term? 2) MBC increased under warming, why is that? What is the response of DOC to warming? Is the increase of MBC due to parameterization? Because MBC under warming was largely overestimated compared with control in 2019. Additionally, the authors came to the conclusion that "Long-term projections reconceptualize soil carbon loss (−2.2%) as arising primarily from enhanced microbial biomass and oxidase activity, reframing carbon decline as a consequence of microbial physiological adaptation rather than substrate depletion alone." How come the carbon decline as a consequence of microbial physiological adaptation rather than substrate depletion alone? I do not think the results in this study support this conclusion. As I mentioned earlier, it is unclear whether vegetation and microbial shifts were considered in long-term project, and how microbial physiological adaptation was considered in the model was unclear. And DOC was not included in the analysis; how did the authors claim that substrate depletion was also responsible for soil carbon response to warming in the long term?*

Response: Thank you for pointing these out. In response, we have substantially clarified the design, scope, and interpretation of the 50-year simulations, and we have revised overly strong causal statements to better reflect what is supported by the model results.

(1) Forcing data, analysis period, and treatment of vegetation and microbial shifts.

We have clarified the long-term simulation design as follows:

Methods (Line 624–634, Page 31): "Unlike previous studies that applied time-invariant forcing to drive long-term scenario analyses (Allison et al., 2010; Hagerty et al., 2014), we repeated the observed daily forcing from 2018 to 2021 as a cyclic sequence to preserve both seasonal and interannual variability in the long-term (50-year) simulations (Ekici et al., 2014). To minimize transient effects associated with initial conditions, we summarize long-term responses using the final decades when state variables approach quasi-steady behavior. Long-term shifts in vegetation and microbial community composition under warming were not considered, owing to the lack of predictive capability for these changes, despite the microbial dormancy-resuscitation dynamics were explicitly simulated. Therefore, the long-term simulations represent a scenario analysis that accounts for seasonal and interannual variability, rather than a forecast that incorporates long-term changes in plant or microbial community composition."

We further emphasized this limitation as follows.

Discussion (Line 428–431, Page 21): "Neither vegetation succession nor microbial community turnover is represented in this study. Consequently, the long-term simulations should be interpreted as isolating the effects of repeated warming and freeze–thaw forcing on soil–microbial processes, rather than predicting ecosystem evolution under climate change."

(2) Increased MBC under warming and the role of DOC/substrate availability

To address concerns regarding increased MBC under warming, we added relative substrate availability to the long-term analysis in Fig. 5e (see below) and cite this metric explicitly in the revised manuscript.

Results (Line 201–205, Page 10–11): "Long-term projections driven by repeated experimental forcing suggest that the steady-state SOC content under warming is 2.2% lower than under control, accompanied by an 18% decline in substrate availability (Fig. 5e). In contrast, MBC increase by 11.5%, while the r_{MBA} remains nearly unchanged (+1.2%). Whole-soil warming exerts a limited impact on hydrolase activities (+1.8%) but leads to a substantially increase in oxidase activities (+23%) (Fig. 5e)."

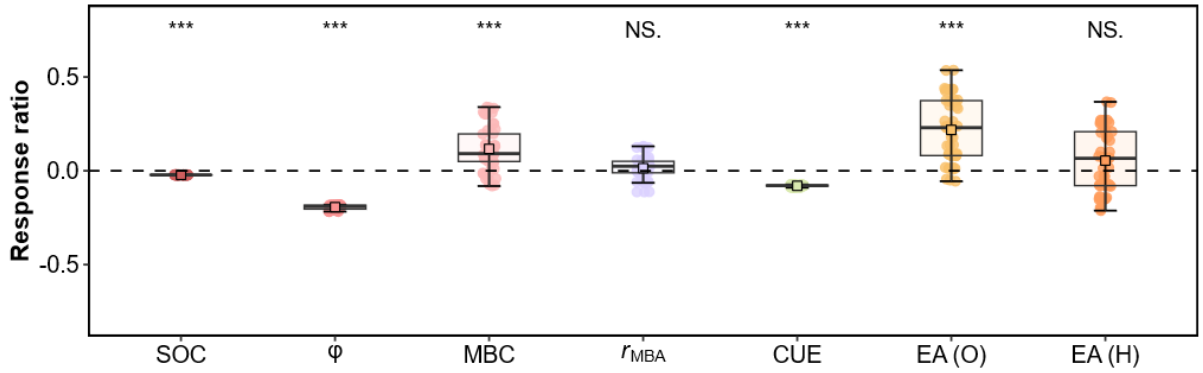

Fig. 5e Long-term (50-year) warming effects on soil process and microbial activity. Soil variables include soil organic carbon (SOC), substrate availability (φ = DOM/(DOM + KsDOM)), and microbial biomass carbon (MBC). Microbial activity is expressed as active fraction (r_{MBA}), microbial carbon use efficiency (CUE), and enzyme activities (EA) of oxidases (O) and hydrolases (H). Soil and microbial responses were projected by MEND-FT model. The dots represent yearly modeled values. Differences between RR values and 0 were tested using the Wilcoxon test. "***" denotes a significance difference at $p < 0.01$; "NS." means not significant ($p > 0.05$).

We also added the long-term simulations using the MEND-old model in Supplementary Fig. S6 (see below) and included the following text.

Results (Line 206–216, Page 11): "To evaluate whether this MBC response is a parameterization artifact, we conducted a structural comparison with the original MEND model (MEND-old; Supplementary Fig. S6). Under the same stationary forcing, MEND-old predicts declining MBC with an increasing r_{MBA}, whereas MEND-FT predicts increased MBC with a near-stable r_{MBA}. Because both models show declining substrate availability under warming, this divergence cannot be attributed solely to parameter calibration. Rather, it reflects the added

freeze–thaw microbial regulation in MEND-FT, which alters long-term microbial allocation and turnover dynamics. These results demonstrate that increased MBC under warming does not arise from increasing substrate availability. Instead, substrate limitation intensifies while microbial biomass increases, indicating a decoupling between biomass size and substrate pools under repeated forcing."

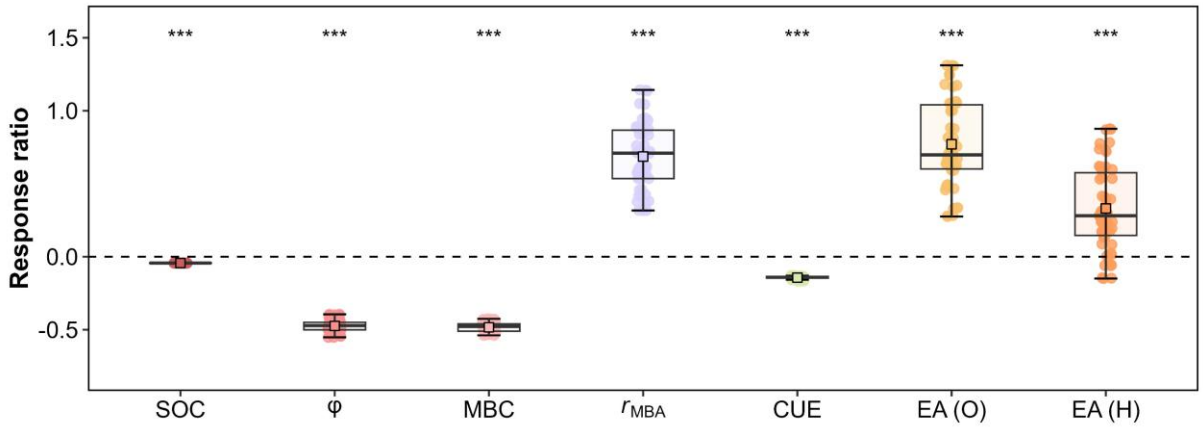

Fig. S6 | Long-term (50-year) warming effects on soil process and microbial activity simulated by MEND-old model. Soil variables include soil organic carbon (SOC), substrate availability (φ = DOM/(DOM + KsDOM)), and microbial biomass carbon (MBC). Microbial activity is expressed as active fraction (r_{MBA}), microbial carbon use efficiency (CUE), and enzyme activities (EA) of oxidases (O) and hydrolases (H). The dots represent yearly modeled values. Differences between RR values and 0 were tested using the Wilcoxon test. "***" denotes a significance difference at $p < 0.01$; "NS." means not significant ($p > 0.05$).

(3) Interpretation of SOC decline and clarification of "microbial physiological adaptation"

We agree with the reviewer that our original wording overstated the causal attribution. Accordingly, we revised the Abstract, Discussion, and Conclusions to present these results as co-occurring patterns linking SOC loss with microbial and substrate changes, rather than making overly strong attributions:

Abstract (Line 29–32, Page 2): "Long-term simulations suggest that the modest soil carbon loss (−2.2%) co-occurs with increased microbial biomass and enhanced oxidase activity, consistent with freeze-thaw-induced microbial physiological regulation rather than substrate depletion alone."

Discussion (Line 379–399, Page 19–20): "Our experiment-model integration provides temporally resolved analyses beyond the limited observations, offering a mechanistic interpretation of the observed SOC loss in alpine grassland under long-term warming (Chen et al., 2023). Under stationary forcing, this decline is associated with higher active microbial

biomass, and a stronger enhancement of oxidase activity relative to hydrolase activity (Fig. 5e). Although MEND-old qualitatively agrees these two mechanisms (Supplementary Fig. S6), the two model formulations imply different microbial strategies that steer ecosystem processes toward a new quasi-steady state over the long run. MEND-FT shows more moderate soil and microbial responses to warming, characterized by a near-stable r_{MBA} (mean RR = 0.01) together with increased total microbial biomass (RR = 0.12) (Fig. 5e), whereas MEND-old predicts declining total biomass (mean RR = −0.48) accompanied by an increasing r_{MBA} (RR = 0.68) (Fig. S6). Consistently, warming-induced amplification of oxidase activity is much weaker in MEND-FT (RR = 0.2) than in MEND-old (RR = 0.8). This demonstrates that the inclusion of freeze-thaw microbial regulation not only influences short-term dynamics but reshapes projected long-term soil responses to warming. Collectively, MEND-FT reframes the SOC decline as emerging from freeze-thaw-induced microbial physiological regulation. This is primarily linked to (i) reduced substrate relative availability, (ii) increased microbial biomass with a near-stable active fraction, and (iii) enhanced oxidase (but not hydrolase) activity. These patterns suggest that microbial physiological regulation, expressed through biomass maintenance and enzyme investment strategies under freeze–thaw conditions, modulates how substrate depletion translates into SOC loss, rather than substrate depletion alone determining the outcome."

Conclusions (Line 456–458, Page 23): "The long-term projection suggests that reductions in quasi-steady state SOC stocks co-occur with enhanced microbial biomass and oxidase activity, and are not explained by substrate changes alone."

A7. *L. 320-444: What statistical analysis approached were used in this study? And what variables were used to force the model? And what are their durations?*

Response: We have now clarified both the statistical analyses used in this study and the variables employed to force the model, together with their durations. Specifically, we revised the following information:

(1) Statistical analysis

Methods (Line 583–589, Page 29): "Warming effects were examined by the response ratio (RR)(Luo et al., 2006; Wang et al., 2022):

$$\mathrm{RR} = ln\left(\frac{X_{Warming}}{X_{Control}}\right) \quad (6)$$

where RR is the response ratio that quantifies the log proportional change between the variables of warming ($X_{Warming}$) and control ($X_{Control}$) samples. The Wilcoxon singed-rank test was applied to access whether RR values differed significantly from zero. Statistical significance is denoted as follows: "***" means $p < 0.001$; "**" is $p < 0.01$; "*" is $p < 0.05$; "NS." is not significant

($p > 0.05$)."

(2) Model forcing variables and their durations

Methods (Line 571–577, Page 28): "The field warming experiment began in June 2018 and continued through September 2021, including control and warming treatments (soil temperature increased by + 4℃ at depths of 0–100 cm), with four replicated plots per treatment (Chen et al., 2024). Soil CO_2 fluxes (soil respiration) were measured on seven dates in 2018, ten dates in 2019 and 2020, and five dates in 2021, all during the growing seasons (May to September). In contrast, measurements of SOC, MBC, microbial C:N and N_{inorg} (the sum of NH_4^+ and NO_3^-) were taken at the end of August in 2019 and 2021 only (Chen et al., 2024)."

Methods (Line 578–583, Page 29): "To further assess the model's ability to represent microbial and enzyme functional traits, we validated it against additionally literature-reported microbial carbon use efficiency (CUE) and enzyme activities (EA) (Zhang et al., 2023). We tested the model's performance in capturing warming-induced changes in EA of hydrolases and oxidases, which drive the SOM decomposition in the MEND model. Measurements of microbial CUE and EA for both oxidases and hydrolases were obtained exclusively in August 2021 (Zhang et al., 2023)."

Methods (Line 590–597, Page 29): "Model forcing data included daily soil temperature, soil moisture, and gross primary production (GPP) from 17 June 2018 to 30 September 2021. Soil temperature and moisture for both control and warming treatments were derived directly from experimental measurements (Fig. S2) (Chen et al., 2024).Soil pH was fixed at 8.4 for both treatments based on field observations (Chen et al., 2024). Daily GPP for the control treatment was obtained from ChinaFLUX (https://www.chinaflux.org/). Gaps in the time series were filled using the long short-term memory (LSTM) model (Hochreiter and Schmidhuber, 1997). The model was first trained on historical data, with 80% randomly allocated for training and 20% for testing, and then used to predict the missing values based on the PyTorch framework (Ren et al., 2022)."

Methods (Line 602–608, Page 30): "We used daily GPP as a flux driver to represent the temporal dynamics of photosynthetic carbon uptake. Daily litter carbon input was parameterized as a fraction of daily GPP based on the plant-to-litter transfer efficiency reported by ref.(Fan et al., 2023), and then partitioned into litter entry pools using the fractions in the model configuration. To represent treatment effects on carbon input, we scaled the daily GPP time series by the observed annual net primary production (NPP) ratio between warming and control plots (Chen et al., 2024), thereby translating treatment-induced productivity differences into varying litter input. "

B. Response to Reviewer #2:

B1. *This manuscript presents a timely study that examines how freeze–thaw cycles and microbial dormancy influence soil C processes in alpine systems. Extending MEND model to MEND-FT by incorporating Freeze-Thaw (FT) influenced microbial dormancy and testing it against field warming experiments on the Qinghai–Tibet Plateau and found the new model performed better on simulating microbial physiology and showed increased warming effects in non-growing season adds great value in understanding how microbial processes regulate alpine soils to warming. The paper is clearly written, and results show are noteworthy. My major concern is that the authors did not clearly explain the linkages between the added new freeze–thaw–dormancy processes in the model and the enhanced performance on CUE, enzyme activity, and Ninorg, etc. I recommend the manuscript to be published after addressing the following major and minor comments I have:*

Response: Thank you for the complimentary comments!

B2. *Major: The manuscript would benefit from a clearer mechanistic explanation linking the newly implemented freeze–thaw–dormancy processes to the model's improved performance in simulating microbial CUE and enzyme production. It is not clear why MEND-FT predicts a lower CUE than MEND-old (Fig. 4a), or why MEND-FT captures the observed decrease in enzyme activity in August 2021 while generally predicting higher enzyme activity under warming elsewhere. Throughout the manuscript, the authors emphasize that model outputs align with observations, but they do not explain the underlying mechanisms driving this agreement. As this is a modeling study, I encourage the authors to present key intermediate variables or fluxes to illustrate how the new processes translate into these outcomes and thereby clarify the causal chain within the model.*

Response: We thank the reviewer for this constructive suggestion and agree that a clearer mechanistic explanation is needed to demonstrate how the newly implemented freeze–thaw–dormancy processes translate into improved simulations of microbial carbon use efficiency (CUE) and enzyme activities. In response, we have strengthened the mechanistic narrative by (i) revising Fig. 4 to explicitly show key intermediate state variables and fluxes, and (ii) adding targeted explanations in the Discussion to clarify the causal chain within the model. We further added Supplementary Figs. S4–S9 to document the process-level pathways by which freeze–thaw regulation alters microbial state and metabolism, and we integrated these results into the revised manuscript.

(1) Revisions to Fig. 4 to expose the causal chain

To make the internal model mechanisms transparent, we revise Fig. 4 to explicitly include model-traceable intermediate state variables and fluxes, thereby clarifying the causal chain linking process representation to emergent model behavior. Specifically, we expanded Fig. 4 to

include (i) carbon partitioning between microbial growth and respiration, (ii) relative substrate availability, and (iii) key intermediate microbial variables regulating enzyme production.

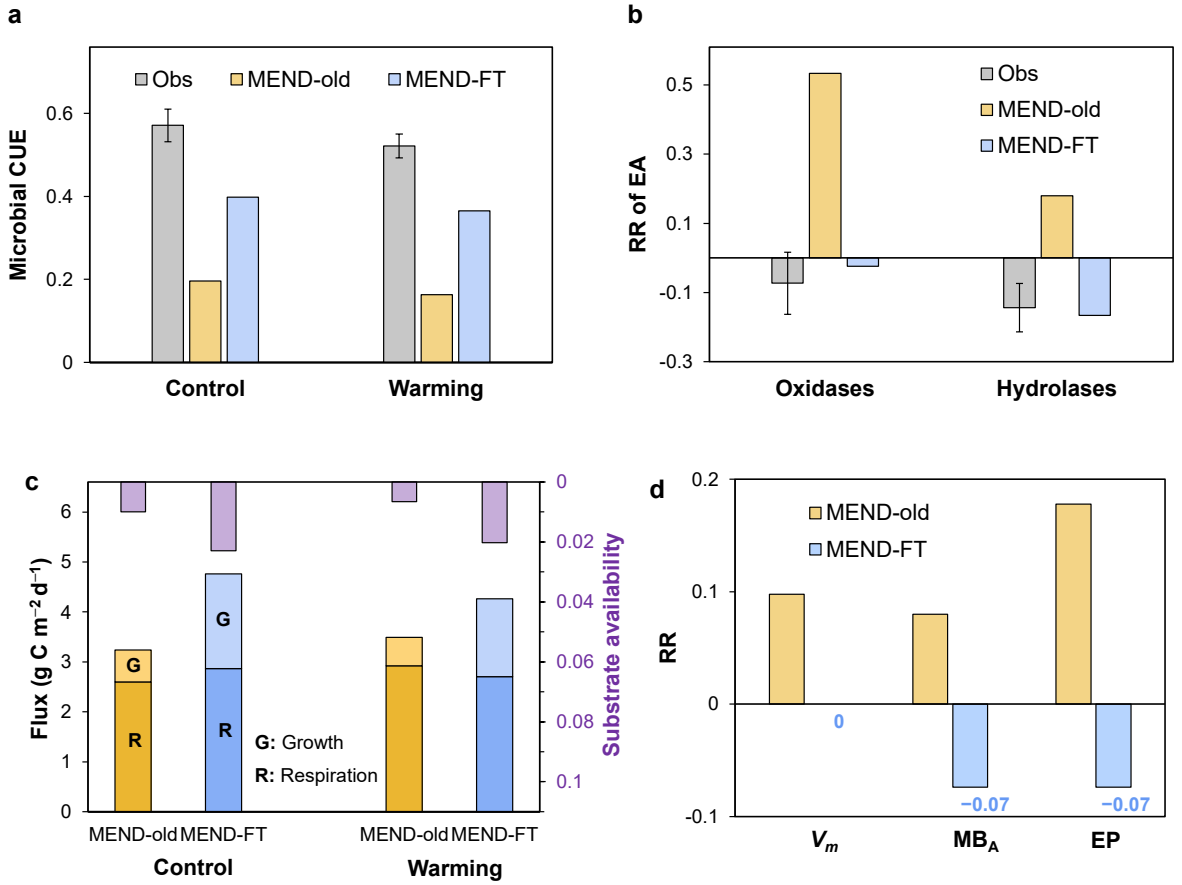

Fig. 4 | Comparison of microbial carbon use efficiency (CUE) and warming effects on soil enzyme activities by two models (MEND-old and MEND-FT) in August 2021. **(a)** Observed versus simulated microbial CUE under control and warming treatments (error bars denote standard deviations). **(b)** Response ratio (RR = $\ln(X_{Warming}/X_{Control})$) of observed enzyme activities (EA) for oxidases and hydrolases, compared with simulated EA from MEND-old and MEND-FT. **(c)** Simulated carbon partitioning showing growth (G) and respiration (R) fluxes, where G + R = U (microbial uptake), together with substrate availability (φ = DOM/(DOM + KsDOM)) for each model under control and warming. Microbial CUE is defined as (U − R)/U = G / (G + R). **(d)** Simulated RR of intermediate variables in August 2021, including biomass-specific maintenance rate (V_m), active microbial biomass (MB_A) and total enzyme production flux (EP), comparing MEND-old and MEND-FT.

(2) Mechanistic explanation for enzyme activity responses in August 2021

To clarify why only MEND-FT captures the observed decrease in enzyme activity in August 2021, we added the following explanation:

Discussion (Line 237–253, Page 12–13): "Only the MEND-FT model reproduced the observed decline in enzyme activities under warming during the thaw period, whereas the

original model (MEND-old) predicted the opposite, i.e., positive responses (Fig. 4b). In both models, enzyme production depends on active microbial biomass and the availability of enzyme-target substrates using the COmpetitive Dynamic Enzyme ALlocation (CODEAL) strategy (Eq. S38 in Table S3) (Sinsabaugh et al., 2014; Jian et al., 2016; Wang et al., 2022; Qi et al., 2023). The key distinction lies in how warming alters the active microbial pool. MEND-FT regulates microbial processes via freeze-thaw-mediated dormancy-resuscitation dynamics. During fully thawed conditions, the freeze-thaw modifier converges between control and warming treatments, resulting in only modest changes in biomass-specific metabolic rates (e.g., maintenance rate V_m; RR ≈ 0; Fig. 4d). Under these conditions, warming shifts microbial state toward a smaller active biomass pool (RR = −0.07) consequently reduces enzyme production (RR = −0.07) at the thaw-period sampling time (Fig. 4d). In contrast, MEND-old lacks freeze-thaw regulation; warming primarily increases V_m (RR = 0.1) and active biomass (RR = 0.08) (Fig. 4d), leading to higher enzyme production (RR = 0.18) and positive enzyme activities responses (Fig. 4b, d). This explicitly demonstrates how the freeze–thaw–regulated microbial dormancy module alters microbial state variables, rather than enzyme kinetics per se, leading to divergent enzyme responses between models."

(3) Mechanistic explanation for different simulated microbial CUE in August 2021

We also clarified the mechanism underlying differences in microbial CUE between MEND-FT and MEND-old. Contrary to our earlier wording, MEND-FT predicts higher CUE than MEND-old, and this difference arises from altered substrate–uptake dynamics rather than direct parameter tuning. We add the following text:

Discussion (Line 321–329, Page 16): "Microbial CUE, which regulates the balance between microbial growth and respiratory losses (Zhang et al., 2023; He et al., 2024), is also better represented in MEND-FT. Compared with MEND-old, MEND-FT simulates higher DOM availability, expressed as higher relative substrate availability (φ = 0.023/0.020 for control/warming vs. 0.009/0.007 in MEND-old) (Fig. 4c). This higher φ increases microbial uptake and growth fluxes while respiration remains comparable between models (Fig. 4c), resulting in higher simulated CUE in MEND-FT (Fig. 4a). Consequently, modeled CUE values increase from 0.20 (control) and 0.16 (warming) in MEND-old to 0.40 and 0.36 in MEND-FT. These values align more closely with the reported mean CUE (0.42) for grassland ecosystems (He et al., 2023), yet remain lower than the ^{18}O-derived estimates (0.57 and 0.52)."

(4) New Supplementary Figs. S4–S9 to provide a clear causal chain

To further substantiate the causal chain across seasons and years, we added Supplementary Figs. S4–S9. These figures are intended to expose intermediate processes and diagnostics variables, and their content and specific revisions are addressed in detail in the corresponding

Responses. Briefly:

- Fig. S4: Dormancy-related flux imbalances in the original MEND model (see Response to **B9**).
- Fig. S5: Aggregated seasonal patterns across all thaw months (June–October) and freeze months (November–May) rather than single representative months (Response to **A5**, **B9**).
- Fig. S6: Divergent long-term microbial strategies between MEND-FT and MEND-old (Response to **A6**, **B16**).
- Fig. S7: Seasonal shifts in N mineralization vs. immobilization balance (Response to **B12**).
- Fig. S8: Seasonal shifts in enzyme concentration and specific activity responses (Response to **B11**).
- Fig. S9: Direct tracking of uptake vs. respiration changes driving CUE decline (Response to **B14**).

We also reflected these additions in the main text as follows:

Discussion (Line 276–279, Page 14): "Our analysis delineates a clear, model-traceable causal chain linking freeze–thaw-regulated microbial dormancy-resuscitation dynamics to shifts in active microbial pools and metabolic rates, subsequent changes in enzyme activities and microbial CUE, altered SOC and nutrient responses (Figs. 4–5; Supplementary Figs. S4–S9)."

B3. *Line 22: This work doesn't seem to involve any genes data, or am I missing something here?*

Response: Thank you for the comment. The phrase "from genes to ecosystems" refers to the scalable design of the modeling framework rather than the specific data streams used in this paper, please see Response to **A2**. We reclaimed this sentence as follows.

Abstract (Line 19–23, Page 2): "Here, we develop a freeze-thaw-enabled microbial ecological model (MEND-FT) and integrate it with multi-year whole-soil warming experiments in an alpine meadow on the Qinghai-Tibetan Plateau to investigate warming effects on soil carbon dynamics from microbial physiology to ecosystem processes."

B4. *Line 51: "substrate availability for decomposition" is not essentially physical soil process, but a biological one.*

Response: We agree that "substrate availability" for decomposition is not a purely physical soil process, but rather an emergent property regulated by both physical constraints (e.g., soil temperature, moisture, and aggregate structure) and biological processes (e.g., microbial uptake, enzyme production, and substrate preference). Our original wording did not sufficiently

distinguish between these controls. Our intent was to emphasize that many existing modeling studies prioritize physical soil processes or bulk substrate pools, while giving limited attention to explicitly modeled microbial physiological regulation. To clarify this distinction and avoid conceptual ambiguity, we have revised the sentence as follows.

Introduction (Line 49–52, Page 3): "Recent modeling efforts addressing carbon-climate feedbacks in cold regions have primarily focused on physical soil processes, such as soil thermal-hydrological dynamics (Gao et al., 2019; Li et al., 2021), or have represented substrate limitation implicitly through bulk SOC availability (Hayes et al., 2014; Liu et al., 2024). However, these models often overlook explicit microbial physiological strategies under repeated freeze-thaw cycles."

B5. *Line 74: Does the approach/framework in this manuscript incorporates genes data?*

Response: Thank you for the comment. While genetic data are not used here, the MEND modeling framework is designed to integrate such information when available. Prior studies have integrated microarray-based gene abundances into MEND by linking gene signals to modeled enzyme pools or activities, thereby using genetic data as quantitative indicators of microbial functional potential (Gao et al., 2020; Guo et al., 2020; Wang et al., 2022; Tao et al., 2024). This capability underpins the framework's extensibility, even though it was not activated in the current application. Please see the detailed explanation provided in the Response to **A2**. We have rewritten the txt in the manuscript as follows:

Introduction (Line 72–78, Page 4): "Given the strong linkages between warming-induced microbial processes and carbon dynamics, a comprehensive approach integrating field observations, laboratory incubations, and process-based modeling is needed. Such a framework enables a mechanistic representation of microbial physiology at the process level, propagates these microbial-enzymatic dynamics to soil C-N pools and fluxes at the ecosystem scale through mass-balance and kinetic formulations, and allows genetic data, where available, to directly constrain enzyme-mediated processes, thereby strengthening mechanistic realism across scales (Gao et al., 2020; Wang et al., 2022; Tao et al., 2024)."

B6. *Line 115-131, How are all these objects weighted in the multi-object weighted calibration, I could not find the values of the different weights wi in the Supplementary Info. Have the authors tested alternative weighting schemes to evaluate sensitivity? The uncertainties or rationale for these weights should be discussed, at least in the Supplementary Information.*

Response: Thank you for pointing this out. We agree that the weight setting should be reported and their rationale and uncertainties discussed. We have now fully reported the weights assigned to each response variable in Tables S7 and expanded Supplementary Text 3.4 to explain the

calibration strategy and the rationale for weight selection. Besides, we conducted a weight sensitivity test using alternative weighting schemes (Table S8).

The added text in Supplementary Text 3.4:

"In this study, the multi-object weighted calibration was performed in two sequential steps. In the first step, ten microbial physiology parameters (r_E, p_{EP}, fp_{EM}; V_g, α, β, γ, K_D, Y_g; and r_0; see Table S5 for their definitions) were optimized using experimental observations of soil respiration (Rs), microbial biomass carbon (MBC), and soil organic carbon (SOC) (Table S7). We assigned weights of 0.5, 0.3, and 0.2 to Rs, MBC, and SOC, respectively, reflecting the substantially larger number of Rs measurements (32 data points) relative to MBC and SOC (2 observations each). In the second step, these calibrated microbial parameters were fixed, and five key inorganic N parameters (VN_{VG}, VN_{im}, VN_{fix}, VN_{nit}, and VN_{denit}; see Table S5 for their definitions) were optimized using observations of microbial C:N ratio, and concentrations of ammonium (NH_4^+) and nitrate (NO_3^-) (Table S7). In this stage, weights of 0.4, 0.3, and 0.3 were assigned to microbial C:N, NH_4^+, and NO_3^-, respectively. We further tested several alternative weighting schemes (W1–W3, Table S8). Across these schemes, the optimal parameter values and objective function values (Table S7) changed only modestly, indicating that the calibration is robust to reasonable variations in weighting (Table S8)."

In addition, we have added the following sentence to the main text:

Results (Line 116–117, Page 6) "Details of the model calibration procedure, a including the weighting of response variables and sensitivity analyses, are provided in Supplementary Text 3.4 and Table S7–S8."

Table S7 and Table S8 are shown below:

Table S7. Experimental data for MEND model calibration and validation

Response variable	Description	Objective function	Data points	Weight
Rs	Soil respiration	$1-R^2$	32	0.5
SOC	Soil organic carbon	MAREt, tolerance = 0.05	2	0.2
MBC	Microbial biomass carbon	MAREt, tolerance = 0.1	2	0.3
Microbial C:N	Microbial C:N ratio	|PBIAS|	2	0.4
NH_4^+	Ammonium concentration	|PBIAS|	2	0.3
NO_3^-	Nitrate concentration	|PBIAS|	2	0.3
Microbial CUE	Microbial carbon use efficiency	For model validation only.	1	—
EPO	Oxidase		1	
EPH	Hydrolase		1	

Note: R^2 is the coefficient of determination (Eq. S74), MAREt is the mean absolute relative error (MARE) with a tolerance (Eq. S77), |PBIAS| is the absolute value of the percent bias (Eq. S75).

Table S8. Optimal parameter and objective function values under different weight schemes.

Parameter	W0	W1	W2	W3
r_0	0.38	0.56	0.46	0.48
r_E	0.03	0.03	0.03	0.03
p_{EP}	0.01	0.01	0.01	0.01
fp_{EM}	2.99	2.65	2.73	2.74
V_g	0.017	0.017	0.017	0.017
α	0.5	0.5	0.5	0.5
K_D	0.29	0.28	0.29	0.29
Y_g	0.31	0.33	0.34	0.35
β	7.4	9.9	9.9	9.7
γ	0.0015	0.0018	0.0019	0.0023
VN_{im}	0.099	0.083	0.087	0.085
VN_{fix}	0.099	0.099	0.099	0.099
VN_{nit}	975	959	972	978
VN_{denit}	0.89	0.66	0.66	0.66
VN_{VG}	1e-6	1e-6	1e-6	1e-6
Response variable	f_{OBJ} (calibration | validation), best = 0			
Rs	0.34 | 0.38	0.34 | 0.37	0.34 | 0.38	0.34 | 0.39
SOC	0.33 | 0.31	0.33 | 0.31	0.33 | 0.31	0.33 | 0.31
MBC	0.21 | 0.60	0.17 | 0.66	0.18 | 0.64	0.18 | 0.64
C:N	0.06 | 0.08	0.08 | 0.07	0.07 | 0.07	0.06 | 0.09
NH_4^+	0.42 | 0.43	0.42 | 0.45	0.42 | 0.45	0.42 | 0.45
NO_3^-	0.29 | 0.15	0.30 | 0.13	0.30 | 0.13	0.29 | 0.13

Note: W0 denotes the weighting scheme used in the main text (weights listed in Table S7). W1−W3 are alternative weight schemes. W1 uses weights of 0.5, 0.25, 0.25 for Rs, MBC, and SOC, respectively; and 0.33, 0.33, 0.33 for microbial C:N, NH_4^+, and NO_3^-, respectively. W2: 0.5, 0.2, 0.3 for Rs, MBC, and SOC, respectively; and 0.33, 0.33, 0.33 for microbial C:N, NH_4^+, and NO_3^-, respectively. W3: 0.5, 0.3, 0.2 for Rs, MBC, and SOC; 0.2, 0.4, 0.4 for microbial C:N, NH_4^+, and NO_3^-, respectively.

B7. *Fig. 3, For MBC and Ninorg, why is the results in 2020 not shown here and why only Augst? Is that the only data found in that existing literature? The authors should state the data availability and span in the "Study site and data compilation" section in the Methods without readers having to going back to the literature to check.*

Response: Sorry for the confusion. The temporal coverage of each response variable in Fig. 3 is constrained by measurement availability. Specifically, microbial biomass carbon (MBC) and inorganic N (N_{inorg}) were not measured in 2020 and were sampled only during late growing-season campaigns in 2019 and 2021. Consequently, these variables are shown only for August in Fig. 3, corresponding to the periods when observations are available. August sampling was

selected because it represents peak biological activity and provides the most consistent basis for comparison across years. We have now explicitly stated the data availability and span in the "Study site and data compilation" section of the Methods.

Methods (Line 571–577, Page 28): "The field warming experiment began in June 2018 and continued through September 2021, including control and warming treatments (soil temperature increased by + 4℃ at depths of 0–100 cm), with four replicated plots per treatment (Chen et al., 2024). Soil CO_2 fluxes (soil respiration) were measured on seven dates in 2018, ten dates in 2019 and 2020, and five dates in 2021, all during the growing seasons (May to September). In contrast, measurements of SOC, MBC, microbial C:N and N_{inorg} (the sum of NH_4^+ and NO_3^-) were taken at the end of August in 2019 and 2021 only (Chen et al., 2024)."

Methods (Line 578–583, Page 29): "To further assess the model's ability to represent microbial and enzyme functional traits, we validated it against additionally literature-reported microbial carbon use efficiency (CUE) and enzyme activities (EA) (Zhang et al., 2023). We tested the model's performance in capturing warming-induced changes in EA of hydrolases and oxidases, which drive the SOM decomposition in the MEND model. Measurements of microbial CUE and EA for both oxidases and hydrolases were obtained exclusively in August 2021 (Zhang et al., 2023)."

In addition, we have revised Fig. 3 caption and as follows:

"**Fig. 3 | Model calibration and validation.** (a) Soil respiration (Rs) calibration and simulated daily Rs values during the growing season (GS) and non-growing season (NGS) under the control treatment. (b) Rs validation using data from the warming treatment. (c) Calibration of microbial biomass carbon (MBC), soil organic carbon (SOC), microbial C:N, and inorganic nitrogen (N_{inorg}) with the control data. (d) Validation of MBC, SOC, microbial C:N, and N_{inorg} using the warming data. Measurements of MBC, SOC, microbial C:N, and N_{inorg} were available only from late August sampling in 2019 and 2021, whereas Rs was measured repeatedly throughout the GS. R^2 values in (a) and (b) denote the coefficient of determination. Error bars are standard deviation. Differences in Rs between treatments were assessed using the Wilcoxon test. "***" denotes a significance difference at $p < 0.01$, "NS." means not significant ($p > 0.05$)."

B8. *Fig. 4, why is only one month of one year (August 2021) was included in here? Is it because you only had microbial data in that particular month? The authors referred all the microbial data they compared to into one sentence from line 437 to 439, they should be clearer about which months and years are the data available there.*

Response: We thank the reviewer for this helpful comment and apologize for the lack of clarity.

The observed microbial data used in Fig. 4, including microbial carbon use efficiency (CUE) and enzyme activities (EA), were available only from a single sampling campaign in August 2021 (Zhang et al., 2023). Comparable microbial measurements were not available for other months or years, which constrained our ability to evaluate model performance for these variables beyond this time point. August was selected because it represents peak microbial activity during the growing season and provides the most informative snapshot for assessing warming effects on microbial physiology. We have explicitly stated this information in the "Study site and data compilation" section of the Methods (see Response to **B7**.) and have revised the Fig. 4 caption accordingly:

"**Fig. 4 | Comparison of microbial carbon use efficiency (CUE) and warming effects on soil enzyme activities by two models (MEND-old and MEND-FT) in August 2021.** (a) Observed versus simulated microbial CUE under control and warming treatments (error bars denote standard deviations). **(b)** Response ratio ($RR = \ln(X_{Warming}/X_{Control})$) of observed enzyme activities (EA) for oxidases and hydrolases, compared with simulated EA from MEND-old and MEND-FT. **(c)** Simulated carbon partitioning showing growth (G) and respiration (R) fluxes, where G + R = U (microbial uptake), together with substrate relative availability ($\varphi = DOM/(DOM + KsDOM)$) for each model under control and warming. Microbial CUE is defined as $(U - R)/U = G / (G + R)$. **(d)** Simulated RR of intermediate variables in August 2021, including biomass-specific maintenance rate (V_m), active microbial biomass (MB_A) and total enzyme production flux (EP), comparing MEND-old and MEND-FT."

B9. *Fig. 5a showed that the rMBA on the rise most of the time during the experimental years even in control scenarios, which left me wondering if the model is not spun up to steady state. Could the authors provide any info on the model spin-up and steady state in the setup?*
In Fig. 5b, rMBA seems to decrease with warming in the short-term experiment, yet in the long-term warming projection (Fig. 5e) it slightly increases. The authors should discuss why the short- and long-term responses differ.
Also again why in Fig.5c-d, Why are only one thaw month and one freeze month shown in Fig. 5c–d? Even if patterns across different years' thaw-freeze months are similar, the authors should consider aggregating all thaw/freeze months or moving other months' results to Supplementary Figures. Also, please clarify in the caption whether the dots represent daily modeled values.

Response: Thank you for the constructive comment. we have clarified the model spin-up strategy, explained the contrasting short- versus long-term r_{MBA} responses to warming, and revised the presentation of freeze–thaw month results to improve generality and transparency.

(1) Model spin-up and steady sate in the setup

We have added a detailed description of model initialization and spin-up in the revised manuscript.

Methods (Line 609–621, Page 30): "Both MEND-old and MEND-FT were initialized using experimentally measured C and N pool sizes (Supplementary Text 2.1), with identical initial conditions applied to the control and warming treatments. Because continuous forcing data were available only from June 2018 onward, the observed forcing was repeated backward to January 2017. The period from January 2017 to September 2018 was used as model spin-up, and simulations from October 2018 to September 2021 were used for analysis. We emphasize that this short-term spin-up approach is scientifically sound for field-level modeling: initializing with measured pool sizes ensures that the model begins close to the observed system state, so that the dynamics reflect realistic seasonal and interannual responses (Kanari et al., 2022). In contrast, global or Earth system models often lack site-specific initial conditions and rely on hundreds to thousands of years of spin-up to bring slow-cycling carbon pools to equilibrium (Dufresne et al., 2013; Liu et al., 2024). At the field scale, a short spin-up suffices to minimize transient effects while capturing relevant microbial and biogeochemical dynamics under well-constrained experimental conditions."

Results (Line 175–181, Page 9): "The gradual increase in the active microbial fractions (r_{MBA}) under control conditions in Fig. 5a does not indicate insufficient spin-up. Instead, it reflects a structural feature of the MEND-old formulation. In MEND-old, dormancy and resuscitation fluxes are regulated primarily by soil moisture, while dormant microbial maintenance respiration proceeds continuously (Supplementary Fig. S4). In seasonally frozen soils, this imbalance leads to progressive depletion of dormant microbial pool and a corresponding increase in r_{MBA} over time."

We have added a new Supplementary Figure (Fig. S4), which illustrate the relative magnitudes of dormancy-related fluxes and their environmental controls in MEND-old, as shown below:

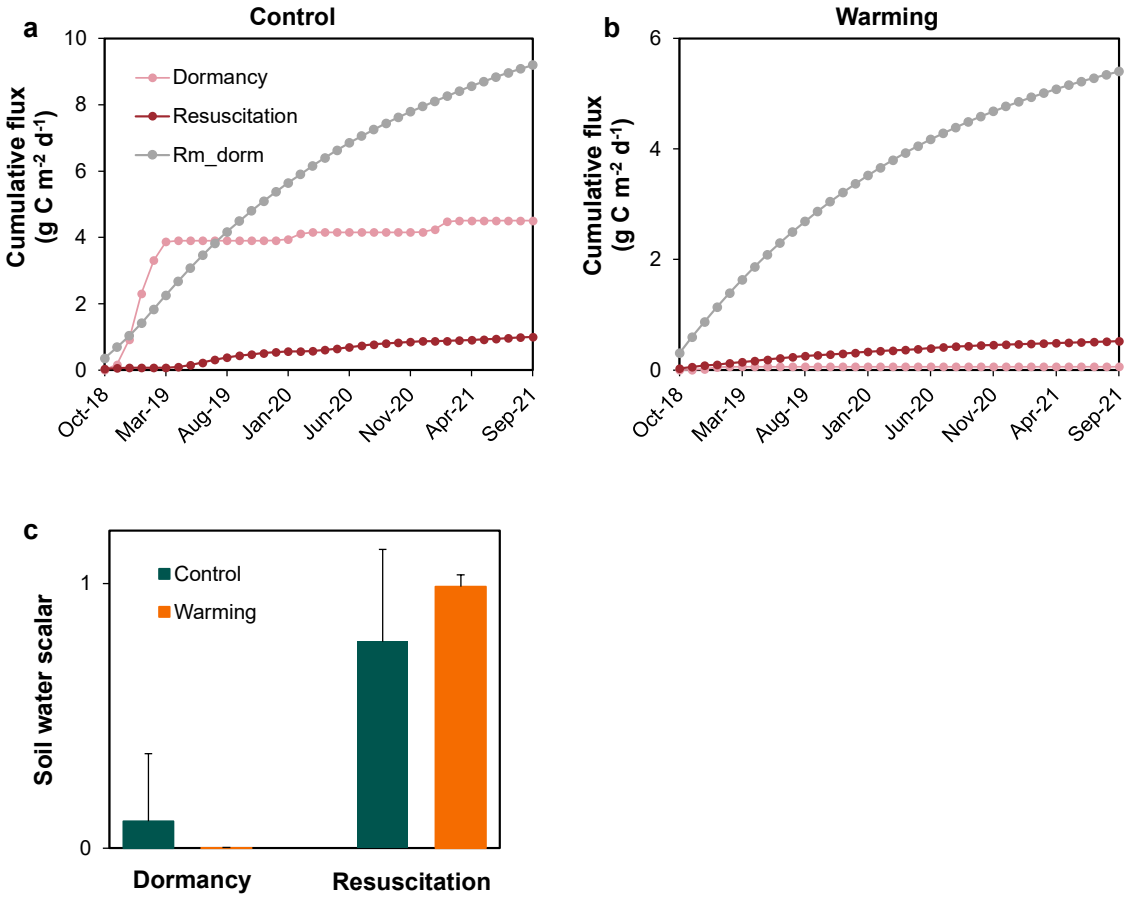

Fig. S4 | Microbial dormancy processes and soil water scalar in MEND-old. (a–b) Cumulative fluxes of dormancy, resuscitation, and dormant microbial maintenance respiration (Rm_dorm) under (a) control and (b) warming treatments. (c) Monthly mean soil water scalar regulating dormancy and resuscitation processes in MEND-old. Error bar means standard deviations (n = 36).

(2) Divergent short- and long-term r_{MBA} responses to warming

Fig. 5b shows r_{MBA} dynamics simulated by MEND-FT, where freeze-thaw regulation explicitly alters transitions between active and dormant microbial states. We expanded the discussion to clarify the short-term and long-term warming responses:

Discussion (Line 257–269, Page 13–14): "In the short-term warming experiment (October 2018–September 2021), MEND-FT predicts a strongly seasonal warming effect on r_{MBA}, with increases during freeze months but decreases during thaw months (Supplementary Fig. S5). This seasonality arises from the combined effects of freeze-thaw constraints on state transitions and warming-induced changes in relative substrate availability. During thaw months, modeled biomass-specific metabolic rates differ only modestly between control and warming treatments

(Supplementary Fig. S5), consistent with the one sampling event in late August 2021 (Zhang et al., 2023). Under these conditions, reduced relative substrate availability (φ) under warming slightly shifts microbial state transitions toward dormancy, resulting in lower r_{MBA} (Supplementary Fig. S5). By contrast, during freeze months, warming relaxes cold-season constraints on microbial activity and state transition, enhancing resuscitation and thus increasing r_{MBA} (Supplementary Fig. S5). When integrated over 50-year scenario analysis, these opposing seasonal effects largely offset, yielding a small net increase in r_{MBA} (RR = 0.01), which differs in sign from the short-term thaw-season response (Fig. 5e)."

(3) Representation of thaw and freeze months in Fig. 5c–d

Regarding Fig. 5c–d, we initially presented one thaw and one freeze month as representative examples to illustrate microbial responses under distinct seasonal states defined by the freeze-thaw cycle. For the top 100 cm soil profile, February is typically fully frozen and was therefore selected as the representative freeze month. September is typically fully thawed and closely corresponds to the late-August period when observations were collected, so it was selected as the representative thaw month.

We agree that showing only one month may limit generality. To address this, we have now aggregated results across all thaw-period months (June to October) and freeze-period months (November to May) and present the results in Supplementary Fig. S5 (see below). We also add the following text in the revised manuscript:

Results (Line 189–193, Page 10): "Beyond these representative months, we aggregated results across all thaw-period month (June to October) and freeze-period months (November to May), as identified by the ALTscalar-based dormancy-resuscitation controls (Supplementary Fig. S5a, b), and presented the corresponding responses in Supplementary Fig. S5c, d. This analysis demonstrates that the patterns shown in Fig. 5c–d are robust across years and seasonal states."

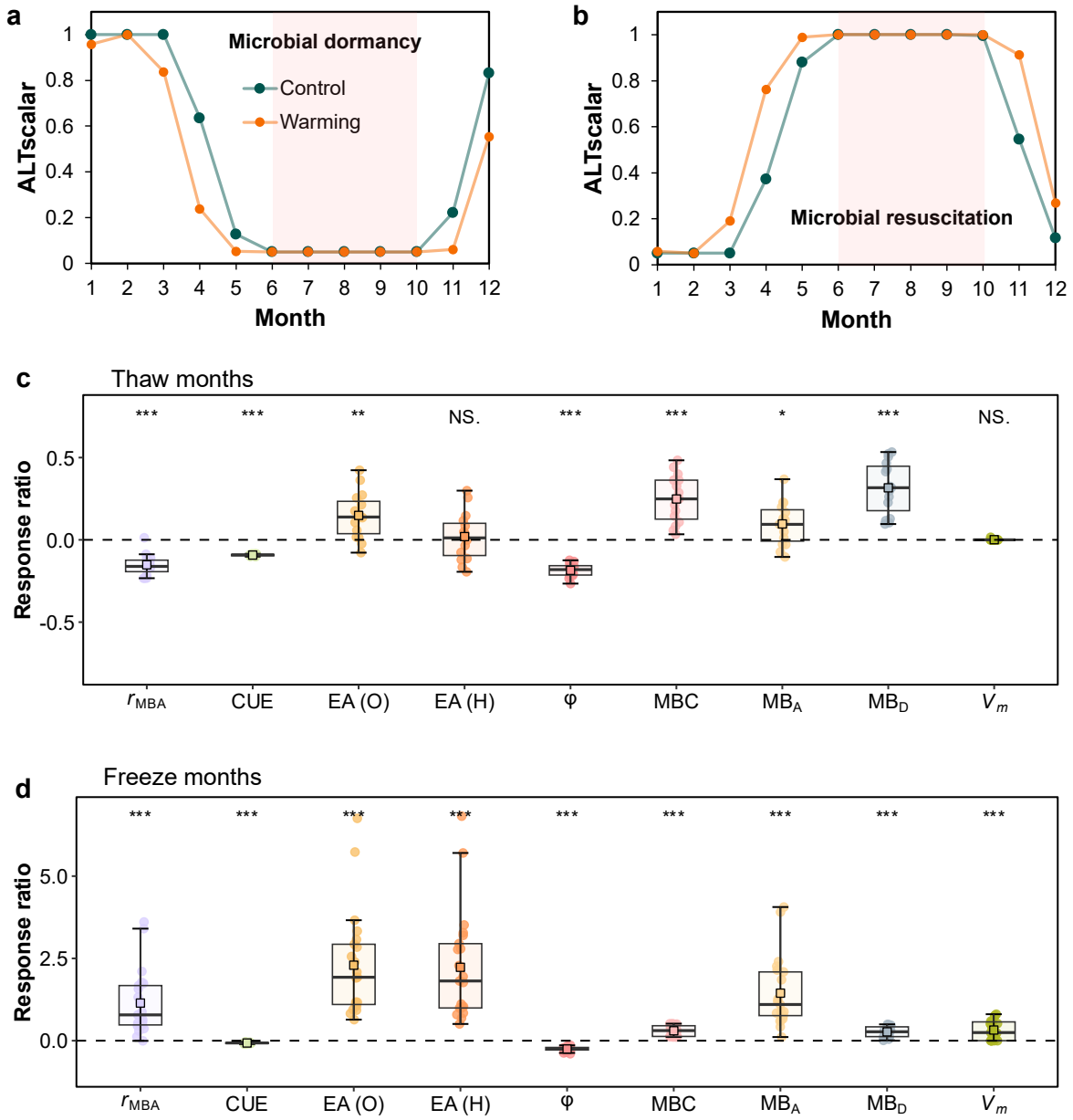

Fig. S5 | Freeze-thaw regulation (a–b) and simulated monthly microbial responses to warming across (c) all thaw-period months (June to October) and (d) freeze-period months (November to May). Variables include active fraction (r_{MBA}), microbial carbon use efficiency (CUE), enzyme activities (EA) of oxidases (O) and hydrolases (H), substrate availability (φ = DOM/(DOM + KsDOM)), microbial biomass carbon (MBC), active microbial biomass (MB_A) and dormant microbial biomass (MB_D), and biomass-specific maintenance rate (V_m). Differences between RR values and 0 were tested using the Wilcoxon test. "***" denotes a significance difference at $p < 0.01$; "NS." means not significant ($p > 0.05$).

Finally, the dots in Fig. 5 c–d represent daily modeled values, while Fig. 5e are yearly modeled values. We have explicitly presented it in Figure caption:

"**Fig. 5 | Active microbial fraction time series and simulated responses to warming. (a–b)** Temporal changes in the active microbial fraction (r_{MBA} = active microbial biomass / active and dormant microbial biomass) simulated by MEND-old (a) and MEND-FT (b). **(c–d)** Warming effects on microbial activity during a representative thaw (c) month and freeze (d) month of the experimental years (2019–2021), quantified by the response ratio ($RR = \ln(X_{Warming}/X_{Control})$). **(e)** Long-term (50-year) warming effects on soil process and microbial activity. Soil variables include soil organic carbon (SOC), substrate availability (φ = DOM/(DOM + KsDOM)), and microbial biomass carbon (MBC). Microbial activity is expressed as active fraction (r_{MBA}), microbial carbon use efficiency (CUE), and enzyme activities (EA) of oxidases (O) and hydrolases (H). Soil and microbial responses were projected by MEND-FT model. The dots in (**c–d**) represent daily modeled values, and dots in (**e**) are yearly modeled values. Differences between RR values and 0 were tested using the Wilcoxon test. "***" denotes a significance difference at $p < 0.001$; "NS." means not significant ($p > 0.05$). "

B10. *Line 204-210, it's unclear and underexplored here, why adding the freeze-thaw dormancy leads to less enzyme production. Here the authors explained as "This improvement is due to the model's representation of enzyme production depending on microbial biomass and substrate availability", but I believe both MEND_old and MEND_FT has this representation. The difference seems to stem from MEND-FT simulating less microbial biomass under warming (Fig. 5b), but the mechanism behind this remains unexplained. Is it due to reduced CUE with warming in MEND-FT? And if so, why does this occur in MEND-FT but not in MEND-old?*

Response: Yes, both MEND-old and MEND-FT represent enzyme production as a function of active microbial biomass and the availability of enzyme-target substrate. We appreciate the reviewer's comment and rewrite the text:

Discussion (Line 234–256, Page 12–13): "While previous applications of the MEND model successfully integrated microbial dormancy and resuscitation in response to soil temperature and moisture in non-frozen soils (Wang et al., 2019; Wang et al., 2021), our adaptation for frozen soils reveals critical interactions between freeze–thaw dynamics and microbial physiological responses. Only the MEND-FT model reproduced the observed decline in enzyme activities under warming during the thaw period, whereas the original model (MEND-old) predicted the opposite, i.e., positive responses (Fig. 4b). In both models, enzyme production depends on active microbial biomass and the availability of enzyme-target substrates using the COmpetitive Dynamic Enzyme ALlocation (CODEAL) strategy (Eq. S38 in Table S3) (Sinsabaugh et al., 2014; Jian et al., 2016; Wang et al., 2022; Qi et al., 2023). T

The key distinction lies in how warming alters the active microbial pool. MEND-FT regulates microbial processes via freeze-thaw-mediated dormancy-resuscitation dynamics. During fully thawed conditions, the freeze-thaw modifier converges between control and warming treatments, resulting in only modest changes in biomass-specific metabolic rates (e.g., maintenance rate V_m; RR ≈ 0; Fig. 4d). Under these conditions, warming shifts microbial state toward a smaller active biomass pool (RR = −0.07) consequently reduces enzyme production (RR = −0.07) at the thaw-period sampling time (Fig. 4d). In contrast, MEND-old lacks freeze-thaw regulation; warming primarily increases V_m (RR = 0.1) and active biomass (RR = 0.08) (Fig. 4d), leading to higher enzyme production (RR = 0.18) and positive enzyme activities responses (Fig. 4b, d). This explicitly demonstrates how the freeze–thaw–regulated microbial dormancy module alters microbial state variables, rather than enzyme kinetics per se, leading to divergent enzyme responses between models."

B11. *Moreover, the decline in enzyme production appears mainly in the growing season, while oxidative enzyme activity increases during thaw and frozen months (Fig. 5). The authors should clarify why enzyme responses differ across seasons and, if possible, show intermediate variables or model outputs to illustrate these contrasting behaviors.*

Response: To clarify the seasonal contrast in enzyme responses, we have added intermediate variables in Supplementary Fig. S8, as shown below:

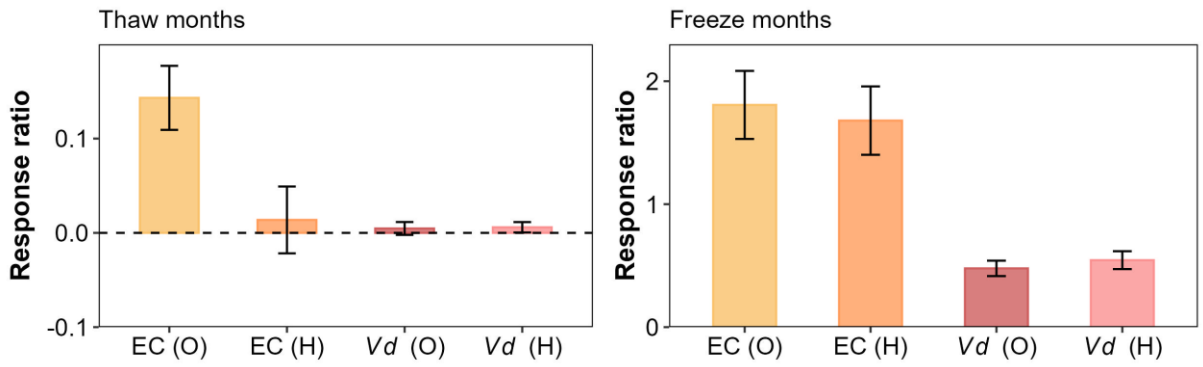

Fig. S8 | Simulated warming effects on enzyme concentration (EC) and specific enzyme activity (*Vd*). Response ratio (RR = $\ln(X_{Warming}/X_{Conrol})$) of EC and *Vd* of oxidases (O) and hydrolases (H) during thaw months (June to October) and freeze months (November to May) simulated by the MEND-FT. Error bar denotes standard error, n = 15.

In addition, we have added a concise mechanistic explanation as follows.

Discussion (Line 362–369, Page 18): "These seasonal patterns are consistent with shifts in active microbial fractions under freeze-thaw dynamics (Supplementary Fig. S5 c–d), highlighting the regulatory role of microbial dormancy. During thaw months, microbial

resuscitation is already near saturation, such that warming induces only minor changes in enzyme concentration and negligible changes in specific enzyme activity (*Vd*) (Supplementary Fig. S8). In contrast, during freeze months, the warming-induced increase in active microbial biomass strongly enhances *Vd* and enzyme concentrations, leading to pronounced increases in enzyme activity (Supplementary Fig. S5 and S8)."

B12. *Line 227-228, It is not clear how the model produces enhanced N mineralization after adding the FT cycle. Both microbial biomass and rMBA decrease with MEND-FT, so how can a smaller active biomass lead to greater mineralization? The authors should examine additional model outputs to clarify this mechanism and, if possible, include supplementary figures showing the relevant processes or fluxes that drive this result.*

Response: Thank you for this insightful comment. We agree that additional model outputs are needed to clarify the underlying mechanism of enhanced N mineralization after adding the freeze-thaw regulation. Although the active fraction [r_{MBA} = MB_A/(MBA + MB_D)] decreases in MEND-FT during thaw months, this does not indicate a decline in active microbial biomass (MB_A). Instead, under warming, MB_A increases, while dormant biomass (MB_D) increases even more due to freeze–thaw dynamics, leading to a lower r_{MBA} during the thaw period. We have clarified this point in Fig. S5 and Response to **B9**.

Regarding N dynamics, we added Fig. S7, which shows that the enhanced inorganic N pools (N_{inorg}) and net N mineralization ($N_{mn\text{-}net}$) in MEND-FT are driven primarily by a weaker warming stimulation of microbial N immobilization (N_{im}) relative to N mineralization (N_{mn}) during thaw months. In particular, the maximum specific microbial N immobilization rate is much less responsive to warming in MEND-FT than in MEND-old, resulting in higher ($N_{mn\text{-}net}$) and larger N_{inorg} pools. The time series of $\Delta N_{mn\text{-}net}$ (i.e., difference between warming and control) further indicates that this net effect varies with freeze-thaw cycles, particularly in the thaw months, where warming relaxes cold-temperature constraints on microbial processes. We have revised the text accordingly.

Discussion (Line 285–290, Page 14–15): "Enhanced simulation of N_{inorg} concentrations (Figs. 3c–d) further suggests that freeze-thaw dynamics influence nutrient limitations, primarily by shifting the balance between N mineralization (N_{mn}) and N immobilization (N_{im}), thereby increasing net N mineralization ($N_{mn\text{-}net}$ = N_{mn} − N_{im}) during thaw months (Supplementary Fig. S7). This aligns with meta-analysis findings that freeze-thaw events could increase soil NH_4^+ levels by promoting N release and disrupting soil aggregates (Ji et al., 2022)."

Figure S7 is shown as below:

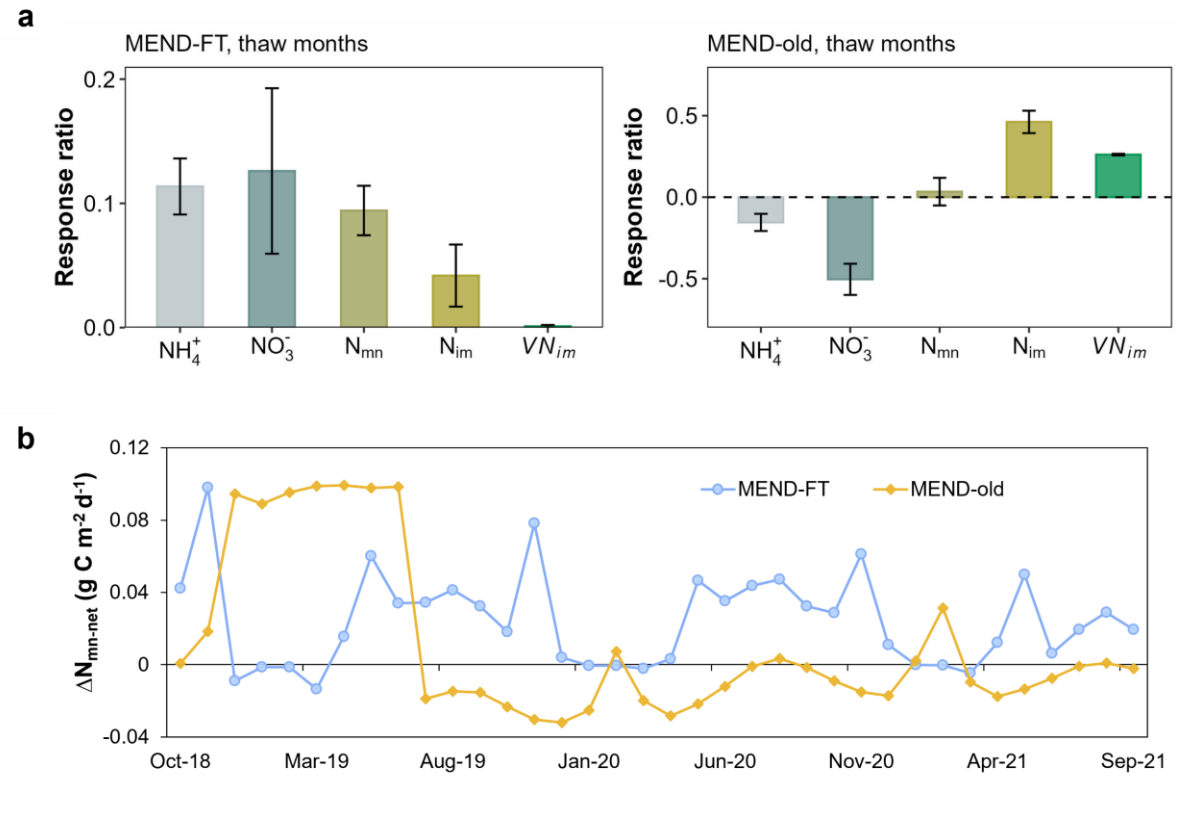

Fig. S7 | Simulated warming effects on inorganic N processes. (a) Response ratio (RR = $\ln(X_{Warming}/X_{Conrol})$) of NH_4^+ and NO_3^- pools, N mineralization (N_{mn}), N immobilization (N_{im}), and the maximum specific microbial N immobilization rate (VN_{im}) during thaw months (June to October) simulated by MEND-FT and MEND-old model. Error bar denotes standard error, $n = 15$. (b) Time series of warming-induced changes in net N mineralization flux ($N_{mn\text{-}net} = N_{mn} - N_{im}$), $\Delta N_{mn\text{-}net}$ denotes the difference of $N_{mn\text{-}net}$ between warming and control.

B13. *Line 239-244, again it is unclear why the new model achieved high CUE here compared to the MEND_old.*

Response: We have revised the text.

Discussion (Line 321–329, Page 16): "Microbial CUE, which regulates the balance between microbial growth and respiratory losses (Zhang et al., 2023; He et al., 2024), is also better represented in MEND-FT. Compared with MEND-old, MEND-FT simulates higher DOM availability, expressed as higher relative substrate availability (φ = 0.023/0.020 for control/warming vs. 0.009/0.007 in MEND-old) (Fig. 4c). This higher φ increases microbial uptake and growth fluxes while respiration remains comparable between models (Fig. 4c), resulting in higher simulated CUE in MEND-FT (Fig. 4a). Consequently, modeled CUE values increase from 0.20 (control) and 0.16 (warming) in MEND-old to 0.40 and 0.36 in MEND-FT.

These values align more closely with the reported mean CUE (0.42) for grassland ecosystems (He et al., 2023), yet remain lower than the ^{18}O-derived estimates (0.57 and 0.52)."

B14. *Line 278-279, Finally the authors explained here the possible reasons for CUE declined, but since this is a modeling study, the authors should be able to track all the changes to show exactly what occurred to decrease the microbial CUE.*

Response: We agree with the reviewer and have clarified the mechanisms underlying the simulated decline in microbial CUE by tracking changes in microbial uptake and respiration in Supplementary Fig. S9 (see below). We also revised the corresponding sentence in the main text.

Discussion (Line 370–376, Page 18–19): "Unlike prior research that reported limited CUE responses to warming based on a single sampling point (Zhang et al., 2023), our analysis over the 3-year experimental period reveals a slight but consistent decline in microbial CUE (Fig. 5c–d). During thaw months, warming increases microbial uptake, but active microbial respiration is stimulated more stronger, leading to a net reduction in CUE relative to the control (Supplementary Fig. S9a). In contrast, during freeze months, both microbial uptake and respiration remain low, and their relative changes under warming are small, consistent with no pronounced reduction in microbial CUE (Fig. S9b)."

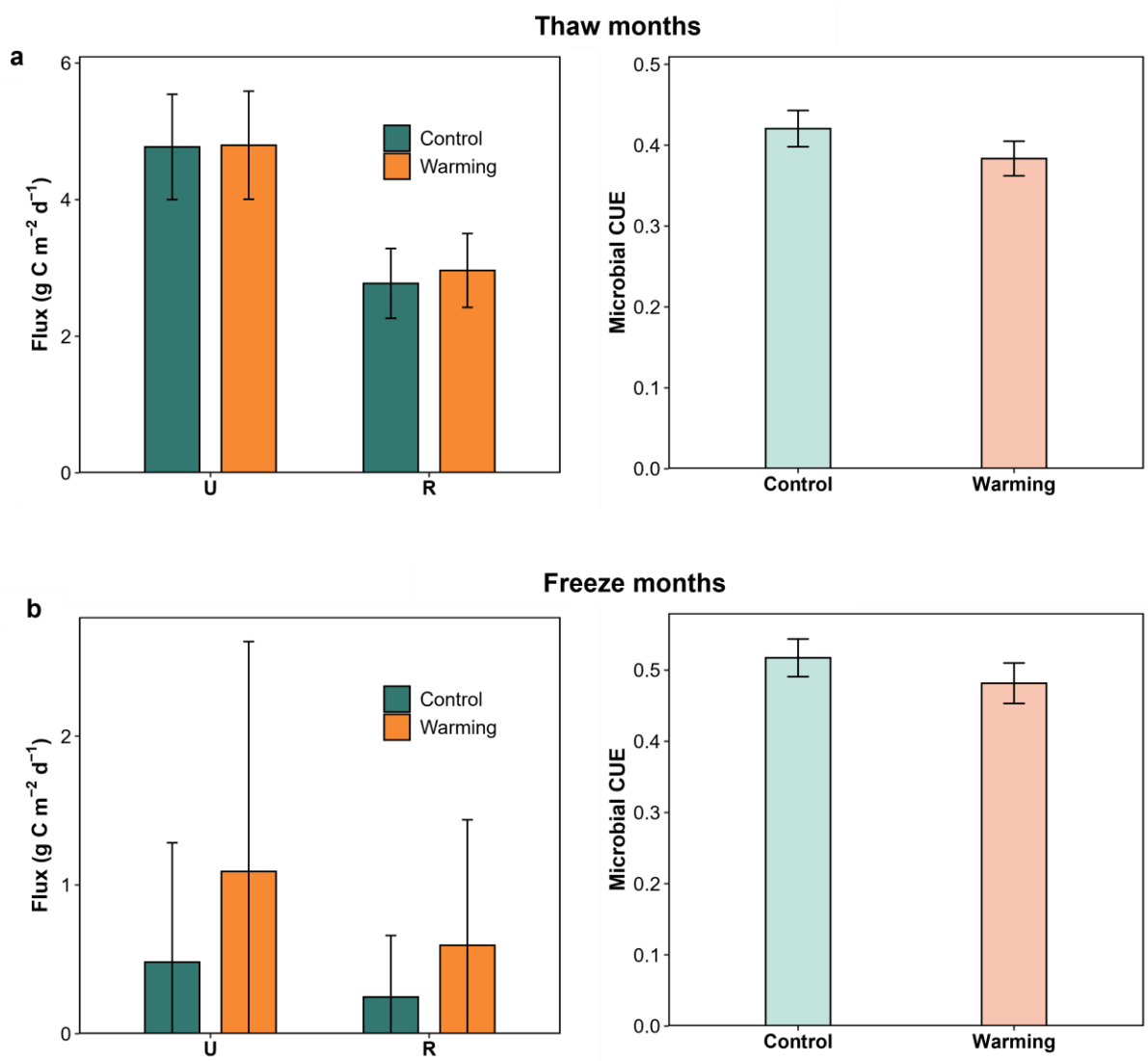

Fig. S9 | Seasonal microbial uptake, respiration and carbon use efficiency (CUE) simulated by the MEND-FT model. Shown are microbial uptake (U) and respiration (R) (left panels, mean ± s.d.), and CUE (right panels, mean ± s.d.) under control and warming treatments. Results are summarized for thaw months (June–October; n = 15) and freeze months (November–May; n = 21). Microbial CUE is calculated as (U − R)/U.

B15. *Line 287-289 Does the MEND-FT model include a dynamic, C-targeting enzyme allocation scheme? What mechanisms allow it to capture the relatively stronger increase in oxidative versus hydrolytic enzymes under warming, while the MEND-old can't?*

Response: Thanks for the insightful comments. To address this concern, we added the following discussion:

Discussion (Line 400–416, Page 20–21): "Both MEND-old and MEND-FT simulate a relatively stronger increase in oxidative than hydrolytic enzyme activities under warming in the long-term simulations (Fig. 5e and Supplementary Fig. S6), consistent with empirical evidence

for enhanced investment in enzymes that degrade recalcitrant substrates like lignin (Feng et al., 2007; Seo et al., 2015; Chen et al., 2020). In the MEND framework, oxidative and hydrolytic enzymes are represented by separate POM (particulate organic matter) decomposition pathways, each with distinct environmental response functions and are pool-size dependent. Because the lignin-like POM pool (POM_O) is larger than the cellulose-like pool (LF_0 = $POM_O/(POM_O+POM_H)$ = 0.61 (Zhang et al., 2023); Table S5) , enzyme production is preferentially routed through the oxidative pathway. The MEND model further applies different soil water potential scalars to these two enzyme groups (see Supplementary Text 1.2.3). Together, these mechanisms drive a stronger increase in oxidative than hydrolytic enzyme activities in the long-term warming. The key distinction in MEND-FT is the incorporation of freeze-thaw controls on POM decomposition. This addition influences the seasonality of enzyme dynamics, as the freeze-thaw cycle affects the microbial community's activity and enzyme production patterns across seasons (Fig. S5). As a result, MEND-FT captures a more dynamic response in oxidative enzyme activities, particularly during freeze-thaw transitions, compared to MEND-old, which lacks this seasonal regulation."

B16. *Line 305-307 Did the authors also conduct long-term projections using MEND-old? If so, did MEND-old predict similar trends in microbial biomass and enzyme activities? Presenting both would clarify whether the freeze–thaw mechanism truly alters long-term outcomes. At least a summary in Supplementary Information would be valuable.*

Response: Thank you for the insightful suggestion. We have conducted parallel long-term projections using MEND-old and added the results to the Supplementary Information (Fig. S6):

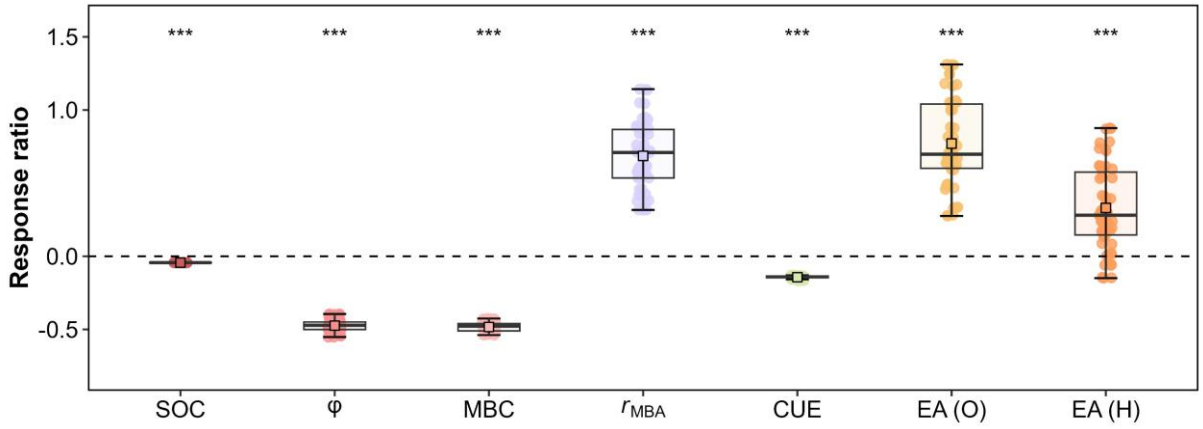

Fig. S6 | Long-term (50-year) warming effects on soil process and microbial activity simulated by MEND-old model. Soil variables include soil organic carbon (SOC), substrate availability (φ = DOM/(DOM + KsDOM)), and microbial biomass carbon (MBC). Microbial

activity is expressed as active fraction (r_{MBA}), microbial carbon use efficiency (CUE), and enzyme activities (EA) of oxidases (O) and hydrolases (H). The dots represent yearly modeled values. Differences between RR values and 0 were tested using the Wilcoxon test. "***" denotes a significance difference at $p < 0.01$; "NS." means not significant ($p > 0.05$).

We have also revised the manuscript text to explicitly incorporate this comparison:

Discussion (Line 383–392, Page 19): "Although MEND-old qualitatively agrees these two mechanisms (Fig. S6), the two model formulations imply different microbial strategies that steer ecosystem processes toward a new quasi-steady state over the long run. MEND-FT shows more moderate soil and microbial responses to warming, characterized by a near-stable r_{MBA} (mean RR = 0.01) together with increased total microbial biomass (RR = 0.12) (Fig. 5e), whereas MEND-old predicts declining total biomass (mean RR = −0.48) accompanied by an increasing r_{MBA} (RR = 0.68) (Fig. S6). Consistently, warming-induced amplification of oxidase activity is much weaker in MEND-FT (RR = 0.2) than in MEND-old (RR = 0.8). This demonstrates that the inclusion of freeze-thaw microbial regulation not only influences short-term dynamics but reshapes projected long-term soil responses to warming."

B17. *Line 330-363: The use of ALTscalar suggests that the model represents soil as a single layer, with freeze–thaw effects scaled by the thawed depth fraction. Could this simplification introduce uncertainties compared to a vertically resolved soil model? Would a multi-layer implementation, with microbial dormancy driven directly by depth-dependent temperature and moisture, produce similar outcomes?*

Response: Thank you for raising this important point. The ALTscalar captures the fraction of the soil profile that is thawed, effectively accounting for vertical heterogeneity in thermal-hydrological conditions without the need to explicitly resolving multiple soil layers. This approach was adopted to balance process representation with parameter identifiability and computational efficiency. One of the additional advantages of the ALTscalar is that it avoids the complex representation of liquid-ice phases of soil water (Bao et al., 2016; Gao et al., 2019), which would be required in a multi-layer model. This single-layer ALTscalar simplification makes the model more tractable by sidestepping the need for detailed phase transitions between liquid and frozen states of water within soil layers.

While a multi-layer implementation, where microbial dormancy and activities are driven by depth-dependent temperature and moisture conditions, could provide a more mechanistic description of these processes, it would substantially increase model complexity and parameter requirements. This increase in complexity does not necessarily translate to improved predictive skill, especially given the uncertainty in accurately parameterizing depth-specific dynamics. Therefore, the ALTscalar approach offers a practical alternative, with fewer parameters and

lower computational cost. That said, a vertically resolved model remains a promising direction for future work, particularly to enhance the mechanistic representation of depth-dependent thermal-hydrological effects. We have added the following sentences:

Discussion (Line 438–441, Page 22): "Moreover, the current MEND framework does not account for a multi-layer structure, so microbial processes are not yet driven by depth-dependent thermal-hydrological conditions. Closing these observational and structural gaps is a promising direction for future work."

B18. *Fig. S3a There is no error bar here?*

Response: We have added the error bar in Fig. S3a:

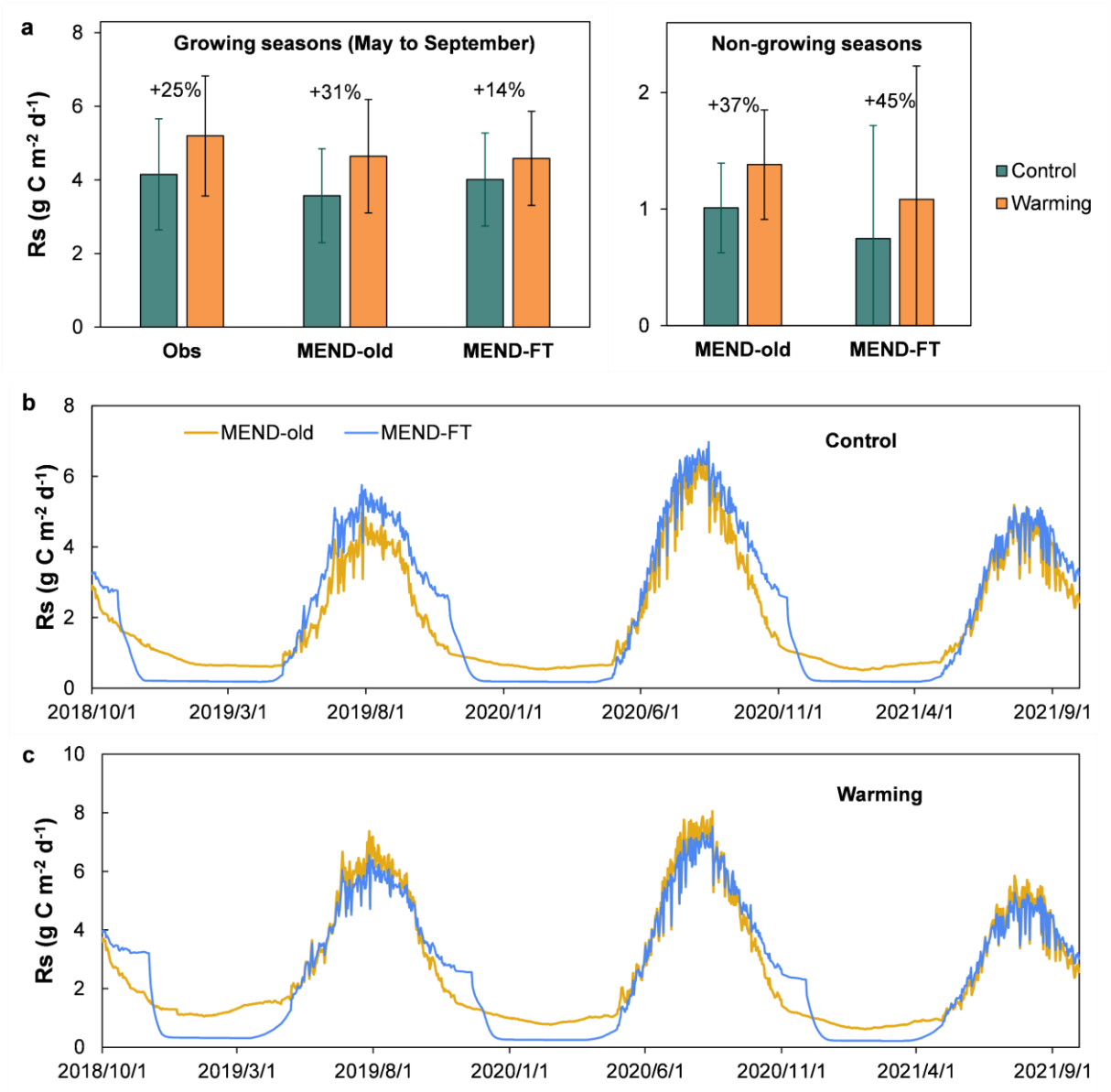

Fig. S3 | Soil respiration (Rs) responses to warming and continuous daily simulations. (a)

Warming effects on Rs during the growing and non-growing seasons. Error bar denotes standard deviation. (b–c) Simulated daily Rs under control (b) and warming (c) treatments.

B19. *Table S7 This table clarifies a bit why many figures show data for only one or two months, but it is never cited in the main text or figure captions. Please reference it explicitly. It also highlights large differences in data frequency between microbial and SOC observations, which may affect the weighting in the multi-objective calibration, which the authors should discuss. Plus, the authors should discuss the uncertainties from relying on such limited microbial data for model calibration and validation.*

Response: Thank you for the comment. We have now referenced Table S7 in the main text:

Results (Line 116–117, Page 6): "Details of the model calibration procedure, a including the weighting of response variables and sensitivity analyses, are provided in Supplementary Text 3.4 and Table S7–S8."

We also expend the discussion about the uncertainties arising from the limited availability of microbial observations.

Discussion (Line 432–441, Page 21–22): "Several priorities stand out for future experiment-model integration. Many alpine field studies report soil C-N variables only during the growing season or at a few discrete time points, and microbial measurements are often limited to a single sampling, including this study (Table S7). Expending observations, particularly of soil C-N fluxes and microbial activity during freeze months, could substantially reduce uncertainty in the parameterization, given this period appears highly sensitive to climate warming (Lei et al., 2024; Lyu et al., 2024). As additional data, processes, and feedback mechanism become available, model inferences may evolve accordingly. Moreover, the current MEND framework does not account for a multi-layer structure, so microbial processes are not yet driven by depth-dependent thermal and hydrological conditions. Closing these observational and structural gaps is a promising direction for future work."

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Microbial dormancy under freeze–thaw cycling regulates alpine soil responses to warming

(COMMSENV-25-4161-A)

Response to Comments from Reviewers

We have addressed the reviewers' comments and questions point-by-point. The original reviewer's comments are *italicized*, and our Response to the reviewer's comments follow. The numbers of lines and pages in the text are referred to the revised version.

Response to Reviewer #2:

1. *This manuscript presents a timely and valuable study examining how freeze–thaw cycles and microbial dormancy regulate soil carbon processes in alpine ecosystems. The authors have undertaken a very careful and thorough revision in response to my previous comments and provided clear, point-by-point responses to all my questions. I appreciate the substantial effort that went into this revision. In particular, the clarification of model assumptions and calibration processes, the much-improved discussion of microbial physiological responses simulated by the old and the new model, and the expanded explanations of the freeze–thaw–dormancy mechanisms have strengthened both the transparency and interpretability of the work.*
Overall, I find that the authors have addressed my previous concerns, and the manuscript now presents a clear mechanistic framework linking freeze–thaw dynamics, microbial dormancy, and soil carbon responses to warming. The integration of experimental observations with the MEND-FT model provides a meaningful contribution to understanding cold-region carbon cycling and its sensitivity to climate change. I am pleased to see the manuscript strengthened through revision and only have two very minor comments for the authors to consider before publication:

Response: We thank the reviewer for the careful evaluation of the revised manuscript and for the encouraging comments on the clarity and mechanistic framework of the study!

2. *Figures S7 and S9 appear to have visible outline artifacts around the panels. Please check the export/rendering settings to ensure consistent formatting.*

Response: Thank you for noting this formatting issue. We have revised Supplementary Figs. S7 and S9, which are shown below:

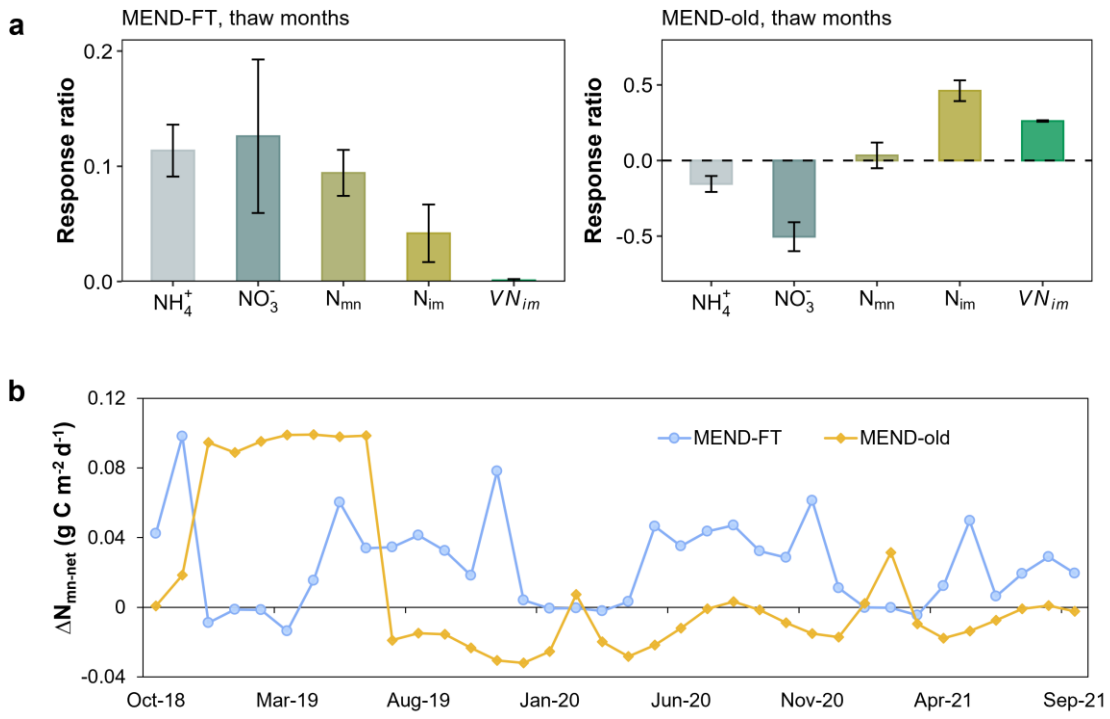

Fig. S7 | Simulated warming effects on inorganic N processes. a Response ratio (RR = $\ln(X_{Warming}/X_{Conrol})$) of NH_4^+ and NO_3^- pools, N mineralization (N_{mn}), N immobilization (N_{im}), and the maximum specific microbial N immobilization rate (VN_{im}) during thaw months (June to October) simulated by MEND-FT and MEND-old model. Error bar denotes standard error, n = 15. **b** Time series of warming-induced changes in net N mineralization flux ($N_{mn\text{-}net} = N_{mn} - N_{im}$), $\Delta N_{mn\text{-}net}$ denotes the difference of $N_{mn\text{-}net}$ between warming and control.

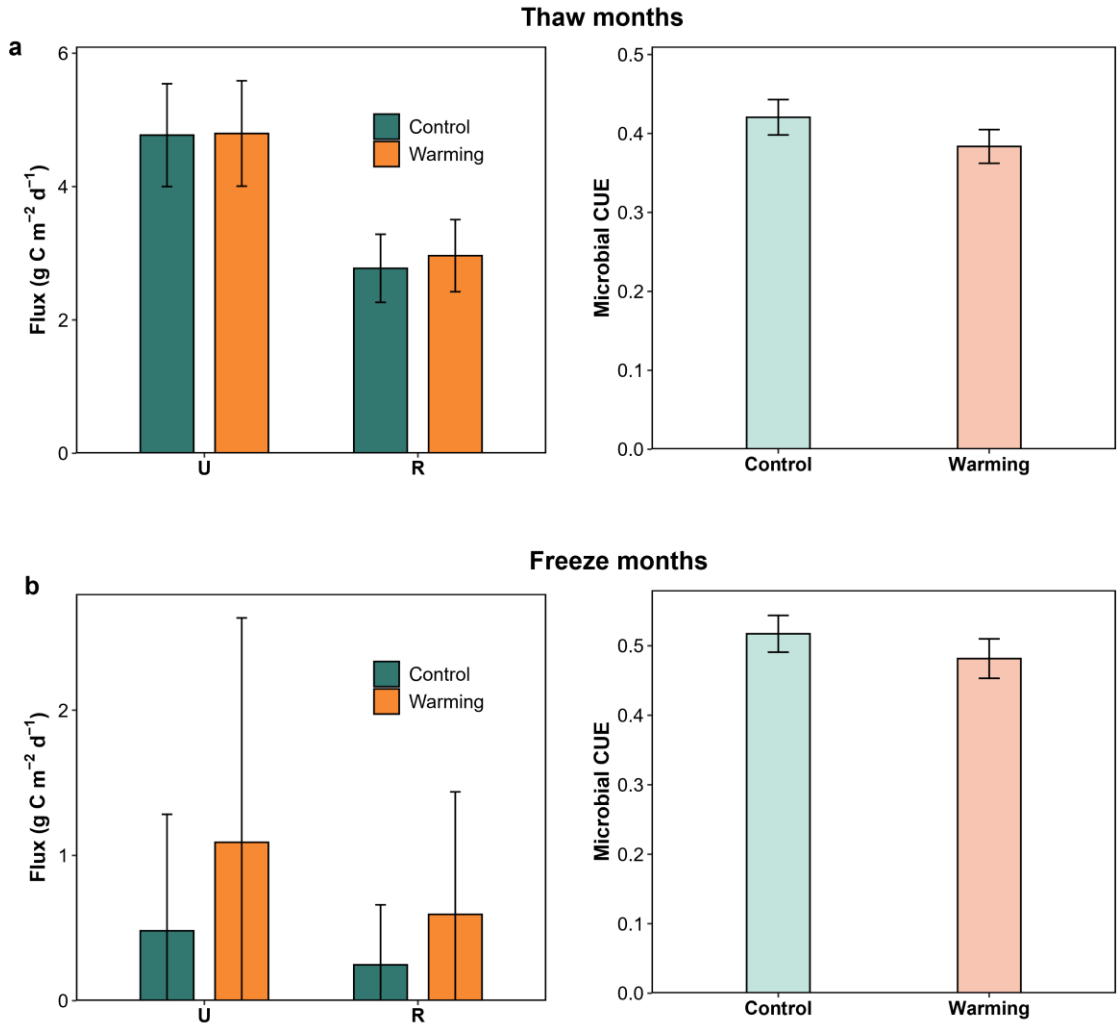

Fig. S9 | Seasonal microbial uptake, respiration and carbon use efficiency (CUE) simulated by the MEND-FT model. Shown are microbial uptake (U) and respiration (R) (left panels, mean ± s.d.), and CUE (right panels, mean ± s.d.) under control and warming treatments. Results are summarized for thaw months (June–October; n = 15) and freeze months (November–May; n = 21). Microbial CUE is calculated as (U − R)/U.

3. *In the Discussion (Lines 370–376), the text suggests that reductions in freeze months are less pronounced than those in thaw months. Based on the figure, both appear to show small net reductions, and the relative difference is not visually clear. Please clarify or quantify this comparison.*

Response: Thank you for pointing this out. We have revised the corresponding text to quantify the comparison:

Discussion (Line 370–377, Page 18–19): "Unlike prior research that reported limited CUE responses to warming based on a single sampling point (Zhang et al., 2023), analysis over the three-year experimental period reveals a slight but consistent decline in microbial CUE (Fig.

5c–d). During thaw months, warming increases microbial substrate uptake, but microbial respiration is stimulated more strongly, leading to a net reduction in CUE relative to the control (0.42 under control vs. 0.38 under warming; Supplementary Fig. S9a). In contrast, during freeze months, both microbial uptake and respiration remain low, and their responses to warming are modest, resulting in only a slight decline in microbial CUE (0.52 vs. 0.48; Supplementary Fig. S9b)."

References:

Zhang, Q., Qin, W., Feng, J., Li, X., Zhang, Z., He, J.-S., Schimel, J.P., Zhu, B., 2023. Whole-soil-profile warming does not change microbial carbon use efficiency in surface and deep soils. Proceedings of the National Academy of Sciences 120, e2302190120.

This manuscript presents a timely study that examines how freeze–thaw cycles and microbial dormancy influence soil C processes in alpine systems. Extending MEND model to MEND-FT by incorporating Freeze-Thaw (FT) influenced microbial dormancy and testing it against field warming experiments on the Qinghai–Tibet Plateau and found the new model performed better on simulating microbial physiology and showed increased warming effects in non-growing season adds great value in understanding how microbial processes regulate alpine soils to warming. The paper is clearly written, and results show are noteworthy. My major concern is that the authors did not clearly explain the linkages between the added new freeze–thaw–dormancy processes in the model and the enhanced performance on CUE, enzyme activity, and Ninorg, etc. I recommend the manuscript to be published after addressing the following major and minor comments I have:

Major: The manuscript would benefit from a clearer mechanistic explanation linking the newly implemented freeze–thaw–dormancy processes to the model's improved performance in simulating microbial CUE and enzyme production. It is not clear why MEND-FT predicts a lower CUE than MEND-old (Fig. 4a), or why MEND-FT captures the observed decrease in enzyme activity in August 2021 while generally predicting higher enzyme activity under warming elsewhere. Throughout the manuscript, the authors emphasize that model outputs align with observations, but they do not explain the underlying mechanisms driving this agreement. As this is a modeling study, I encourage the authors to present key intermediate variables or fluxes to illustrate how the new processes translate into these outcomes and thereby clarify the causal chain within the model.

Line 22: This work doesn't seem to involve any genes data, or am I missing something here?

Line 51: "substrate availability for decomposition" is not essentially physical soil process, but a biological one.

Line 74: Does the approach/framework in this manuscript incorporates genes data?

Line 115-131, How are all these objects weighted in the multi-object weighted calibration, I could not find the values of the different weights w_i in the Supplementary Info. Have the authors tested alternative weighting schemes to evaluate sensitivity? The uncertainties or rationale for these weights should be discussed, at least in the Supplementary Information.

Fig. 3, For MBC and Ninorg, why is the results in 2020 not shown here and why only Augst? Is that the only data found in that existing literature? The authors should state the data availability and span in the "Study site and data compilation" section in the Methods without readers having to going back to the literature to check.

Fig. 4, why is only one month of one year (August 2021) was included in here? Is it because you only had microbial data in that particular month? The authors referred all the microbial data

they compared to into one sentence from line 437 to 439, they should be clearer about which months and years are the data available there.

Fig. 5a showed that the rMBA on the rise most of the time during the experimental years even in control scenarios, which left me wondering if the model is not spun up to steady state. Could the authors provide any info on the model spin-up and steady state in the setup?

In Fig. 5b, rMBA seems to decrease with warming in the short-term experiment, yet in the long-term warming projection (Fig. 5e) it slightly increases. The authors should discuss why the short- and long-term responses differ.

Also again why in Fig.5c-d, Why are only one thaw month and one freeze month shown in Fig. 5c–d? Even if patterns across different years' thaw-freeze months are similar, the authors should consider aggregating all thaw/freeze months or moving other months' results to Supplementary Figures. Also, please clarify in the caption whether the dots represent daily modeled values.

Line 204-210, it's unclear and underexplored here, why adding the freeze-thaw dormancy leads to less enzyme production. Here the authors explained as "This improvement is due to the model's representation of enzyme production depending on microbial biomass and substrate availability", but I believe both MEND_old and MEND_FT has this representation. The difference seems to stem from MEND-FT simulating less microbial biomass under warming (Fig. 5b), but the mechanism behind this remains unexplained. Is it due to reduced CUE with warming in MEND-FT? And if so, why does this occur in MEND-FT but not in MEND-old?

Moreover, the decline in enzyme production appears mainly in the growing season, while oxidative enzyme activity increases during thaw and frozen months (Fig. 5). The authors should clarify why enzyme responses differ across seasons and, if possible, show intermediate variables or model outputs to illustrate these contrasting behaviors.

Line 227-228, It is not clear how the model produces enhanced N mineralization after adding the FT cycle. Both microbial biomass and rMBA decrease with MEND-FT, so how can a smaller active biomass lead to greater mineralization? The authors should examine additional model outputs to clarify this mechanism and, if possible, include supplementary figures showing the relevant processes or fluxes that drive this result.

Line 239-244, again it is unclear why the new model achieved high CUE here compared to the MEND_old.

Line 278-279, Finally the authors explained here the possible reasons for CUE declined, but since this is a modeling study, the authors should be able to track all the changes to show exactly what occurred to decrease the microbial CUE.

Line 287-289 Does the MEND-FT model include a dynamic, C-targeting enzyme allocation scheme? What mechanisms allow it to capture the relatively stronger increase in oxidative versus hydrolytic enzymes under warming, while the MEND-old can't?

Line 305-307 Did the authors also conduct long-term projections using MEND-old? If so, did MEND-old predict similar trends in microbial biomass and enzyme activities? Presenting both would clarify whether the freeze–thaw mechanism truly alters long-term outcomes. At least a summary in Supplementary Information would be valuable.

Line 330-363: The use of ALTscalar suggests that the model represents soil as a single layer, with freeze–thaw effects scaled by the thawed depth fraction. Could this simplification introduce uncertainties compared to a vertically resolved soil model? Would a multi-layer implementation, with microbial dormancy driven directly by depth-dependent temperature and moisture, produce similar outcomes?

Fig. S3a There is no error bar here?

Table S7 This table clarifies a bit why many figures show data for only one or two months, but it is never cited in the main text or figure captions. Please reference it explicitly. It also highlights large differences in data frequency between microbial and SOC observations, which may affect the weighting in the multi-objective calibration, which the authors should discuss. Plus, the authors should discuss the uncertainties from relying on such limited microbial data for model calibration and validation.