Responses to Reviewer #2

General comment
The effects of global change on nutrient release from litter layers are certainly an actual and valid research objective in northern ecosystems. The study presented by Hagedorn et al. contains interesting data, which seem to merit publication. However, the analysis of different ecotones with different vegetation will always lead to highly significant results. This means that the presentation of the data needs considerable improvement as the authors seem to be partly lost in data. They should consider shortening the text and removing some approaches, which do not add much information to the study.

Response: Thanks for the evaluation, the constructive comments, and the careful read. We incorporated all comments in the revised manuscript. We have removed one of the Figures (Fig. 7). Following the suggestions of the reviewer we have largely removed and rephrased the discussion of "overflow respiration. We agree with the reviewer that litter quality and its processing are likely to vary across ecotones. However, we believe our findings make two important contributions to the understanding of plant-soil interactions across treelines: (1) The results demonstrate that treelines act as a 'natural boundary' for microbial processing and nutrient cycling, and (2) they highlight that the higher nutrient release in forests compared to tundra has implications for vegetation dynamics—a factor often overlooked in treeline ecology.

Specific comments
The font is too small to allow easy reading of the PDF printout.
I would prefer continuous line numbering.
Response: We apologize but we formatted the manuscript according to the guidelines.

L38-41: Awkward statement! Microbial biomass and microbial residues also need to be mineralized for releasing nutrients.
Response: We followed the suggestion of the reviewer and rephrased the statement as follows:
"In the initial phase, plant detritus is mineralized to CO_2 and inorganic nutrient forms or converted into microbial biomass (Berg and McClaugherty, 2020). Subsequently, nutrients can be released upon microbial residue decomposition."

L59: There is too much focus on overflow respiration in the current manuscript, which occurs mainly when high concentrations of low molecular weight organic substances are available to microorganisms. The authors should consider extracellular polymeric substances (EPS), fungal vacuoles and bacterial storage components, such as poly-hydroxybutyrate, as reasons for stoichiometric variability of soil microorganisms. Also, the presence or absence of Mn and Cu has often strong effects on lignin decomposition in litter layers.
Response:
- **"overflow respiration"**: We agree with the reviewer that the term "overflow respiration" is misleading and unlikely to occur. Nonetheless, it is used in key review publications on how microbes adjust their ecophysiology to low nutrient contents in decomposed organic matter (Zechmeister-Boltenstern et al., 2015, Mooshammer et al., 2014; Manzoni et al., 2021). In the revised manuscript, we are using the term "overflow respiration" only once in the Discussion, where we think that this is actually not the underlying process.
"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 degradation."

- Low molecular weight organic substances: We agree with the reviewer that such a study could profit from the analysis of LMW-substances but this would go beyond the scope of this study. We provide data on Mn in litter layers (SI Table 3)

L97-100: Awkward statement! Rephrase!
Response: Thanks, we rephrased the objectives as follows:
"In addition, we studied the responses of microbial ecophysiology to the range of litter layer characteristics across the two treelines by (i) analyzing C:N:P ratios in microbial biomass, (ii) measuring the activity of extracellular enzymes hydrolyzing organic C, N, and P compounds, (iii) determining the metabolic quotient (qCO_2) as well as the use of ^{13}C-labelled glucose-6-phosphate (G6P) by microorganisms, and (iv) quantifying net P mobilization or immobilization from the added G6P."

L143-144: I do not understand the reason for this initial leaching.
Response: Clarified by writing: *"Following an initial leaching to standardize moisture conditions and remove nutrients released upon sample storage and processing, litter layer samples were incubated for two weeks in a climate chamber at 15°C and leached on a weekly basis to precondition the litter samples (Canali and Benedetti, 2006)."*

L152-153: Please, give the range of NaOH molarity.
Response : We provide the range as 0.05 to 0.1M NaOH

L189: Brookes et al. (1985) and Vance et al. (1987) used 0.5 M K2SO4 for extracting mineral soil at a ratio of 1 to 4 (soil to extractant). The current authors extracted litter at a ratio of 1 to 20 (litter to extractant) with 0.05 M K2SO4. This deviation from the original references is based on previously published work in determining microbial biomass in litter, which should be cited in all fairness.
Response: We have added the reference of Makarov et al. (2015) in the revised manuscript as suggested (Line 190)

L191, L219, L226: remove "Corp", "Inc", and "Limited"!
Response: These words have been deleted in the revised manuscript.

L193: The kEC, kEN, and KEP values are not factors. The kEC value of 0.45 has been proposed by Wu et al. (1990), which should be cited.
Response: We have changed "factor" to "extraction efficiency coefficient".
In addition, "Wu et al. (1990)" has been added in the revised manuscript.

L215: The formula should be given.
Response: Thank you for the suggestion. The formulas are added to the revised manuscript.

"$$Activity\ (nmol\ g^{-1}\ h^{-1}) = \frac{Net\ Fluorescence \times Buffer\ volume\ (ml)}{Emission\ coefficient \times Homogenate\ volume\ (ml) \times Time\ (h) \times Soil\ mass\ (g)} \quad [1]$$

$$Net\ Fluorescence\ = \left(\frac{Assay - Homogenate\ control}{Quench\ coefficient}\right) - Substrate\ control \quad [2]$$

$$Emission\ coefficient\ (fluorescence\ nmol^{-1})\ = \frac{Standard\ fluorescence}{\left[\frac{Standard\ concentration\ (nmol) \times Assay\ volume\ (ml)}{Volume\ of\ standard\ (ml)}\right]} \quad [3]$$

$$Quench\ coefficient\ = \frac{Quench\ control - Homogenate\ control}{Standard\ fluorescence} \quad [4]$$"

L243 and throughout the manuscript: The metabolic quotient is defined as basal respiration / microbial biomass C (Anderson and Domsch, 1990) and should not be used for the microbial use of a freshly added substrate.

Response: We estimate the metabolic quotient for the litter layer, which had been in the field for extended time (at least 10 months). Therefore, the litter layer had been colonized by microbial communities before we started our incubation study. For the added glucose-6-phosphate, we use the term 'substrate-use efficiency'

Tables 1, 2, 3 and 4: The decimal numbers should be restricted to two, not in bold, non-significant numbers should be presented as NS.
Response: We reduced the decimal numbers, and effects of p>0.10 as NS. We still provide values of p<0.10 (not only restricted it to the commonly used p<0.05). Nevertheless, in the manuscript, we only speak from significant at p<0.05.

L284-287: This is not a Results statement. Move to Materials and Methods or the Discussion section!
Response: We moved it to the Introduction.

L306-309: It is impossible for me to get a clear information out of this poorly lay-outed
Response: We apologize for the low quality in the pdf. The figure documents the net mineralization of C, N, and P during 12 weeks. It provides information about the temporal patterns, the differences between the elements and between the main sites (tundra and tree canopy). We have re-formatted the Figure.

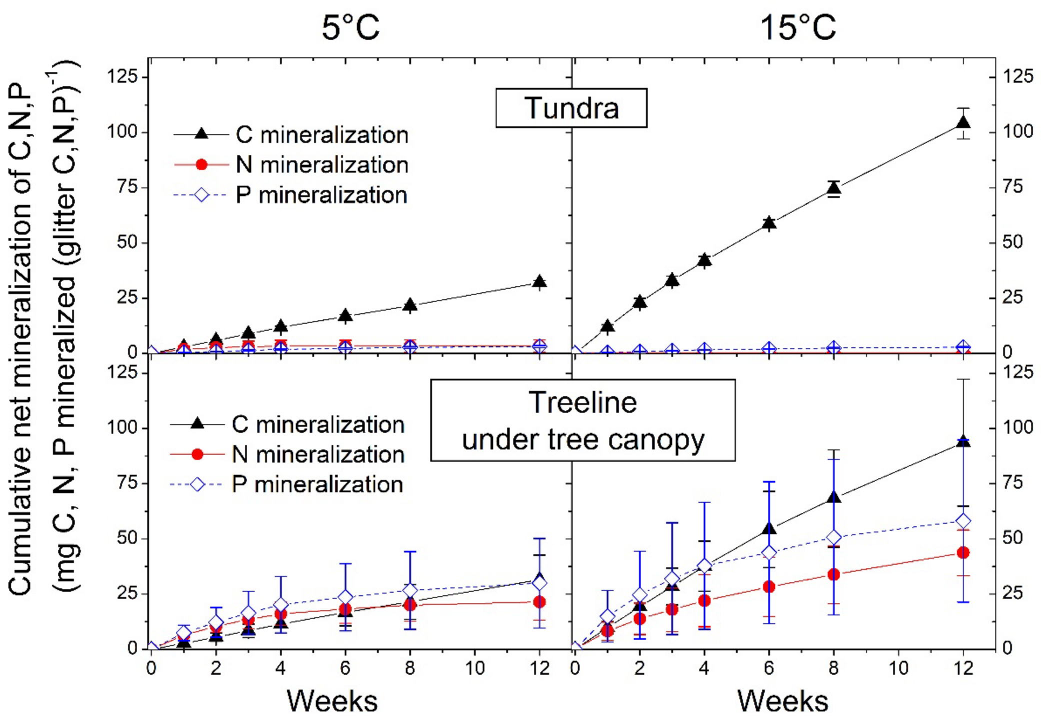


Figure 1. The data of the endpoints should be given in a table.
Response: In the Legend, we write: *"Cumulative values after 12 weeks of all vegetations types along the elevation gradient in the Khibiny mountains and South-Urals are shown in Figure 2."*

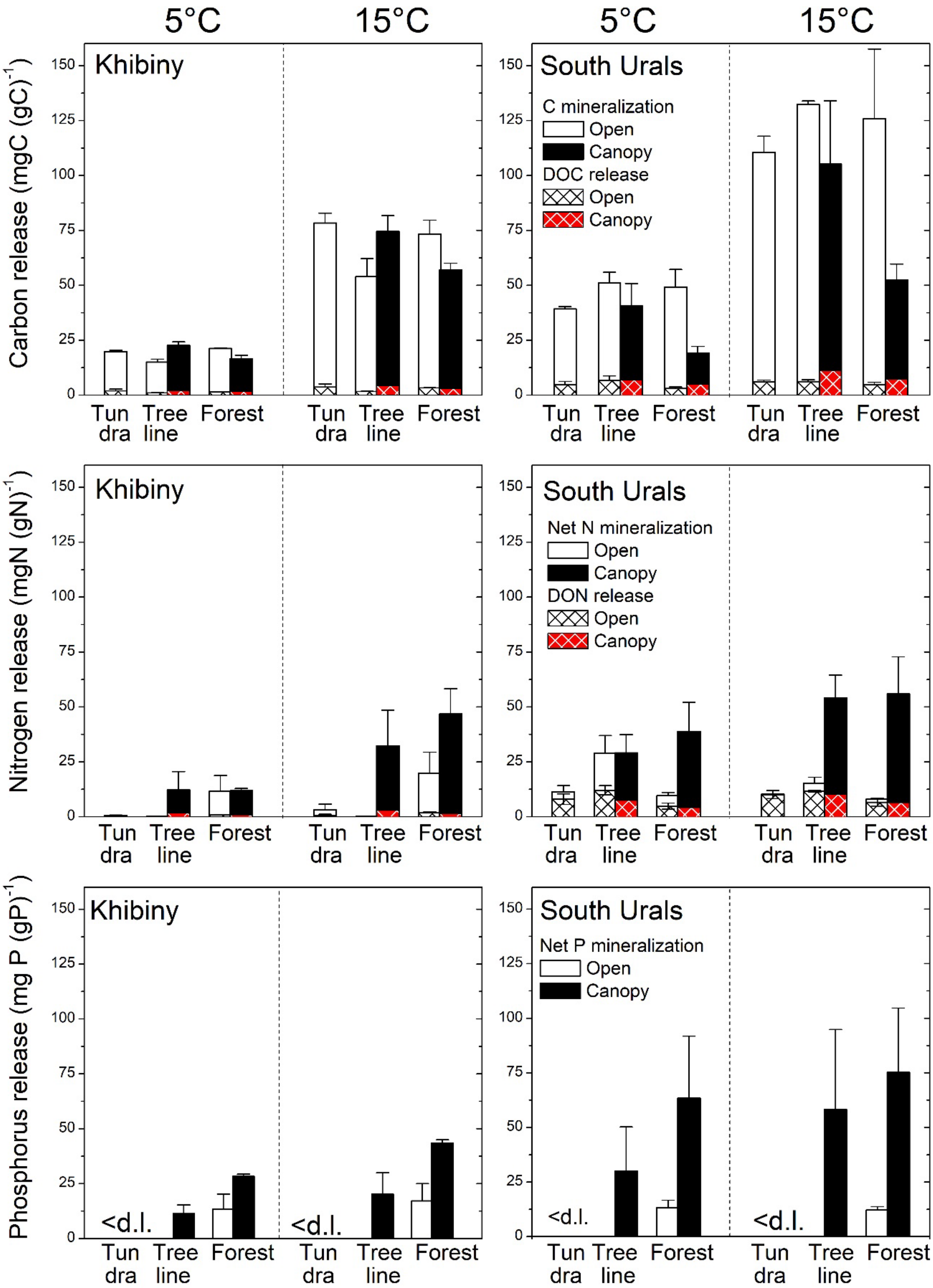
L311-314: This is not a Results statement. Move to Materials and Methods or the Discussion section!
Response: We moved the reference Introduction.

L325-329: Also, the layout of Figure 2 is poor. It does not make sense to adjust C, N, and P release to an identical scale. In addition, the figure contains excessive legends.

Response: We apologize for the low quality in the pdf. We have removed some of the legends, changed the color code and increased line sizes.

In our opinion, it makes sense to use identical scale by referring to the masses of each element, which allows a comparison of net mineralization rates among the elements (sensu Weintraub & Schimel, 2003; SBB). For instance, a smaller net N than C mineralization implies that released N during decomposition is immobilized.

See revised Figure 2:



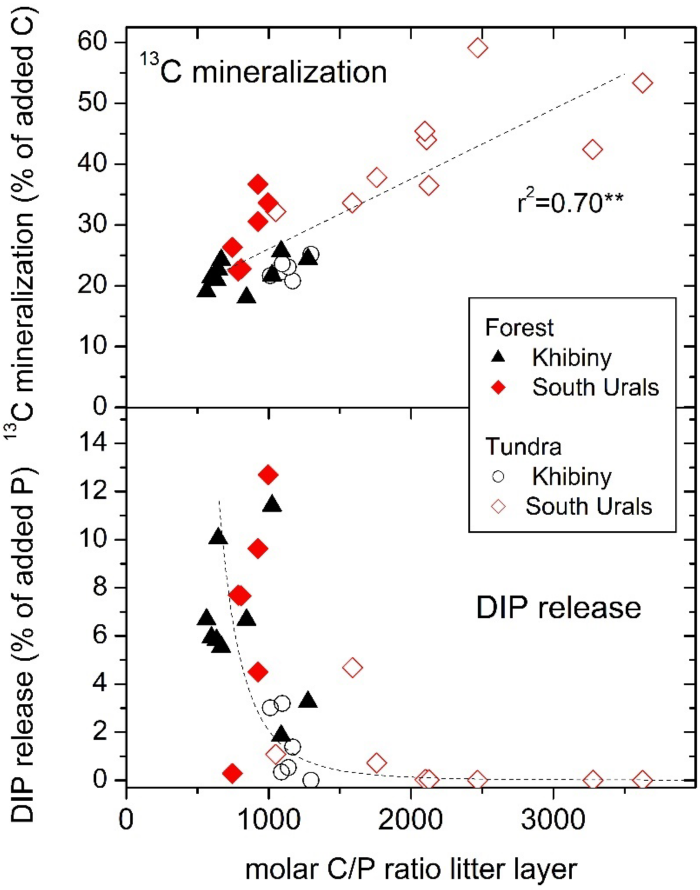
L326: I miss information on the DOC/DON ratio as quality index for the measurements.
Response: In the revised manuscript, we provide DOC/DON ratios by writing:
"The molar DOC:DON ratio of released DOM ranged between 23.5 under tree canopy and 168 in the tundra ($p_{Elevation} < 0.001$)."
In the manuscript, we kept discussion about DOM short in order to avoid extending further the manuscript.

L341-3??: I have doubts that these presentation of correlation coefficients is valid as the data are presumably not normally distributed as those presented in Figure 7.
Response: In Figure 3, we now use the Spearman Rank correlation coefficient, which is independent from the distribution of data. In the mixed effect model we log-transformed all data, accounting for the non-normal distribution of data.

L406-4??: Q10 values of MBC, MBN, and MBP should be removed.
Response: We think that it is an important information that MBC, MBN, and MBP were not temperature sensitive.

L423-425: Figure 7 should be removed.
Response: We have removed Figure 7. Mineralization of ^{13}C from the added G6P is now presented in the former Figure 8 together with net released DIP (following suggestions of reviewer 1).

L426-428 (former Figure 8): It is not possible to distinguish the site-specific symbols using a greyscale print-
out.
Response: we have redrawn the Figure:



L435-436: Trivial statement! Remove!
Response: While the changes of organic constituents and stoichiometry is an expected outcome of the study, we think that the statement is needed as the litter quality changes are the reasons for the changes in nutrient release across the treeline ecotone. In the revised manuscript, we add explanation for the stoichiometric differentiation as follows:
"Consistent with our hypothesis, the composition of organic constituents in the litter layer changed and C:N:P ratios strongly decreased with the shift in plant life forms and species from tundra to forest (Table 1). 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 reicnforce 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 N_2-fixation which is a critical mechanism for N accumulation in Arctic ecosystems (Rousk et al., 2017).

L462-464: Awkward statement rephrase!
Response: we have revised the section about P immobilization by discussing the G6P experiment more in detail:
"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). Again, there was complete net retention of the added P from the easily mineralizable G6P in tundra litter, albeit to a lesser extent in tree canopy litter. Our findings could have been influenced by sorption of mineralized phosphate (Brödlin et al., 2019); however, this seems improbable in the purely organic litter layer, where negatively charged organic matter does not sorb negatively charged PO_4^{3-}. Thus, phosphate mineralized either from the litter layer itself or from the added G6P must have been immobilized, potentially within microbial biomass, as observed in organic layers with low P availability (Siegenthaler et al., 2024)."

L490, L491, L502: "microbial biomass" not just "microbial"!
Response: changed

L579: Again, there is too much focus on overflow respiration. It is possible but cannot be clearly concluded from the current data.
Response: we have removed the term "overflow respiration". Instead we wrote:
"Microbial ecophysiology paralleled these changes, including a more efficient use of organic matter by microorganisms during decomposition with smaller litter C:N:P ratios in the forest compared to tundra."

L584-587: This statement is not a Conclusion. I miss a clear "take-home" message.
Response: we rephrased the sentence as follows:
"The study highlights that litter stoichiometry has a greater influence on net N and P mineralization than a 10°C temperature gradient, suggesting that the indirect effects of climate warming through plant species shifts are more critical for N and P cycling than the direct effects of temperature increases."

References

Asplund, J., and Wardle, D. A.: The impact of secondary compounds and functional characteristics on lichen palatability and decomposition, J. Ecol., 101, 689–700, https://doi.org/10.1111/1365-2745.12075, 2013.

Berg, B., and McClaugherty, C.: Decomposition as a process–some main features in plant litter: decomposition, humus formation, carbon sequestration (Cham: Springer International Publishing), 13–43, https://doi.org/10.1007/978-3-030-59631-6_2, 2020.

Canali, S., and Benedetti, A.: Soil nitrogen mineralization. In: Bloem, J., Hopkins, D.W., Benedetti, A. (Eds.), Microbiological Methods for Assessing Soil Quality. CABI, Wallingford, UK, pp. 23–49, 2006.

Elser, J. J., Fagan, W. F., Kerkhoff, A. J., Swenson, N. G., and Enquist, B. J.: Biological stoichiometry of plant production: metabolism, scaling and ecological response to global change, New Phytol., 186, 593-608, https://doi.org/10.1111/j.1469-8137.2010.03214.x, 2010.

Fetzer, J., Moiseev, P., Frossard, E., Kaiser, K., Mayer, M., Gavazov, K., and Hagedorn, F.: Plant-soil interactions drive nitrogen and phosphorus dynamics in an advancing subarctic treeline, Glob. Chang. Biol., 30, e17200, https://doi.org/10.1111/gcb.17200, 2024.

Makarov, M.I., Malysheva, T.I., Menyailo, O.V., Soudzilovskaia, N.A., Van Logtestijn, R.S.P., and Cornelissen, J.H.C.: Effect of K_2SO_4 concentration on extractability and isotope signature ($\delta^{13}C$ and $\delta^{15}N$) of soil C and N fractions, Eur. J. Soil Sci., 66, 417–426, https://doi.org/10.1111/ejss.12243, 2015.

Manzoni, S., Chakrawal, A., Spohn, M., and Lindahl, B. D.: Modeling microbial adaptations to nutrient limitation during litter decomposition, Front. For. Glob. Chang., 4, 1–23, https://doi.org/10.3389/ffgc.2021.686945, 2021.

Mooshammer, M., Wanek, W., Zechmeister-Boltenstern, S., and Richter, A.: Stoichiometric imbalances between terrestrial decomposer communities and their resources: Mechanisms and implications of microbial adaptations to their resources, Front. Microbiol., 5, 1–10, https://doi.org/10.3389/fmicb.2014.00022, 2014.

Rousk, K., Degboe, J., Michelsen, A., Bradley, R. and Bellenger, J.-P.: Molybdenum and phosphorus limitation of moss-associated nitrogen fixation in boreal ecosystems. New Phytol, 214, 97-107. https://doi.org/10.1111/nph.14331, 2017

Siegenthaler, M.B., McLaren TI, Frossard E, and Tamburini, F.: Dual isotopic (^{33}P and ^{18}O) tracing and solution ^{31}P NMR spectroscopy to reveal organic phosphorus synthesis in organic soil horizons, Soil Biol. Biochem., 197, 109519, https://doi.org/10.1016/j.soilbio.2024.109519, 2024.

Weintraub, M.N., and Schimel, J.P.: Interactions between carbon and nitrogen mineralization and soil organic matter chemistry in arctic tundra soils, Ecosystems, 6, 129-143, https://doi.org/10.1007/s10021-002-0124-6, 2003.

Wu, J., Joergensen, R.G., Pommerening, B., Chaussod, R., and Brookes, P.C.: Measurement of soil microbial biomass C by fumigation-extraction-an automated procedure. Soil Biol. Biochem., 22, 1167-1169, https://doi.org/10.1016/0038-0717(90)90046-3, 1990.

Zechmeister-Boltenstern, S., Keiblinger, K. M., Mooshammer, M., Peñuelas, J., Richter, A., Sardans, J., and Wanek, W.: The application of ecological stoichiometry to plant-microbial-soil organic matter transformations, Ecol. Monogr., 85, 133–155, https://doi.org/10.1890/14-0777.1, 2015.