

We thank both reviewers for their careful, thoughtful, and constructive evaluation of our manuscript. We greatly appreciate the positive overall assessment of the study and the recognition of its contribution to understanding soil organic matter dynamics and warming-induced carbon losses in subalpine soils. The reviewers' comments were very helpful in identifying areas where the manuscript could be improved, particularly with regard to clarity of presentation, methodological transparency, precision in interpretation, and a clearer discussion of the scope and limitations of the incubation experiment.

In response, we revised the manuscript throughout to improve readability, strengthen the links between vegetation history, microbial dynamics, and SOM turnover, clarify key aspects of the experimental design and analytical methods, expand the discussion of priming, and more explicitly acknowledge the mechanistic nature and limitations of the study.

We believe that the revised version is now clearer in its methodological description, more cautious and precise in its interpretation, and more explicit about the mechanistic scope and limitations of the study. We hope that the changes made adequately address the concerns raised and that the revised manuscript is now suitable for publication. Below, we provide a detailed point-by-point response to all comments and indicate where corresponding changes were made in the revised manuscript.

The **line numbers** cited below refer to the manuscript version with tracked changes.

#### Reviewer 1:

*This manuscript presents a well-designed and carefully executed laboratory incubation study investigating the effects of temperature, litter input, and microbial community dynamics on soil organic carbon turnover in subalpine forest and pasture soils. The combination of respiration measurements,  $\delta^{13}\text{C}$  tracing, priming calculations, and PLFA-based microbial analyses provides a robust and comprehensive framework to address soil carbon–climate feedbacks. The results are clearly presented and well discussed in the context of existing literature. Overall, the study makes a valuable contribution to the understanding of SOM dynamics under warming in alpine and subalpine ecosystems. The manuscript is of high quality, and I only suggest minor revisions aimed at improving clarity, methodological transparency, and precision in interpretation.*

*1) Line 35: Link between vegetation shifts and SOM dynamics: The section discussing vegetation changes (treeline advance, shrub encroachment, abandonment of pastures) is thorough and well supported by literature. To further strengthen this part, the link between vegetation-driven changes (e.g. litter quality and quantity) and the observed SOM and microbial dynamics could be made more explicit. A short synthesis paragraph highlighting these mechanistic connections would improve coherence.*

**We agree and thus added a short synthesis paragraph after the vegetation-change section (L. 48ff.) to more explicitly link vegetation-driven changes (litter quantity/quality) to the expected microbial and SOM responses in our incubation framework.**

*2) Line 46: The statement that a 10 °C temperature increase can double or triple soil respiration is well established, but in alpine and subalpine soils temperature responses are often non-linear and constrained by substrate availability and microbial adaptation. While this complexity is addressed roughly in this passage, a slightly more precise wording would improve this part.*

**We agree and revised this section. In the new version, the previous broad formulation has been replaced by more precise wording (L. 62ff) that explicitly frames the two- to threefold**

response as a first-order approximation, and clarifies that realized temperature responses depend on substrate availability, substrate quality, accessibility of organic matter, microbial acclimation, and community shifts under warming. We also explicitly note that warming responses in alpine and subalpine soils can be non-linear and may weaken over time. This more carefully reflects current understanding and directly addresses the reviewer's concern.

*3) Line 113: The collection of approximately 30 kg of mineral soil from a depth of 5-10 cm implies substantial soil disturbance. It would be helpful to clarify whether this amount was obtained by pooling multiple spatially distributed subsamples or by excavating a single location. This information is relevant for assessing representativeness and spatial heterogeneity.*

**The samples were taken from one single location at an area of approximately 1 m<sup>2</sup>, we added this information to the text (L. 146).**

*4) PLFA Analysis: Please clarify whether recovery rates were used to assess extraction efficiency in the PLFA analyses.*

**PLFA quantification was performed using an internal standard (PC19:0), and an additional control standard (D<sub>39</sub>C<sub>20</sub> acid) was added before methylation. These standards were used to control quantification and derivatization performance. In addition, extraction efficiency was assessed at least partly through recovery of the added intact PLFA standard, and methodological comparisons in our earlier study helped optimize yields. Based on internal laboratory experience and tests, recovery of the standard was generally around 80%, but in some cases was lower, occasionally in the range of 50–60%. We also note that method development and improved yields were discussed in Zosso and Wiesenberg, J. Microbiol. Methods (2021), where different methodological approaches were compared. As these methods are described in this paper, we do not explicitly mention the assessment of the extraction efficiency in the revised manuscript.**

*5) The 360-day incubation period is appropriate to capture both short-term and longer-term dynamics. However, it remains unclear how frequently samples were taken during the incubation. Sampling frequency maybe better move from Supplement to main manuscript.*

**We agree and added this to the method section (L. 169-171), while keeping the detailed schedule in the Supplement. Shortly, in the beginning of the incubation we had a higher sampling frequency for the respired CO<sub>2</sub> of about 3 days, at later stages of 2 weeks. Soil samples were destructively sampled at 6 points throughout the incubation.**

*6) Some sentences are very long, occasionally affecting readability. Maybe shorten sentences to improve readability.*

**We shortened and split several sentences in the text to improve readability.**

*7) The large number of reported percentages and time points could be summarized more clearly in overview tables or schematic figures.*

**We improved readability and interpretative structure, and we also added relative abundance information for PLFAs in Supplementary Fig. S1, which helps contextualize the absolute PLFA concentration data.**

## Reviewer 2 (Jérémy Puissant):

*This manuscript is well described, detailed, and clearly written. I enjoyed reading it, and it represents a valuable contribution to the field by providing mechanistic insights into how warming influences carbon losses from subalpine soils and how microbial processes may modulate the timing of these responses. Several points would benefit from clarification before publication. In particular, given the limited site replication and the nature of the incubation experiment, some conclusions—especially those related to vegetation effects and temperature optima—could be interpreted more cautiously. I therefore suggest adding a short “limitations of the study” paragraph to clarify the scope and generality of the findings, especially with respect to points 3–5 below. The aim of these comments is not to downplay the results, but rather to more precisely define what this study contributes to the field and to clearly state its limitations, so that its mechanistic insights can be interpreted appropriately and provide a robust basis for future work. The comments below focus on several key points that would benefit from detailed clarification and response, as they influence the interpretation and scope of the conclusions.*

### *Major comments*

#### *1) Soil moisture control and incubation conditions*

*Additional details on soil moisture control would help ensure that the higher litter-induced respiration (LIR) observed at 16.5 °C compared to 20.5 °C reflects biological responses rather than methodological effects.*

*Please clarify: How soil moisture was controlled throughout the experiment; The target soil moisture used (gravimetric water content or %WHC); How often soil moisture was checked and adjusted; Whether differences in vapor pressure deficit among temperature treatments were considered; Whether a pre-incubation period was used to stabilize respiration before applying temperature treatments and litter addition; Whether soils were air-dried and rewetted or kept field-moist and stored cold prior to incubation (and for how long). These details are important for interpreting temperature effects on respiration.*

### **The revised manuscript now clarifies that:**

- **soils were stored for 10 months in a cool, dark room in covered buckets that allowed air circulation while avoiding complete air drying (L. 148-150)**
- **the experiment included a two-week pre-incubation conditioning phase before litter addition (L. 160f.)**
- **soil moisture was adjusted to field capacity at the start of pre-incubation (L. 161)**
- **soil moisture was checked gravimetrically at least biweekly and adjusted when necessary (L. 161f.)**
- **vials containing water were placed inside each jar to reduce evaporation and minimize differences in headspace humidity among temperature treatments (L. 162f.)**
- **the core design was intentionally mechanistic, with seasonal and daily temperature fluctuations omitted (L. 152ff.)**

#### *2) Link between hypotheses and discussion*

*The Introduction presents three main research questions/hypotheses, but these are not always explicitly revisited in the Discussion. Structuring the Discussion more clearly around these hypotheses, or explicitly stating whether each is supported, would improve readability.*

*Priming is mentioned in two hypotheses but is only briefly discussed, despite being a central*

*component of the results. A more explicit treatment of priming in the Discussion would strengthen the manuscript.*

**We agree and revised the Discussion so that the hypotheses are revisited explicitly and priming is discussed much more clearly. The revised manuscript now states that the second hypothesis was confirmed, but that the temperature effect on priming was expressed mainly in the early phase rather than as a sustained increase over the full incubation; the third hypothesis was only partly confirmed, because pasture rather than forest showed slightly higher overall priming; and the first hypothesis was only partly supported, because cumulative LIR peaked at intermediate rather than highest temperature. In addition, priming now receives a dedicated and much more explicit treatment in the Discussion, especially regarding its timing, transience, and coupling to litter-derived respiration and microbial biomass.**

**Priming discussion: Paragraph starting L. 386; L. 438ff.; L. 472; L. 480ff.; L. 526ff.; L. 553ff.**

**Hypothesis 1 revisited: L. 499ff.**

**Hypothesis 2 revisited: L. 394ff.**

**Hypothesis 3 revisited: L. 441ff.**

### *3) Duration and realism of the incubation experiment*

*A one-year incubation at constant temperatures represents a strong treatment for subalpine soils. The 12.5 °C treatment corresponds to growing-season temperatures, whereas these ecosystems normally experience long periods of cold temperatures and snow cover. It would be helpful to clarify that this experiment is primarily intended for mechanistic or hypothesis testing, rather than for direct simulation of field conditions, and to discuss how the results should be interpreted in relation to natural climate scenarios.*

**We clarified this point in both the Methods (L. 152ff.) and Discussion (L. 508ff.). The revised manuscript now explicitly states that the incubation design was intended to improve mechanistic understanding of SOM decomposition and microbial dynamics during the growing season and under future warming, and that seasonal effects and daily temperature changes were omitted deliberately to simplify the design. We also now state that the 360-day period is longer than the local growing season and may correspond roughly to about three growing seasons, and we explicitly acknowledge the artificial nature of the design and omission of other fresh organic inputs such as root exudates and seasonal litterfall. In the Discussion, we further clarify that the experiment primarily resolves process-level responses during biologically active phases rather than reproducing full field conditions with snow cover and seasonal transitions.**

### *4) Interpretation of litter-induced respiration (LIR)*

*Cumulative litter-induced respiration ( $LIR = R(L+) - R(L-)$ ) integrates both litter-derived  $CO_2$  and priming-induced SOM mineralization. Because priming is strong, transient, and temperature-dependent (Fig. 4), the statement (line 388) that “the LIR optimum was consistent across both soils ... suggesting that microorganisms decomposing the litter operate near their physiological optimum at this temperature” implicitly interprets LIR as litter decomposition.*

*Without explicitly presenting isotopically partitioned litter-derived  $CO_2$  fluxes, the observed LIR maximum at 16.5 °C cannot be uniquely attributed to a physiological optimum of litter-*

*decomposing microorganisms. It may instead reflect differences in priming dynamics, microbial biomass persistence, carbon use efficiency, stress-related community shifts at 20.5 °C, or the integration of short-lived respiration pulses in cumulative fluxes. Tempering this interpretation and, if possible, presenting cumulative priming and/or litter-derived CO<sub>2</sub> fluxes would help clarify the underlying mechanisms.*

**We agree that LIR represents the net effect of litter addition and therefore includes both litter-derived CO<sub>2</sub> and priming-induced changes in native SOM mineralization. In the original manuscript, the wording in Section 4.3 was too strong because it implicitly treated cumulative LIR as a proxy for litter decomposition alone. In the revised manuscript, we therefore clarified in the Methods that LIR integrates both components (L. 208f.), while priming was quantified using isotopic partitioning of respired CO<sub>2</sub>. Our results show that litter contributed strongly to respiration immediately after addition, as indicated by the strong initial <sup>13</sup>C enrichment of respired CO<sub>2</sub> in litter-amended treatments (Fig. 3b), whereas priming was short-lived, peaking within the first week and declining to near zero by about day 28 (Fig. 4). At the same time, we agree that Fig. 3b does not explicitly show partitioned cumulative fluxes. We therefore revised the Discussion to interpret the LIR maximum more cautiously as the result of combined litter mineralization, transient priming, microbial biomass dynamics, and possible stress effects at 20.5 °C, rather than as evidence for a physiological optimum of litter decomposers alone (L. 460ff.; L. 472f.).**

#### *5) Site replication and ecosystem effects*

*Only two sites (one forest and one pasture) are included. As a result, site-specific effects are confounded with ecosystem type. It would therefore be helpful to acknowledge more explicitly that conclusions regarding vegetation effects are based on limited replication and should be interpreted cautiously.*

**We agree and made this limitation explicit in two places. First, when discussing the priming and vegetation-history contrasts (L. 397ff.), we now state that the comparison is based on one forest and one pasture site, and that site-specific properties cannot be fully separated from vegetation effects. Second, in the broader implications section (L. 543ff.), we explicitly note that the vegetation-related differences should be interpreted cautiously because vegetation cover and site identity were not independently replicated. We hope this addresses the reviewer's concern directly and more explicitly than in the previous version.**

#### *6) PLFA interpretation*

*PLFA results are reported as absolute concentrations (μg g<sup>-1</sup>), but presenting relative abundances (%) would help support statements regarding changes in community composition. Interpretations of cyclopropyl PLFAs as stress indicators could also be phrased more cautiously, as increases in cyclopropyl fatty acids can reflect stationary phase, nutrient limitation, or general stress, not exclusively heat stress.*

**We agree and addressed this point in two ways. First, to better support interpretation of microbial community composition, we added the relative abundances of the PLFA groups in the Supplementary Material (Fig. S1) and now refer to them in the Results (L. 317–337). Second, we revised the Discussion to interpret cyclopropyl PLFAs more cautiously. Instead of treating them as exclusive indicators of heat stress, we now**

**describe them as reflecting a broader microbial response that may include physiological stress, reduced growth efficiency, and accelerated depletion of readily available substrates, particularly at 20.5 °C (L. 460ff.).**

*Minor comments and clarifications*

*Line 23: Gobiet et al. do not directly demonstrate SOM vulnerability to climate change; please revise or update the reference.*

**We changed this in the revised manuscript and now cite Chersich et al., J. For. Sci. 61, 2015. (L. 23)**

*Line 47: Please clarify what is meant by “classical theory” (cite explicitly) or rephrase.*

**The revised manuscript no longer uses the vague formulation “classical theory.” Instead, it now refers to a “common first-order approximation” and then explains the factors that modify realized temperature responses. This is clearer and more precise (L. 62ff.).**

*Line 115: Please clarify the sampling strategy (field replication vs pooling).*

**We addressed this by explicitly stating that the soil was sampled at a single location for each site over an area of approximately 1 m<sup>2</sup> and at 5–10 cm depth (L. 146), followed by sieving, root removal, homogenization, and storage clarification.**

*Line 409: Forest stand age is given, but pasture age/history is not; please clarify.*

**We addressed this by adding information that the pasture has been present for at least approximately 160 years, and likely longer, consistent with the long history of pasture use in the region (L. 139f.). This complements the forest stand age information and improves comparability between the two land covers.**

*Lines 342 and 439: Avoid acronyms such as “L–” in the Discussion; spelling out terms would improve readability.*

**We have replaced the acronyms in the discussion to improve readability (L. 375; L. 405; L.537).**