

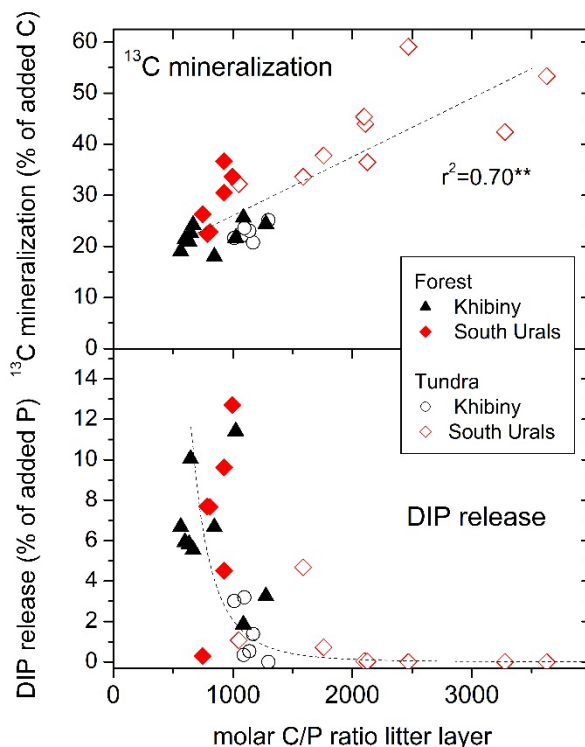
Responses to Reviewer #1

This is a nice paper about microbial C, N and P cycling in the litter layer of forest and tundra ecosystems. I have two suggestions and some rather minor comments.

Response: thanks a lot for your positive evaluation, the constructive comments, and the careful read. We incorporated all comments in the revised manuscript.

Reviewer: Concerning Fig. 8, I suggest adding a second panel that shows the percentage of respired ^{13}C (derived from glucose-6-phosphate). Based on this figure, the authors could discuss the loss of C in comparison to the loss of P.

Response: Thanks for the constructive suggestion. We have added a second panel to Figure 8 (now Figure 7 in the revised manuscript), clearly documenting that DIP release from added glucose-6-phosphate decreased with increasing molar C/P ratios despite an increase in ^{13}C mineralization along this trajectory.



These findings were used to discuss P immobilization, for example: “*This conclusion is supported by the experiment tracking the fate of glucose-6-phosphate (G6P). While 20–50% of the ^{13}C -labeled G6P was mineralized within 3 days in the litter layer, only a small fraction of the added P was released as phosphate (Figure 7).*”

Reviewer: It seems that the authors conclude that the main driver of the differences in microbial element cycling between forest and tundra is the difference in litter stoichiometry between these two ecosystems. This raises further questions. For example, about how the plants are able to acquire higher nutrient contents in the forest than in the tundra, and specifically how the forests are able to build up a larger N stock than the tundra ecosystems. I think that the (formation of the) larger N stock in the forest ecosystems is very relevant for the discussion of microbial N cycling in these ecosystems.

Response: Good comment and interesting aspect. Our additional data on N and P pools show that while the total N and P pools exhibit a modest increase from tundra to forest ecosystems,

the more pronounced changes occur in the inorganic forms of N and P (Table S2), which show a clear upward trend along this trajectory.

In the revised, the explanation for the stoichiometric patterns in the layer across treeline are addressed at the beginning as follows:

“One reason for the pronounced change in litter stoichiometry is the species-specific stoichiometric homeostasis of plant tissues (Elser et al., 2010). For instance, lichens and mosses in the tundra typically have lower nutrient concentrations compared to vascular plants in forests (Asplund and Wardle, 2013). Plant-soil feedbacks may reinforce the stoichiometric differences between tundra and forest vegetation, as the smaller C:N:P ratios in forest litter contribute to higher nutrient content in soil organic matter, thereby increasing nutrient availability (Fetzer et al., 2024). Additionally, tree roots and associated mycorrhizae enhance weathering and nutrient mining. While these processes primarily affects P rather than N, enhanced P availability—coupled with molybdenum mobilized in the rhizosphere—can promote N₂-fixation which is a critical mechanism for N accumulation in Arctic ecosystems (Rousk et al., 2017).”

Reviewer: I would like to read some lines of text about this, including a short outlook about how this might change in response to global warming and what might happen with these element ratios when the treelines shift.

Response: We add a short outlook about the potential consequences of global warming by writing in the last sentence of the Conclusion: *“We suggest that the pronounced shift in net N and P mineralization across treelines leads to a positive ‘litter feedback’, where forest expansion driven by a warming climate will tighten the C-to-nutrient ratios in decomposing organic matter compared to tundra, which will in turn accelerate nutrient cycling and enhance nutrient availability. This potentially promotes the productivity of the advancing forest.”*

As the discussion (and paper) is already relatively long, we tried to keep these additional aspects as short as possible.

Further comments

L.50-60 the transport of nutrients (nitrogen and phosphorus) into decomposing litter by fungi is also be very important during the initial decomposition stages.

Response: Thanks, we added: *“[...] (4) and fungi translocate nutrients from the soil into the nutrient-poor litter layer via their hyphae alleviating nutrient imbalances (Spohn and Berg, 2023).”*

L. 59 Whether “overflow respiration“ really occurs in soil microorganisms is debatable. It might be a process that can only be observed under extreme conditions in the lab.

Response: We agree with the reviewer and with reviewer#2 that “overflow respiration” is a misleading term and that we used it too often in the manuscript. Nevertheless, “overflow respiration” is used as a mechanistic term in key review publications on how microbes adjust their ecophysiology to low nutrient contents (Zechmeister-Boltenstern et al., 2015, Mooshammer et al., 2014; Manzoni et al., 2021). and we have to refer to it. We think that “overflow respiration” in natural systems can rather be understood as a simple balance between C and nutrients during microbial processing of litter, where C is lost as CO₂ while nutrients are recycled by microbial communities (when nutrient supply is limited). In the revised manuscript, it is removed from the Introduction and only presented once in the Discussion

L. 71 and 81 It is not clear what exactly “litter quality” is. This term requires an explanation.

Response: We agree that litter quality (as soil quality) is a difficult term. We changed it to *“changes in stoichiometry and organic constituents in the litter layer”*

L. 95 In hypothesis 1, the term “litter quality” requires an explanation.

Response: clarified to: *“due to decreasing C:N:P ratios.”*

L. 98 What is “microbial functioning”? This sounds a little awkward. I guess the authors mean microbial ecophysiology or something related.

Response: Thanks: We have changed the term to *“microbial ecophysiology”*

L. 105 see above

Response: changed

Section 2.1 It would be helpful to see a map and a few photos of the sites.

Response: We have added a map and few photos of the tundra and forest sites to the Supplementals (see end of the response letter). Adding it to the main manuscript would further increase the manuscript which is already long.

L. 218/219 Was the glucose-6-phosphate uniformly labelled or was it only labelled in one C position?

Response: We added that the glucose-6-phosphate was uniformly labelled.

L. 125 How was this done randomly. Please explain.

Response: We simply threw a ruler to sample the litter layer.

Fig. 1 The label on the y-axis is not clear. This should be improved and further explained in the caption.

Response: We have revised the label on the y-axis:

“Cumulative net mineralization of C,N, and P (mg C,N,P mineralized (glitter C,N,P)⁻¹)”

Furthermore, in the Figure captions we now add an explanation: *“Amounts of mineralized C, N, and P are related to the masses of C, N, and P in the litter layer, respectively, to allow comparisons between the three elements.”*

Fig. 3 and corresponding text. Please indicate whether these ratios are based on mass or number of moles. It is confusing that in some parts of the text, it is indicated that these are molar ratios while in other parts the authors simply refer to ratios.

Response: We used molar ratio in all our data evaluation and report is as molar when values are given. However, when we discuss the ratio in general, we did not add “molar” each time as it does not matter whether the ratio is given on a mass or molar basis.

Fig. 8 I suggest to add a second panel to the figure that shows the percentage of respired ¹³C (derived from glucose-6-phosphate). This would allow the authors to discuss loss of C in comparison to loss of P.

Response: Thanks for the suggestion. We have added another panel, clearly documenting that while ¹³C mineralization from glucose-6-phosphate increased with the molar C/P ratio, DIP release decreased.

Fig. 450/451 This sentence is not entirely clear. Specifically it is not clear what exactly “decreasing C:N:P ratios” refers to and what “effects” refers to.

Response: We have rephrased the sentence as follows: *“This could indicate that in the tundra with high litter C:N:P ratios microorganisms mineralized C in excess to acquire nutrients, a*

mechanism that has been named as “overflow respiration” (Mooshammer et al., 2014). However, we rather relate the apparent positive relationship between C:N:P ratios and C mineralization to a changing composition in organic constituents along the same trajectory. For instance, while the litter layer under tree canopies had the lowest C:N:P ratio, it also contained the highest contents of lignin, which is more resistant to decomposition.”

L. 455 replace “mineral” by “inorganic”

Response: changed

L. 470 Microbial mineralization of what? I guess glucose-6-phosphate but it might be good to clarify this.

Response: clarified

L. 474 It seems that rather the lab studies are biased (or artificial) because they exclude nutrient import and export.

Response: We agree that lab experiments can be more artificial. In our statement, we refer to the potential loss of organic N and P; litter bag studies do not allow to identify which form of the nutrient (organic or inorganic) had been lost. We rephrase the sentence:

“One obvious reason could be that in litter-bag studies the export of nutrients through soil fauna and the leaching of nutrients in organic forms is not considered.”

L. 484 is mineralized (not becomes mineralized)

Response: changed

L. 502 Please replace “very likely” by “which might have been” (since this is rather speculative).

Response: changed accordingly

L. 505 see above. What is “microbial functioning”?

Response: Changed to “*microbial ecophysiology*”

L. 552 remove “in the tundra”.

Response: Deleted and rephrased

L. 556 This is not correct. While the litter has a lower nutrient content in the tundra than in the forest, 100% of the nutrients are released in both ecosystems after several decades

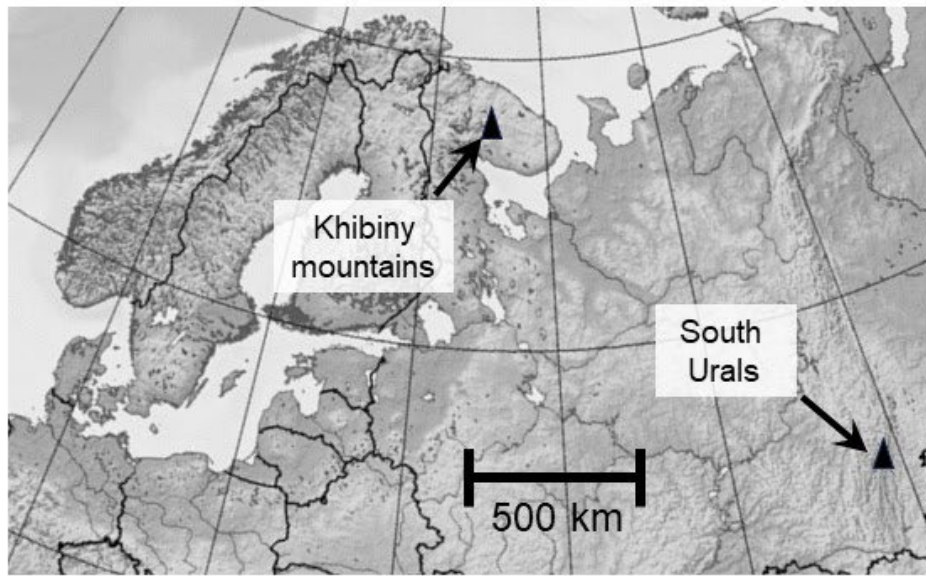
Response: We addressed this as follows:

We wrote: *“Furthermore, N and P immobilized in microbial biomass and incorporated into soil organic matter become eventually released during mineralization of microbial necromass and/or SOM with lower C-to-nutrient ratios (see for N; Knops et al., 2002).”*

L. 572 remove “worldwide”

Response: removed

S1 Study sites



Khibiny mountains

South Urals

Figure 1: Study sites and photographs of the studied tundra and forest ecosystem in the Khibiny mountains and South Urals.

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