Title: Quantifying rhizosphere priming effects on soil organic matter decomposition in situ in a subarctic ecotone using natural abundance radiocarbon
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We appreciate the comments from the two reviewers. In this revised manuscript, we have implemented the suggested changes and we have addressed all the comments below. We would like to acknowledge the inputs of the reviewers which have improved the manuscript.

RC2 Comments:

This is a technically ambitious and genuinely novel field study. Using a ~2700-year-old, ^{14}C-depleted peat substrate as an *in situ* tracer to partition rhizosphere-primed versus "ancient" CO_2 efflux is a very interesting way for field quantification of rhizosphere priming effects, complementing (rather than duplicating) the lab-based ^{13}C-labelling. The pairing with an independent girdling manipulation is a strong design feature, and the treatment of atmospheric contamination (Keeling-plot correction and sensitivity analysis) are commendable examples of rigorous, transparent radiocarbon methodology.

Thank you. We sincerely appreciate this in-depth and well-crafted review that has led to a substantial improvement in our paper. We also hope this approach is adopted as a valuable contribution to the research field. The comments and questions have been addressed point-by-point with responses in blue and corrections or direct quotes in red.

Main comments

1- I agree with Reviewer 1's assessment and specific comments. The figures do need revision for colour-independent interpretability, the terminology issued must be double checked. I would add that the manuscript is internally inconsistent on this last point, and that the "enriched/depleted" imprecision recurs beyond the lines Reviewer 1 flagged.

We have altered the text accordingly and made changes throughout the text; indeed we have extended this beyond RC1's suggestion for the Results section. For example, in the Abstract we now state: "As expected, carbon dioxide (CO_2) and dissolved organic carbon (DOC) from root ingrowth cores were significantly ~~enriched~~ higher in ^{14}C (contemporary carbon) compared to CO_2 and DOC from root exclusion cores (peat carbon), allowing partitioning of carbon mobilisation between heterotrophic (peat substrate) and recent autotrophic (plant) sources."

Examples from the Results section are found in response to RC1.

2- The girdling treatment had likely waned by the time of sampling. Root biomass showed no significant girdling effect by 2021, and the authors themselves attribute this to compensatory understorey rhizosphere development over the (unplanned) fourth growing season. This means Hypothesis 2 was not actually tested under the conditions assumed by the original design. The "no significant girdling effect" result cannot be interpreted as evidence against a role for canopy ectomycorrhizal hosts, only as evidence that the girdling manipulation itself had become confounded by regrowth. This is discussed in the Discussion but should be reflected more explicitly in the abstract/conclusion, where the current phrasing risks being read as a clean negative result rather than an inconclusive one.

Unfortunately, due to the COVID-19 pandemic, we were unable to travel internationally for fieldwork during the planned year, and the girdling treatment was likely beyond peak effectiveness. It is true, the lack of difference in girdle vs. controls cannot be interpreted as evidence against the role of canopy forming ectomycorrhizal hosts and we have made this clear in the Abstract and Conclusion. Now, in the Abstract, we have rephrased this as:

"The lack of a significant girdling effect neither supported nor rejected our specific hypothesis that the presence of ectomycorrhizal roots and their associated mycelium increase SOM decomposition…"

In the conclusion, it now reads as: "We found predominantly positive priming (p = 0.01) of the ancient peat substrate across mountain birch forest and woolly willow stands, even after girdling. However, there was also substantial variability, potentially consistent with the existence of microbial 'hotspots' ~~and 'hot moments'~~. By contrast, we could not identify any statistically significant contrasts in RPEs between the two vegetation communities, nor any effect of girdling. For the latter, it is possible that compensatory rhizosphere development by the understorey plants reduced the importance of C supply by the canopy-forming species, but we were unable to reject or confirm our hypothesis of the influence of ectomycorrhizosphere on priming processes through the girdling treatment"

3- The peat-derived (Δpeat) end-member is a single, once-measured laboratory value applied uniformly to ~40+ field cores across 4 years and heterogeneous microsites, with no propagation of its uncertainty into the mixing-model outputs, and no empirical test of whether it remained representative after 4 years of in situ decomposition. Given that the authors already ran a valuable sensitivity analysis for the Δair end-member, a parallel sensitivity analysis varying Δpeat within a plausible range would substantially strengthen confidence in the robustness of the RPE estimate.

The bulk peat was a 150-g representative sample from the large, well homogenized batch of Uddjaure peat used as a substrate for the incubated soil cores – we have added this detail in the Methods section 2.4. Prior to constructing the cores, the

peat was repeatedly mixed, turned over thoroughly, and passed through a 4-mm sieve. Incubations of three 50-g samples at field capacity were used to collect the $^{14}CO_2$ content of respiration in one composite sample. This has also been clarified in the Methods section.

In terms of the sensitivity of using a single analytical value of Δ_{peat}, please refer to a new section in Appendix B named "*Insensitivity of rhizosphere priming effect ratios to the value of the ancient peat ^{14}C end-member*." Here, we show algebraically why the value of the Δ_{peat} end-member does not influence the RPE ratio.

First, we calculate the fractional contributions of peat-derived CO_2 flux to the total CO_2 flux from the inclusion (f_{peat_IN}) and exclusion (f_{peat_EX}) cores based on a two-source isotopic mixing equation (equation 3 in the main text):

$$f_{peat\_IN} = \frac{(\Delta_{resp\_IN} - \Delta_{recent})}{(\Delta_{peat} - \Delta_{recent})} \quad (3a)$$

$$f_{peat\_EX} = \frac{(\Delta_{resp\_EX} - \Delta_{recent})}{(\Delta_{peat} - \Delta_{recent})} \quad (3b)$$

Where Δ_{resp} is the $^{14}CO_2$ content of respiration from the entire soil core, Δ_{peat} is the $^{14}CO_2$ content of respiration from the peat and Δ_{recent} is the ^{14}C content of the recently photosynthesised carbon (%modern), which we assume is equal to the ^{14}C signature of the atmosphere in 2021. The absolute peat-derived CO_2 flux (umol m^{-2} s^{-1}) for the inclusion (F_{peat_IN}) and exclusion (F_{peat_EX}) cores is then calculated based on equation 4:

$$F_{peat\_IN} = F_{total\_IN} \times f_{peat\_IN} \quad (4a)$$

$$F_{peat\_EX} = F_{total\_EX} \times f_{peat\_EX} \quad (4b)$$

Where F_{total} is the total measured CO_2 flux (umol m^{-2} s^{-1}) from the cores. We define the rhizosphere priming effect ratio (RPE) (equation 5) as the ratio between the absolute peat-derived CO_2 flux from the inclusion cores (F_{peat_IN}) and the absolute peat-derived CO_2 flux from the exclusion cores (F_{peat_EX}):

$$RPE = \frac{F_{peat\_IN}}{F_{peat\_EX}} \quad (5)$$

Substituting 4a and 4b into equation 5:

$$RPE = \frac{F_{total\_IN} \times f_{peat\_IN}}{F_{total\_EX} \times f_{peat\_EX}} \quad (6)$$

Replacing f_{peat_IN} and f_{peat_EX} in equation 6 with equations 3a and 3b gives:

$$RPE = \frac{F_{total\_IN}}{F_{total\_EX}} \times \frac{(\Delta_{resp\_IN} - \Delta_{recent})}{(\Delta_{peat} - \Delta_{recent})} \times \frac{(\Delta_{peat} - \Delta_{recent})}{(\Delta_{resp\_EX} - \Delta_{recent})} \quad (7)$$

Simplifying:

$$RPE = \frac{F_{total\_IN}}{F_{total\_EX}} \times \frac{(\Delta_{resp\_IN} - \Delta_{recent})}{(\Delta_{resp\_EX} - \Delta_{recent})} \quad (8)$$

Thus, while the absolute peat-derived CO_2 flux (F_{peat}) is sensitive to the value of D_{peat} the RPE ratio is not.

4- The root-severing validation (Appendix A) is a reasonable proof-of-concept but should be explicitly caveated as an approximation, not a direct validation of the subarctic peat-core system.

We have edited the wording in the Methods to: "Method validation for an estimate of the longevity of the root and rhizosphere respiration after root severing can be found in Appendix A." Additionally, we have added a caveat in Appendix A with your suggestion that "the root-severing validation, undertaken in the UK, provides proof-of-concept, but should be considered an approximation, not a direct validation of the subarctic peat-core system."

5- The mesh-based ingrowth/exclusion design cannot distinguish whether priming arises from ectomycorrhizal enzymatic SOM mining, rhizodeposition-stimulated saprotrophic activity, or root-associated fauna. Yet the hypotheses are framed specifically around ectomycorrhizal roots and extra-radical mycelium. Given that the willow understorey also contains an ECM species (Betula nana), the canopy vs. understorey ECM contrast is not clean. This should be acknowledged as a limit on mechanistic attribution, not just a footnote about uncontrolled species composition.

We thank RC2 for these comments, which are insightful. We have therefore reviewed the framing of the hypotheses in the Introduction (lines 101 to 104; see below) but believe that this phrasing remains valid (referring to "presence of roots and associated organisms").

"Therefore, we hypothesize 1) that presence of roots and associated organisms increases SOM decomposition (positive priming), and 2) that this priming effect is greater in ungirdled plots (with intact ectomycorrhizal shrubs and canopy trees) than in girdled plots, with only understorey vegetation."

However, regarding more detailed attribution of priming to specific components of the ectomycorrhizosphere (e.g. ectomycorrhizal enzymatic SOM mining, rhizodeposition-stimulated saprotrophic activity, or root-associated fauna), we accept the limits of our approach here and note that this raises interesting follow-up questions for future research. We have therefore addressed RC2's comments in the Discussion by stating "We could not control the species composition of root

ingrowth into the cores, nor the accompanying soil biota (including both microorganisms and potentially soil micro- and meso-fauna). For this reason, the community-level mean RPE may integrate both positive and negative effects associated with different organisms within the mycorrhizosphere."

We would also like to note that, in the willow plots, although *Betula nana* was present within the 4-m diameter plot area, the perimeter of the plots was trenched to exclude other ECM hosts.

6- All CO_2/DOC/^{14}C measurements come from one week in July 2021. The discussion's invocation of "hot moments" (temporal heterogeneity) is not directly testable with this design. Only spatial heterogeneity ("hotspots") is empirically demonstrated. I'd recommend softening this language.

The reviewer is correct that we are unable to differentiate between hot moments and hotspots in our design. We have now phrased this as speculation and a possibility for future research to note that it is not specifically tested. In the concluding paragraph, it now reads: "We found predominantly positive priming (p = 0.01) of the ancient peat substrate across mountain birch forest and woolly willow stands, even after girdling. However, there was also substantial between-plot variability, ~~potentially~~ and we speculate that this is consistent with the existence of microbial 'hotspots and/or hot moments.'

Specific comments

L. 106–110: Six birch pairs and five willow pairs are stated here, but Fig. 2/Table B1 show willow girdled n = 4.

Thank you to the reviewer for spotting this error. Figure 1 remains the same with the correct number of plots, but we have added that "one replicate sample was compromised from the willow girdling treatment during processing for radiocarbon analysis, leaving the willow girdling treatment to n = 4." in the Methods section.

L. 130–136 / (L. 179–186): Please clarify whether the bulk peat and the incubation-derived Δpeat sample were homogenized/composited to ensure the same ^{14}C signature applies across all field cores, and report whether any replicate ^{14}C measurements of the peat batch were made. As it stands, Δpeat is a single analytical value (with only counting-statistics uncertainty, ±0.36) used as a fixed parameter in Eq. 3 across ~40 cores over 4 years; given the acknowledged possibility of shifting lability/age of the decomposing peat pool (rows 437–441), I recommend a sensitivity analysis analogous to the one already performed for Δair, testing plausible bounds on Δpeat.

We thank the reviewer for this comment again, as it is important to consider. We believe we have responded sufficiently to this issue under main comment #3 above.

L. 152–156: Please note explicitly that this is a single sampling date/growing season, which limits the ability to draw conclusions about temporal variability as opposed to purely spatial variability.

Agreed. We have therefore added a paraphrased version of this disclaimer to the Methods section 2.3.

L. 168–171: The CO_2 build-up/incubation duration differed substantially between ingrowth (2–4 h) and exclusion cores (up to 12 h). Please discuss whether this differential incubation time could itself introduce a systematic artifact (e.g., progressive substrate-pool switching, changing headspace conditions) that covaries with treatment, independent of the biological priming effect being tested.

The reviewer correctly notes the contrasting build-up/collection periods for CO_2 in the ingrowth and exclusion cores. This was an inevitable facet of the experimental approach (with much lower respiration rates in the exclusion cores compared with ingrowth cores), as we noted in lines 169-171. Following this line we have therefore added:

"Regarding possible effects of the contrasting CO_2 sampling periods for the ingrowth and exclusion cores, temperature changes within the boxes were minimised, but it is possible that substrate pools could have shifted through time. For the latter, we do not consider that systematic effects on the ^{14}C signature of metabolised substrates are likely over the timescales involved here, although we cannot definitively rule out this possibility."

L. 179–186: Please state the temperature and moisture conditions during the in vitro peat incubation relative to in situ field conditions at Abisko during the July sampling window, since basal respiration rate and community composition are temperature/moisture sensitive and could affect how representative this laboratory Δpeat value is of actual field decomposition.

Daily average temperatures for the month of July 2021 ranged from 8.7 to 17.2 °C in Abisko, with a daily average of 13.4 °C for the week of sampling. We already state in Methods Section 2.4 that "[t]he peat soil was incubated between ~11 and 16 °C from August 2017 to achieve a basal rate of respiration." This is a comparable range and average to the *in situ* daily temperatures. Additionally, soil temperatures of the cores were monitored at 5-cm depth before collection, which ranged between 8.3 and 18 °C, and had an overall mean of 11.8 °C. We do not, therefore, consider that this would dramatically alter the ^{14}C signature of the peat end member, and, as stated previously and demonstrated with a mathematical proof, the peat end member remains inconsequential. Supplementary to this, we sampled respired $^{14}CO_2$ from a mesocosm experiment (unpublished), using the same peat substrate, in the experimental garden in Abisko (n = 15), after four years *in situ*, and found a

striking similarity in Δresp compared with the *in vitro* value (thus *in vitro*: 78.65+/-0.36 % modern ^{14}C; mesocosm experiment in Abisko: 78.32 +/-2.46 % modern ^{14}C). This greatly supports our confidence in representativeness of the laboratory (*in vitro*) Δ_{peat} value adopted in our calculations here.

Data for air temperature from the Swedish Polar Research Secretariat in Abisko can be found here:

https://www.polar.se/en/research-support/open-data/data-from-abisko-scientific-research-station/

L. 336: This is a strong claim. I suggest tempering it in light of the limitations above (small n, single time-point, confounded girdling treatment by year 4, single-measurement peat end-member).

We do not agree entirely with the reviewer concerning 'small n'; as a main effect across all groups (vegetation types and girdling treatment), we have n > 20. Regarding the single time point we have amended the text, following this suggestion, to state:

"Differences in the peat-derived component of respiration between the rooted and non-rooted cores revealed impacts of roots and associated biota on rates of decomposition; although our results are based on sampling during just one week in July 2021, they provide the clearest test yet of rhizosphere priming under field conditions."

It is a moot point to include a disclaimer related to the peat end-member argument, as demonstrated by the mathematical proof provided earlier. We have more explicitly stated that girdling experiment neither confirms nor refutes ectomycorrhizal influences on priming. The difference between the mesh exclusion cores and ingrowth cores remains viable and still supports our conclusions.

L. 385–415: This paragraph does a good job acknowledging the girdling-effect waning issue. An equivalent, explicit caveat could be added to the abstract/conclusion, since as currently phrased these sections could be read as reporting a clean negative result for the girdling hypothesis rather than an inconclusive/confounded one.

Thank you for this helpful comment. This point has been addressed as stated above. We have altered the text in the Abstract and Conclusion accordingly.

Statistics: A large number of fixed-effect significance tests are reported across multiple response variables (Tables B1–B4) without correction for multiple comparisons. I don't think this changes the paper's main conclusions, but a sentence acknowledging this (and perhaps highlighting which effects remain robust

under a more conservative threshold) would strengthen the statistical rigour of the paper.

Following the Benjamini-Hochberg false discovery rate procedure (see reference below), we used a step-by-step evaluation and found that 11 tests pass a correction for multiple testing in contrast to the original 12. Differences in the amount of the willow ancient CO_2 efflux between exclusion peat cores succumbed to corrections for multiple testing. Differences in the RPE in relation to the intercept remain robust. This information is detailed in the Results, and ordered p-values are addressed on in the Discussion. Table B5 is included in the Appendix B to demonstrate the critical threshold (i/15 * 0.05).

Order	Benjamini-Hochberg Threshold	p-value	Test
1	0.003	0.001	Root Biomass Mesh
2	0.007	0.001	Willow CO_2 flux Mesh
3	0.010	0.002	Birch CO_2 flux Mesh
4	0.013	0.002	Willow Recent CO_2 flux Mesh
5	0.017	0.002	Birch Recent CO_2 flux Mesh
6	0.020	0.005	RPE Ratio (Without Outlier)
7	0.023	0.006	Willow $^{14}CO_2$ Mesh
8	0.027	0.006	$DO^{14}C$ Willow Mesh
9	0.030	0.009	$DO^{14}C$ Birch Mesh
10	0.033	0.01	RPE Ratio
11	0.037	0.028	Birch $^{14}CO_2$ Mesh
12	0.040	~~0.046~~	~~Willow Ancient CO_2 flux Mesh~~

Reference:

Yoav Benjamini, Yosef Hochberg, Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing, Journal of the Royal Statistical Society: Series B (Methodological), Volume 57, Issue 1, January 1995, Pages 289–300, https://doi.org/10.1111/j.2517-6161.1995.tb0203