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Peer Review File

Symptoms of a Changing Carbon Cycle: a Breakpoint Increase in Soil Respiration in a Northern Hardwood Forest

Corresponding Author: Dr Angela Possinger

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

Version 0:

Decision Letter:

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Dear Dr Possinger,

Your manuscript titled "Symptoms of a Changing Carbon Cycle: a Breakpoint Increase in Soil Respiration in a Northern Hardwood Forest" has now been seen by 3 reviewers, whose comments are appended below. You will see that they find your work of some potential interest. However, they have raised quite substantial concerns that must be addressed. In light of these comments, we cannot accept the manuscript for publication, but would be interested in considering a revised version that fully addresses these serious concerns.

We hope you will find the reviewers' comments useful as you decide how to proceed. In addition, we want you to work on the following editorial thresholds:

-Provide compelling new evidence into the increase in soil respiration in Northern Hardwood Forest.
-Remove speculative discussion and support the conclusions with experimental evidence to prove the CO2 increase is not related to change in the experimental condition (e.g. changed measurement timing, measurement during warmer periods or warmer daytime etc.)

Should additional work allow you to address these criticisms, we would 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,

Somaparna Ghosh, PhD
Associate Editor,
Communications Earth & Environment

EDITORIAL POLICIES AND FORMAT

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• Ecological, evolutionary & environmental sciences
• Life sciences
An updated and completed version of our Reporting Summary must be uploaded with the revised manuscript
You can download the form here:
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For your information, you can find some guidance regarding format requirements summarized on the following checklist: (https://www.nature.com/documents/commsj-phys-style-formatting-checklist-article.pdf) and formatting guide (https://www.nature.com/documents/commsj-phys-style-formatting-guide-accept.pdf).

REVIEWER COMMENTS:

Reviewer #1 (Remarks to the Author):

General comments

This manuscript describes an analysis of soil respiration (Rs) in Hubbard Brook, a long-term research site subject to extensive manipulations and monitoring over decades; the ~20 years of data analyzed here are one of the longest such records in the world. The authors find a substantial increase in Rs since about 2015, much larger than can be explained by direct changes in drivers such as temperature, moisture, pH, etc., and suggest that this is consistent with (hypothesized) increases in plant production and belowground allocation. This ms is well written and interesting, figures are clear, and generally the analysis is methodologically sound.

There are some problems. The results have a few shaky extrapolations and expository material that it would be better to move to the discussion; the analytical code isn't available; ref 41 could be explained and used better. For all of these, see specific comments below.

It would be good to explore other possible reasons for the Rs increase in a little more depth. First, the authors suggest that increasing productivity and/or belowground allocation could be driving Rs changes, but this is difficult to square with stable leaf litter and LAI (71-72). Are there any data suggesting productivity changes? If this is a regional trend (ref 41), is it reflected in any of the many Rs/Rh global datasets, or remote sensing data? Second, I'm sure the authors have considered this carefully, but I must ask: could *any* protocol or equipment changes have induced a spurious trend? See #7 below; obviously extending the observations into November wouldn't do it…but if that's the same time when you shifted GCs (line 422), that would make me nervous. (Yes, I see 423-424, thanks.)

In summary, this is well done and interesting study reporting a close to unique dataset and an intriguing if ultimately unexplained finding. It needs minor to moderate revisions for clarity, to be a bit more conservative in places, and to improve reproducibility.

Specific comments
1. Line 1: perhaps simply "A breakpoint increase…" or "An unexplained breakpoint increase…"?
2. L. 43: clarify that this is a hypothetical (i.e., that you didn't measure belowground allocation)

3. L. 187-191: this is quite an extrapolation and belongs in the discussion, not results
4. L. 196: perhaps "see Figure 1 in ref 41" to help readers. This thesis chapter is a crucial point of comparison, and I'm surprised the authors aren't giving it more prominence; agreed, seems like the control plots' Rs in this study had a decadal (at least) increase of similar magnitude
5. L. 292-299: seems more appropriate for the discussion?
6. L. 361-367: this is nicely laid out and convincing (i.e., the *potential* for this to account for the observed changes is convincing)
7. L. 417: this protocol change in 2015, right when the breakpoint is subsequently found, makes me nervous
8. L. 555-557: I don't know CEE policies but do not, in 2024, view this as acceptable (although I appreciate the authors' offer); analytical code should be default be available for reviewers and readers. Please support open and reproducible science by permanently depositing your code

Reviewer #2 (Remarks to the Author):

Soil respiration plays a pivotal role in control the net carbon balance of ecosystems. Under the background of climate change (such as global warming, increasing CO2 concentrations, altered precipitation patterns, and reduced sulfur deposition), the authors endeavors to identify significant shifts in soil-atmosphere carbon feedbacks through the lens of soil respiration. Long-term in-situ observations are indispensable for ecological research. Using a long-term in-situ soil respiration measurements from 2002 to 2020, the authors have revealed a marked increasing in soil respiration rates since 2015 in the Hubbard Brook Experimental Forest, particularly pronounced in the months of July and August. This increasing was driven by interactions among below-ground C allocation, soil microbial communities, and soil organic C bioavailability, and cannot be explained directly by temperature, moisture, or pH change alone. These discoveries are innovative and stand to inject fresh insights and impetus into the study of environmentally sensitive biomes, including boreal forests and alpine coniferous forests. Despite the great interest brought by this manuscript, there are several concerns that need to be addressed prior to its publication.

Firstly, the authors ascribe the post-2015 increase in soil respiration to an enhancement in belowground carbon inputs, a claim that requires more robust evidence. The manuscript is devoid of data on rhizosphere carbon input or fine-root biomass. The researchers have solely referenced data from Fahey et al. (2005), which is now two decades old. It would be beneficial if the authors could update this data by conducting new tests in alignment with the methodology of Fahey et al. Additionally, as the authors note, the rapid increasing in soil respiration predominantly occurs during the peak growing season (July and August), coinciding with a decrease in nitrogen availability in this forest (lines 76-78). During this period (July and August), the enhanced rhizosphere input may stimulate the decomposition of old soil organic matter (SOM) to meet the nitrogen demand, thereby "prime" soil respiration. Thus, if data such as soil isotope signals can be provided to substantiate that the carbon derived from plant or microbial sources in SOC has significantly altered since 2015, it would be instructive.

Secondly, following the author's method of proof, it is inevitably that any increase in rhizosphere carbon input would necessarily impact soil microbial processes, including the microbial heterotrophic C mineralization. The authors have indeed documented an enhancement of soil microbial heterotrophic C mineralization post-2015 (Fig 4c). However, the trend of the heterotrophic C mineralization rate from 2015 to 2020 does not coincide with the rise in soil respiration (Fig 3), and it has even exhibited a non-significant decrease. Additionally, the microbial biomass C has not significantly changed since 2015 (Fig 4a). Consequently, i must question whether the increase in soil respiration observed since 2015 is genuinely attributed to heterotrophic respiration (or prime).

Thirdly, it is noteworthy that the increasing trend in soil respiration since 2015 corresponds with the enhanced microbial C mineralization rate in the litter layer (Fig 4c, Oi, Oe,). This suggests that the enhanced decomposition of the litter layer could be a contributing factor to the increased soil respiration. According to Supplementary Table 2, the carbon pool in the forest floor appears to have diminished since 2014, potentially explaining the accelerated decomposition of the litter. Consequently, it appears that the increasing in soil respiration since 2015 in this study may actually reflect variations in leaf litter respiration. Of course, it is my understanding that the soil respiration measured in this study encompasses the respiration from the litter layer (Oi, Oe, Oa).

Fourthly, the significance of the proportion of root respiration (termed soil autotrophic respiration) within soil respiration is particularly noteworthy, especially during the period of rapid growth. It is imperative to acknowledge the contribution of soil autotrophic respiration to overall respiration, despite the author's attribution of root respiration to belowground carbon allocation in the discussion (refer to lines 357-359). It should be noted that an increase in root respiration is distinct from an increase in belowground carbon allocation; these are two separate processes influencing soil carbon dynamics. The evidence presented in the manuscript suggests that enhanced root respiration is likely another critical factor contributing to the observed increase in total respiration.

Lastly, why is the trend of specific respiration in Fig. 4d different from that in Extended Data Fig. 5a. I am also keen to understand the relevance and role of this particular dataset (specific respiration or ln specific respiration) within the context of this study.

Reviewer #3 (Remarks to the Author):

A.R. Possinger and colleagues present a long-term record of soil respiration data from Hubbard Brook. The dataset is highly interesting as it covers multiple sites in the catchment area and soil CO2 efflux jumps to a higher level in the range 2013-18. Supportive data include a time series of microbial biomass and efficiency measurements from organic layer and mineral soil samples and C stock data of the organic layer for a sub-sample (the Ca-treated part of the watershed). The data suggest that the increase in soil CO2 efflux is paralleled by increasing microbial biomass and mineralization rates. The results are very solid as they apply for all landscape positions and both treatments (control, Ca applied). Annual cumulative CO2 efflux patterns are not related to mean annual temperatures or precipitation patterns – hence another mechanism then simple temperature sensitivity must be the cause of the observed increase in soil respiration.

Authors argue (discussion) that increased below ground transfer of C likely is the cause of the soil CO2 efflux increase. Overall, this interpretation is plausible, or likely, but it remains highly speculative. The reason is that a lot of important information towards an improved process understanding is missing. It was not assessed how autotrophic respiration, an equally important soil CO2 source as heterotrophic respiration, had developed over time, nor were fine root biomasses or above ground litter inputs measured – but they all are key elements in the discussion. Soil C-stocks were assessed only of a subsample and only of the forest floor. It cannot simply be assumed that all the microbially respired CO2 arises only from the forest floor. Furthermore, it does not become clear in the text if there is already a decrease in forest floor carbon, or not, and if such a decrease is related to the jump in soil respiration rates. From supplementary table S2 it seems that there is a general C stock decrease in the forest floor over time, but not necessarily related to the increase in soil respiration from 2015 onwards. How does this fit together? Only increased belowground C allocation is provided as explanation for the increased soil CO2 efflux, but there might be alternative explanations as well. What about wintertime snow-cover and soil temperatures – was there any change? Other limiting factors or other than N nutrient availability might have changed and increased microbial biomass and respiration. Accordingly, the breakpoint in CO2 efflux indeed may be seen a symptom of a changing C cycling, but the causes remain open. The results might be discussed in view of other long-term datasets e.g from Harvard forest etc. Is there a similar trend visible?

There also are some methodological issues regarding the CO2 efflux data interpretation – please see below.

Detailed comments:

When I look at Figures 2 and 3, I wonder how they fit together. Fig 2 shows cumulative growing season CO2 fluxes - and here the fluxes during the three most recent three years were more than double as high than the previous ones. Fig 3 shows all CO2 flux rates – but here this pattern does not emerge, or at least by far not as strong as in Fig 1. I also do not understand why Fig 3 does not show all the measurements together in one panel (a) and the solely July-August data in a second one (b). The current procedure showing one fraction (May-June + Sept-Oct) and the other fraction (Jul-Aug) in separate panels is confusing – one may ask why there is a breakpoint in panel a, if there is obviously no difference in fluxes throughout the time series. How can fluxes become negative in panel a? Why are there some years (a) and one year (b) data missing in this figure – but cumulative sums are shown in Fig 1? Figure 2: How can it be that there appear two dots in single years if the graph is supposed to show only a single cumulative flux per year? (panels reference/high elevation 2013-2017 and panel Ca-treated/high elevation 2016).

Data analysis could be improved at one particular point. Currently, variations in soil CO2 effluxes are tested against variations in mean air or mean soil temperatures. However, due to the rather low frequency of CO2 measurements (1 monthly measurement during the growing season), it is not granted that the actual flux measurements took place at mean (monthly) air or soil temperature. It easily can happen that fluxes are measured during a cold or heat spell, and then for sure they do not relate to a mean (monthly) temperature. I therefore suggest testing the measured daily soil respiration rates exactly against the soil temperatures, that occurred exactly during the CO2 efflux measurement (and not against a monthly temperature mean). Even within single days, measurement time can play a role. Measurements early in the morning may produce much lower CO2 efflux rates than in late afternoon when soils are warmer. Only after such a more detailed analysis against real soil temperatures during CO2 efflux measurements is done, it can be guaranteed that there was no influence of temperature on the observed soil respiration trends.

Growing season (May-Oct) total soil CO2 efflux estimates (Fig.2) are generally low with 1-2 tons C per ha. This may be a result of underestimated flux rates (Fig.3) due to the long chamber closure during CO2 flux measurements. As a result, quantitatively, the heterotrophic component of the annual flux estimates would be lower than soil C inputs implying increasing soil C-stocks over time. This might be mentioned somewhere.

The method to assess mineralization rates is rough. Soil samples were left for 10 days at room temperature to accumulate CO2. Room temperature will greatly influence respiration rates. Was room temperature always stable? Already a few degrees change can have large effects on fluxes. How were samples stored and how was air sampled and CO2 measured?

Was there a general correlation between microbial biomass and respiration during all the years?

DIC: Extended Data Fig. 4 and text. Was the break-point calculated separately for DIC, or was just the breakpoint for CO2 efflux used here? It would be interesting if the DIC data suggests a similar break-point as the CO2 data, or not. If there is no DIC break-point, then it does not make much sense splitting the DIC dataset exactly in 2015.

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Version 1:

Decision Letter:

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Dear Dr Possinger,

Your manuscript titled "Symptoms of a Changing Carbon Cycle: a Breakpoint Increase in Soil Respiration in a Northern Hardwood Forest" 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,

Somaparna Ghosh, PhD
Associate Editor,
Communications Earth & Environment
Consulting Editor,
Communications Sustainability

REVIEWERS' COMMENTS:

Reviewer #1 (Remarks to the Author):

The authors have done an excellent job responding to and addressing my previous concerns. The additional analyses, more conservative language, and reorganized discussion all make for a substantially improved manuscript--nice job. I'm pleased to recommend this for publication.

Reviewer #2 (Remarks to the Author):

After the previous round of revisions, the explanation regarding the sudden increase in soil respiration, which was my primary concern, has been significantly strengthened. Additionally, the scientific rigor of the methodology has been sufficiently improved. Therefore, I have no other major revision suggestions.
However, some details regarding scientific accuracy need further enhancement by the authors.
For example:
Line 38-39: “have increased by more than 100% between 2015 and 2020” – I think this description somewhat unscientific because the increase in this context is a continuous process, and using a simple average value is not entirely accurate.
Line 67: “at HBEF has increased by 0.25 °C decade-1” – This seems inconsistent with Fig1 (a).
Line 348-349: “Increased root-to-rhizosphere C flux” – I believe this is the authors' hypothesis, and such wording may lead readers to assume it is an established fact.

Reviewer #3 (Remarks to the Author):

Authors performed a thorough revision. Concerns regarding measurement errors could be ruled out and the revised manuscript reads exceptionally well. Limitations of the study are now clearly acknowledged. I suggest publication of this important study. Few last comments for fine-tuning, see below.

L289: microbial carbon use efficiency is related to microbial growth, not to microbial biomass. You may use the term “microbial substrate use efficiency” instead. “specific respiration” is also a bit unclear (although defined once). Maybe better use “biomass specific respiration”. (The actual term respiration per biomass would be “metabolic quotient”)

Figure 6: I would add an additional arrow from box “Belowground C allocation” that goes directly through the soil surface to “total soil respiration”. This arrow would mean the autotrophic soil respiration component (which also results from C below ground allocation) and could also have increased.

L841-845: if the annual respiration values are potentially massively underestimated due to the measurement procedure, it does not make sense to directly relate them to correctly estimates of C inputs. The few lines with direct C input – output comparison might therefore better be removed.

Line Numbers refer to the manuscript.pdf with marked chanches!

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Manuscript: Symptoms of a changing carbon cycle: a breakpoint increase in soil respiration in a northern hardwood forest

Revision 1: Response to Review

Note: throughout the response, line numbers are indicated for the revised manuscript <u>without</u> tracked changes

Overview:

We thank the reviewers for their thorough, thoughtful reviews that helped us think through our observations and strengthen our interpretations regarding a sudden increase in soil respiration at Hubbard Brook Experimental Forest. We have conducted new analyses and made extensive revisions to the manuscript, and we feel (and hope) that the manuscript is greatly improved as a result. The reviewers' requests for careful consideration of any plausible methodological explanation for our observations strengthened our confidence in the observations and reinforced the importance and potential implications of the rapid increase in soil respiration. In the revised version, we integrated this important supporting information into a new **Supplementary Methods** section in the Supplementary Information (lines 22-72 in the SI).

An additional major change was to revise the discussion section to be more consolidated, synthetic, and easier-to-follow. We expanded and clarified our rationale of potential explanations for our observations of an increase in soil respiration, including a more constrained discussion of proposed below-ground C allocation as a driver of the increase in soil respiration (e.g., lines 343-345 in the revised manuscript), expansion on other plausible drivers (e.g., lines 378-397, and lines 400-422), discussion of relevant data available to address these potential drivers, and new work needed when available data are lacking (e.g., lines 389-397, regarding root respiration).

We also substantially improved the presentation of growing season cumulative soil respiration (in the revised **Fig. 2**) and respiration rate patterns (in the revised **Fig. 3**). We believe that these changes address the reviewers' primary concerns of 1) the need for greater evidence for an increase in soil respiration in a Northern hardwood forest and 2) addressing concerns about possible methodological artifacts, outlined in greater detail below:

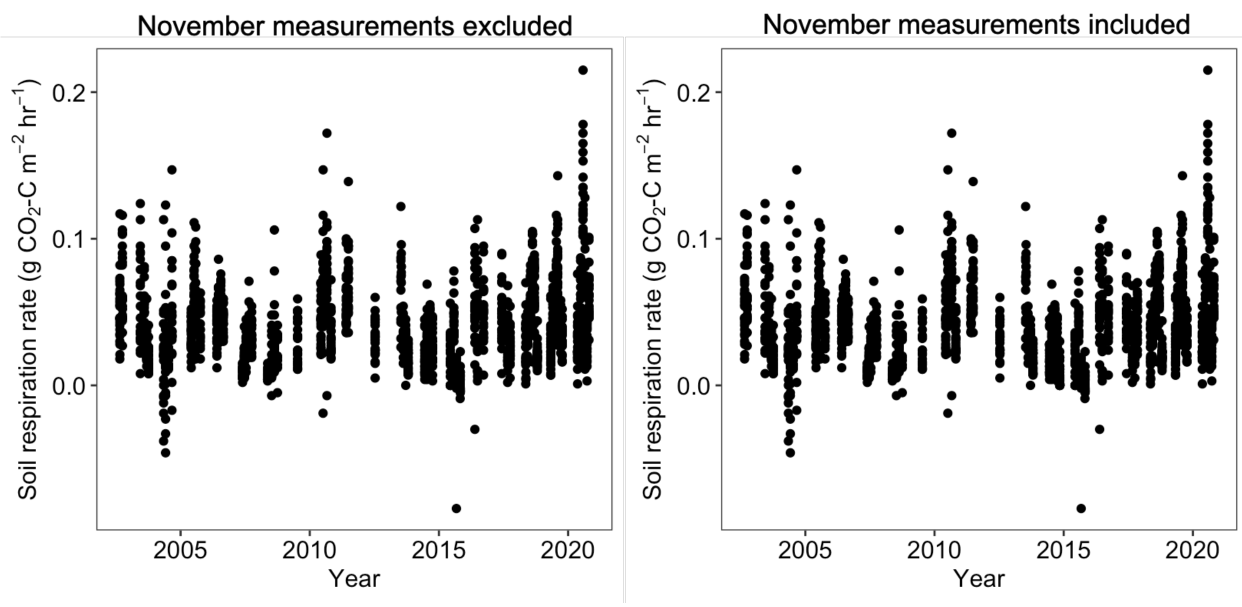
1) **Supporting evidence for the observed increase in soil respiration:** We made several improvements to the manuscript that strengthen the supporting evidence for our observed increase:

- Changes to **Fig. 2** (inclusion of all data points in addition to an overall mean) and **Fig. 3** (inclusion of respiration rates from all months, May-October in contrast to July-August only). These changes help to more clearly illustrate the increase across elevations and watersheds;
- Significant re-organization of the Results and Discussion, which allowed for clearer distinction between observations directly from this study versus. interpretation based on data synthesis and supporting references;
- Improved and expanded integration of other datasets (primarily Mann et al., 2024) that reinforce the observations at Hubbard Brook, and discussion of other regional long-term soil respiration measurements.

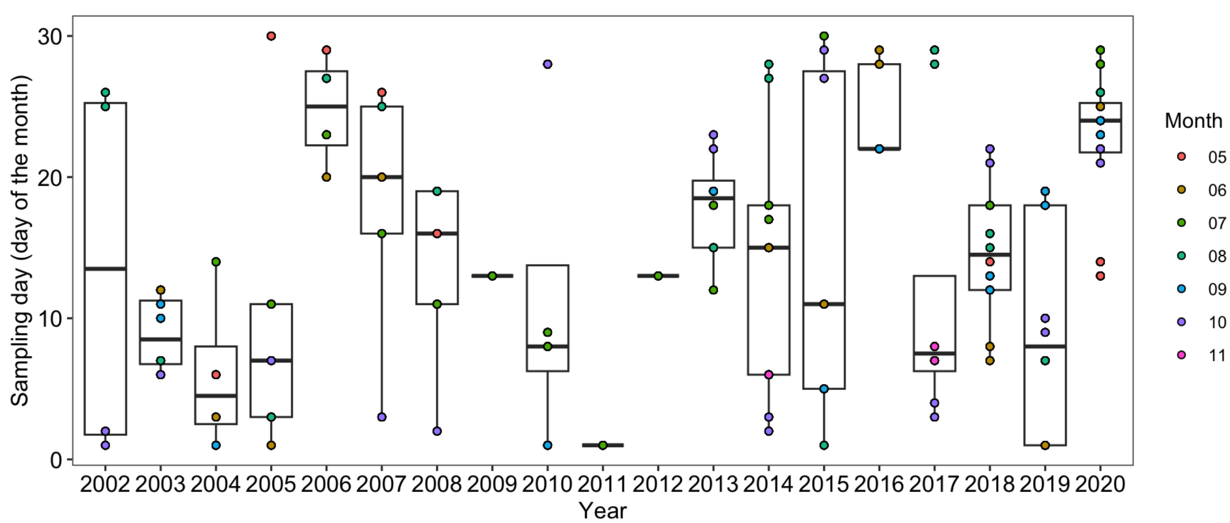
2) **Assessment of possible methodological bias in long-term measurements:** We agree that the nature of the sudden increase in soil respiration lends itself to double- and triple-checking that a methodological change or other experimental artifact was not the cause. In the following response, we provide more detail on several additional methodological checks, and have incorporated key points from below into a new section in the supplementary material in the revised manuscript (**Supplementary Methods,** lines 22-72). Our response here focuses on the concerns of changed measurement timing, measurement during warmer periods or warmer daytime, instrument change and addition of November measurements.

- Changed measurement timing:
  - Sampling months: *In-situ* trace gas measurements were collected from May-October throughout the time series. November measurements were added in 2014 (11-06-2014), but were only collected again once, in 2017. November measurements were not incorporated into growing season cumulative measurements. For the revised manuscript, we double-checked code and it does indeed exclude November. Please see lines 208-209 in code file "CEE_CO2_DataImportProcessing_GitHub" available in the Github repository: https://github.com/arp264/HBEF_soil_respiration. In addition, we checked if including November respiration rate measurements drove the observed increase in respiration rate (manuscript **Fig. 3**), and determined that November rates were not particularly influential because (1) there are only 2 timepoints (2014 and 2017) and (2) the soil respiration increase is mainly driven by July/August rates (as highlighted in the manuscript) (manuscript **Fig. 3**; **Response Fig. 1**; new **Supplementary Fig. 10**).
  - Sampling day of month: We checked if there was a systematic change in the timing of sampling within a month over the long-term record. There is no directional pattern in sampling that would affect this relationship (e.g., consistently earlier sampling in fall, consistently later sampling in spring) (**Response Fig. 2**, new **Supplementary Fig. 11**).
  - Measurement during warmer periods (e.g., warmer times within a day): We are not aware of any systematic changes in the timing of measurements within a given day that would result in a consistent increase in soil respiration rates over time. However, in the revised manuscript, we expanded on our exploration of potential differences in soil temperature over the long-term record by filtering soil temperatures to a reasonable daily sampling window (10am-4pm). Regarding potential time-of-day effects, we do not have exact measurement times for respiration measurements available, but recognizing the potential impact of diurnal temperature variation in these measurements, have expanded our assessment of potential Q_{10} effects in the revision based on the trend in minimum, maximum, and mean soil temperature within the measurement time window (10am-4pm) in July and August (when most of the increase in soil respiration occurred). Based on our new analysis of ranges in soil temperature within a given sampling day, we found a slightly higher average increase in minimum soil temperature (compared to mean soil temperature) and revised our estimation of potential temperature effects (2-5% based on mean; 4-12% based on minimum). This information is included in the text in the revised version (lines 249-264). It

should be noted that our soil temperature measurements are not exactly co-located with the soil respiration collars and thus represent a potential maximum direct influence of soil temperature given the possible ranges at the site. This has now been clarified more strongly in the revised text (lines 253-257).



Response Figure 1 (New Supplmentary Fig. 10). All soil respiration rates (all collars, elevations, and watersheds combined), showing pattern with and without November measurements (in 2014 and 2017 only).



Response Figure 2 (new Supplementary Fig. 11). Day of month soil respiration measurements were collected over the time series used in this study.

- Trace gas sampling and instrumentation:
  - Collar effects: Ni and Groffman (2018) assessed potential effects of wear and tear on PVC collars in this study (and in similar permanent collars in Baltimore, MD)

by installing new collars and comparing with the collars that had been previously installed for a longer period. This test was also conducted at the Multiple Element Limitation in Northern Hardwood Ecosystems (MELHNE) experiment (Mann et al., 2024). Neither collar test showed an installation effect; i.e. the older collars matched the new ones. This detail about collar checks is now mentioned in the **Methods** section (manuscript lines 467-477) and in the new **Supplementary Methods** section (SI lines 47-59).

- Instrument effects: We thank Reviewer 1 for highlighting the question about instrument changes. It is an important question that we considered carefully during initial analyses and expand on more thoroughly in the revised manuscript. While the instrument shift does occur at a similar time to the average increase in soil respiration, we considered several factors related to instrument changes and conclude that the increase is not due to this change in GC instrument model: (1) sampling trip standards and certified external calibration standards were used to verify that instrument bias did not affect gas measurements over time; (2) both GC instruments use the same mechanism of detection and detection type; (3) a change in GC instrumentation would likely result in a step-change (e.g., a baseline shift) rather than a consistent increase over the 2015-2020 period; (4) our observations are broadly consistent with Mann et al. (2024), who reported an overall increase in ambient soil respiration at HBEF and nearby sites using an *in-situ* Li-COR 8100 Soil Respiration System (Licor Biosciences, Lincoln, NE) rather than gas sampling and GC analysis. This information is referenced in the main text (lines 473-475) and included in the new **Supplementary Methods** (lines 61-72).

In summary, our assessment of possible effects of changes in the timing of measurements suggests that these changes had no effect on our observations of an increase in soil respiration. We have added text to the revised manuscript to highlight these methodological checks as described above and summarized in the new **Supplementary Methods** section.

Editorial Policies and Format: In the revised manuscript, we have updated formatting to match Communications Earth and Environment style as outlined in the Communications Journals Style and Formatting Guide.

Response to Reviewer Comments:

Reviewer #1 (Remarks to the Author):

General comments

This manuscript describes an analysis of soil respiration (Rs) in Hubbard Brook, a long-term research site subject to extensive manipulations and monitoring over decades; the ~20 years of data analyzed here are one of the longest such records in the world. The authors find a substantial increase in Rs since about 2015, much larger than can be explained by direct changes in drivers such as temperature, moisture, pH, etc., and suggest that this is consistent with (hypothesized) increases in plant production and belowground allocation. This ms is well written and interesting, figures are clear, and generally the analysis is methodologically sound.

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It would be good to explore other possible reasons for the Rs increase in a little more depth. First, the authors suggest that increasing productivity and/or belowground allocation could be driving Rs changes, but this is difficult to square with stable leaf litter and LAI (71-72). Are there any data suggesting productivity changes? If this is a regional trend (ref 41), is it reflected in any of the many Rs/Rh global datasets, or remote sensing data? Second, I'm sure the authors have considered this carefully, but I must ask: could *any* protocol or equipment changes have induced a spurious trend? See #7 below; obviously extending the observations into November wouldn't do it…but if that's the same time when you shifted GCs (line 422), that would make me nervous. (Yes, I see 423-424, thanks.)

In summary, this is well done and interesting study reporting a close to unique dataset and an intriguing if ultimately unexplained finding. It needs minor to moderate revisions for clarity, to be a bit more conservative in places, and to improve reproducibility.

Response: We thank the reviewer for the helpful and thoughtful comments and suggestions. We appreciate the positive feedback on the study overall, and especially which elements might be most interesting or need further expansion. Here, we summarize our responses to the overarching questions raised, and expand on specific line comments below:

- Is productivity increasing, given stable LAI and leaf litter?
    - Response: This is a great point, and one that is an important assumption in the proposed below-ground allocation concept. To our knowledge, the metrics that would indicate a change in GPP and that align with similar timescales to soil respiration data are limited to LAI, leaf litter, and above-ground living tree biomass (shown in **Fig. 1**) at Hubbard Brook. Flux tower data from Hubbard Brook (Kelsey et al., 2023) do not include an estimate of GPP. Evapotranspiration, however, increased rapidly at Hubbard Brook beginning around ~2011 (Green et al., 2021), and while the cause of this change is not resolved, an increase in GPP would be consistent with this increase. While the increase in ET was mentioned in the original text, we have expanded on the evapotranspiration observations as a potential connection to overall ecosystem changes in carbon balance, highlighted in the revised version in the discussion, lines 367-369. In addition, there are some indications that GPP might be increasing at Bartlett Experimental Forest (Bartlett, NH; close to Hubbard Brook), as indicated by flux tower measurements, but these data are unpublished. As such, in the revised manuscript, we re-organized the logic leading up to the statement of the need for future work that captures these interrelated carbon cycle processes, lines 369-376.

- Are there supporting observations for regional trends from soil respiration datasets and/or remote sensig?
    - Response: We thank the reviewer for this suggestion! We explored the current version (V5) of the Global Soil Respiration Database (SRDB) (Jian et al., 2021), used in Bond-Lamberty et al. (2018) to show global increases in Rs and Rh. The spatial coverage of the Northeastern US region (e.g., latitude 40 to 48 degrees and longitude -75 to -63 degrees) in the SRDB is sparse; most of the observations in the region come from Harvard Forest (Harvard, MA). We added text to the discussion on this limited data availability in the region and the needs to synthesize what data are available, and to expand on soil respiration measurements (lines 307-321). We investigated whether published remote sensing estimates for the Northeast US covered the period of observed Rs increases (~after 2015), and to our knowledge, published remote sensing-derived data mainly cover periods prior to 2015 (e.g., Huang et al., 2020). However, as more updated estimates become available, we will integrate these complementary observations into ongoing work.

- Protocol changes: could there be methodological artifacts?
    - Response: We appreciate the reviewer raising this question, and we agree that with a long-term dataset there is more potential for protocol or equipment changes that could introduce artifacts or biases in the measurements. In the revision, we added further detail regarding possible measurement artifacts in the main manuscript and a new section in the Supplementary Material (lines 22-72). As this point was raised by other reviewers as well, we combined our response to methodological checks in the above section "Assessment of possible methodological bias in long-term measurements."

Specific comments

1. Line 1: perhaps simply "A breakpoint increase…" or "An unexplained breakpoint increase…"?

Response: We appreciate this suggestion! While the simpler title has some benefits, one of the points we wanted to emphasize in the introduction and framing was the assessment of soil respiration as an integrative "symptom" of many co-occurring environmental change factors, which is why this term was used in the title. We'd like to retain the title for this reason, and swapped out the word "signal" for "symptom" in the first paragraph of the introduction (line 58) to emphasize this point further.

2. L. 43: clarify that this is a hypothetical (i.e., that you didn't measure belowground allocation)

Response: Thank you for the comment. This concern has been clarified in the text:

Original text: Rather, our observations are consistent with increasing plant allocation of C belowground in response to changing climatic and soil conditions.

Revised text: We suggest that these observations are consistent with a hypothetical increase in plant allocation of C belowground in response to changing climatic and soil conditions.

3. L. 187-191: this is quite an extrapolation and belongs in the discussion, not results

Response: Agreed! This section highlighting the potential magnitude of the observed increases was moved entirely to the Discussion (lines 279-296 in the revised manuscript). Throughout the manuscript, we have revised the Results and Discussion, which was originally prepared in a more integrative short-format style, to a more standard "Results" and "Discussion" format.

4. L. 196: perhaps "see Figure 1 in ref 41" to help readers. This thesis chapter is a crucial point of comparison, and I'm surprised the authors aren't giving it more prominence; agreed, seems like the control plots' Rs in this study had a decadal (at least) increase of similar magnitude

Response: We appreciate the reviewer's suggestion. We've added the specific reference to the figure shown in Mann et al. (2024) in the text. Since our original submission, the Mann et al. work was published in a peer-reviewed paper, and as such, we've expanded our inclusion of these measurements significantly into the text. While the observations of Mann et al. (2024) point to an increase in soil respiration, the paper is not focused on ambient increases in respiration as such, and shows limited data and interpretation related to these trends. In contrast our observations are unique in the longer-term duration (required to detect the rapid and high-magnitude breakpoint increase), as well as the pairing to microbial C cycling metrics (biomass, mineralization assays, etc.). However, the observations of Mann et al. (2024) are important context for the potential occurrence of this phenomenon as a potential broader regional trend, and we substantially expanded the discussion of its applications in the modified discussion (lines 298-321).

5. L. 292-299: seems more appropriate for the discussion?

Response: Agreed! This section (in which we discussed pH controls) was moved entirely to the discussion in a new section focusing on pH, moisture, and temperature as potential causes of the observed changes in soil respiration flux (lines 324-332, 402-422 in the revised manuscript).

6. L. 361-367: this is nicely laid out and convincing (i.e., the *potential* for this to account for the observed changes is convincing)

Response: We appreciate the positive feedback on this section!

7. L. 417: this protocol change in 2015, right when the breakpoint is subsequently found, makes me nervous

Response: We agree with the reviewer. We noticed this shift as well and considered the impact of the instrument prior to data analysis and interpretations. We revisited the question in the revised manuscript in the new **Supplementary Methods** section. Regarding this point specifically, we noted in the revised **Supplementary Methods** text:

While the instrument shift does occur at a similar time to the average detected increase in soil respiration, we considered several factors related to instrument changes and conclude that the increase is not due to this change in GC instrument model: (1) sampling trip standards and certified external calibration standards were used to verify that instrument bias did not affect gas measurements over time; (2) both GC instruments use the same mechanism of detection and detector type; (3) a change in GC instrumentation would likely result in a step-change (e.g., a baseline shift) rather than a consistent increase over the 2015-2020 period; (4) our observations are consistent with Mann et al.[2], who reported an overall increase in ambient soil respiration at HBEF using an *in-situ* Li-COR 8100 Soil Respiration System (Licor Biosciences, Lincoln, NE) rather than gas sampling and GC analysis.

8. L. 555-557: I don't know CEE policies but do not, in 2024, view this as acceptable (although I appreciate the authors' offer); analytical code should be default be available for reviewers and readers. Please support open and reproducible science by permanently depositing your code

Response: We appreciate the reviewer raising this point. The code was uploaded as a supplementary file in the original submission, but it looks as though it didn't make its way to a more accessible location for review - apologies! For the revision, code has been deposited in a publicly accessible repository, and the data availability statement has been revised as follows:

Original text:
Data processing, breakpoint analysis, and statistical analysis code are available on request from the authors during the review process and will be available via a certified repository upon publication.

Revised text:
Data processing and statistical analysis code are publicly available at https://github.com/arp264/HBEF_soil_respiration.

Reviewer #2 (Remarks to the Author):

Soil respiration plays a pivotal role in control the net carbon balance of ecosystems. Under the background of climate change (such as global warming, increasing CO2 concentrations, altered precipitation patterns, and reduced sulfur deposition), the authors endeavors to identify significant shifts in soil-atmosphere carbon feedbacks through the lens of soil respiration. Long-term in-situ observations are indispensable for ecological research. Using a long-term in-situ soil respiration measurements from 2002 to 2020, the authors have revealed a marked increasing in soil respiration rates since 2015 in the Hubbard Brook Experimental Forest, particularly pronounced in the months of July and August. This increasing was driven by interactions among below-ground C allocation, soil microbial communities, and soil organic C bioavailability, and cannot be explained directly by temperature, moisture, or pH change alone. These discoveries are innovative and stand to inject fresh insights and impetus into the study of environmentally sensitive biomes, including boreal forests and alpine coniferous forests. Despite the great interest brought by this manuscript, there are several concerns that need to be addressed prior to its publication.

Firstly, the authors ascribe the post-2015 increase in soil respiration to an enhancement in belowground carbon inputs, a claim that requires more robust evidence. The manuscript is devoid of data on rhizosphere carbon input or fine-root biomass. The researchers have solely referenced data from Fahey et al. (2005), which is now two decades old. It would be beneficial if the authors could update this data by conducting new tests in alignment with the methodology of Fahey et al. Additionally, as the authors note, the rapid increasing in soil respiration predominantly occurs during the peak growing season (July and August), coinciding with a decrease in nitrogen availability in this forest (lines 76-78). During this period (July and August), the enhanced rhizosphere input may stimulate the decomposition of old soil organic matter (SOM) to meet the nitrogen demand, thereby “prime” soil respiration. Thus, if data such as soil isotope signals can be provided to substantiate that the carbon derived from plant or microbial sources in SOC has significantly altered since 2015, it would be instructive.

Secondly, following the author's method of proof, it is inevitably that any increase in rhizosphere carbon input would necessarily impact soil microbial processes, including the microbial heterotrophic C mineralization. The authors have indeed documented an enhancement of soil microbial heterotrophic C mineralization post-2015 (Fig 4c). However, the trend of the heterotrophic C mineralization rate from 2015 to 2020 does not coincide with the rise in soil respiration (Fig 3), and it has even exhibited a non-significant decrease. Additionally, the microbial biomass C has not significantly changed since 2015 (Fig 4a). Consequently, i must question whether the increase in soil respiration observed since 2015 is genuinely attributed to heterotrophic respiration (or prime).

Thirdly, it is noteworthy that the increasing trend in soil respiration since 2015 corresponds with the enhanced microbial C mineralization rate in the litter layer (Fig 4c, Oi, Oe,). This suggests that the enhanced decomposition of the litter layer could be a contributing factor to the increased soil respiration. According to Supplementary Table 2, the carbon pool in the forest floor appears to have diminished since 2014, potentially explaining the accelerated decomposition of the litter. Consequently, it appears that the increasing in soil respiration since 2015 in this study may actually reflect variations in leaf litter respiration. Of course, it is my understanding that the soil respiration measured in this study encompasses the respiration from the litter layer (Oi, Oe, Oa).

Fourthly, the significance of the proportion of root respiration (termed soil autotrophic respiration) within soil respiration is particularly noteworthy, especially during the period of rapid growth. It is imperative to acknowledge the contribution of soil autotrophic respiration to overall respiration, despite the author's attribution of root respiration to belowground carbon allocation in the discussion (refer to lines 357-359). It should be noted that an increase in root respiration is distinct from an increase in belowground carbon allocation; these are two separate processes influencing soil carbon dynamics. The evidence presented in the manuscript suggests that enhanced root respiration is likely another critical factor contributing to the observed increase in total respiration.

Lastly, why is the trend of specific respiration in Fig. 4d different from that in Extended Data Fig. 5a. I am also keen to understand the relevance and role of this particular dataset (specific respiration or ln specific respiration) within the context of this study.

Response: We thank the reviewer for the thoughtful and constructive feedback on the manuscript. In the revised version of the manuscript, we have substantially changed the Discussion section to address the points raised about proposed explanations for the observations, as well as expanded on our discussion of additional measurements that could help unravel mechanisms. Below, we highlight specific points raised in the review with examples of additions or edits to the manuscript text in the revised version.

(1) Proposed mechanism of enhancement in belowground carbon inputs requires more robust evidence:

- Response: We appreciate the reviewer highlighting this point. We agree, and substantially revised the Discussion section to clarify this mechanism as a proposed hypothesis in the context of other possible influences on carbon bioavailability in turnover, integrating available root biomass data as described below. But, throughout the manuscript, we also clarified that this is a "proposed, "hypothesized," or "plausible" mechanism, and that other explanations for increased bioavailability of soil carbon were possible (or likely to be co-occurring); for example in the Abstract lines 42-44, and in the Discussion in lines 343-345, lines 359-361, and lines 378-397. Overall, our goal was to expand our rationale, but also constrain our language to more accurately represent this proposed mechanism.

  For example, the shift to more constrained language was emphasized in the abstract:

  Abstract:

  Original text lines 39-44: This rapid increase in soil C turnover (25-40 g CO_2-C m^{-2} growing $season^{-1}$) equates to a 25-55% decrease in the forest floor C stock at Hubbard Brook on a decadal timescale. The increase in soil respiration cannot be explained directly by temperature, moisture, or pH change alone. Rather, our observations are consistent with increasing plant allocation of C belowground in response to changing climatic and soil conditions.

  Revised text lines 39-44: The increase in soil respiration cannot be explained directly by temperature or pH change alone. We also found that heterotrophic microbial C mineralization and microbial biomass C also increased rapidly since ~2015, pointing towards an increase in the bioavailability of organic C substrates. We suggest that these observations are consistent with a hypothetical increase in plant allocation of C belowground in response to changing climatic and soil conditions.

(2) Lack of below-ground root or rhizosphere flux data, particularly that following methodology of Fahey et al (2005):

- Response: We absolutely agree that root-related measurements in general are needed to support the proposed mechanism. Unfortunately, time series measurements of root properties and autotrophic respiration are not available that can be meanginfully compared to long-term soil respiration measurements. We have highlighted this limitation in the revised Discussion in lines 378-397. In addition, we also addressed this concern by constraining our language relating to the proposed belowground C allocation mechanism throughout the manuscript, as noted in the response above. We also agree that an update to the Fahey et al. (2005) carbon budget is timely and important, and proposed updates to carbon cycle synthesis are ongoing at HBEF, but outside the scope of the present work. However, we emphasize that we primarily used the Fahey et al. (2005) estimates as benchmark to consider the potential magnitude or implication of the observed increases in soil respiration in the context of our current best estimate of the total carbon budget, rather than a direct comparison of carbon budget estimates *per se*. To clarify this point, we revised the Discussion text to state:

Lines 359-361: In addition to evidence of these consistent spatial and temporal trends, we considered whether plant-microbial-soil feedbacks could be a plausible cause of increasing soil respiration in the context of the forest C budget, given prior estimates of root-rhizosphere C fluxes[23].

(3) Stable isotope data to help resolve plant or microbial sources, particularly to test the hypothesis that belowground plant C allocation could be an SOM priming response:

- Response: This is a great suggestion, and we agree that this information would be a helpful data point to help resolve potential changes in plant-derived C allocation belowground. Unfortunately, there are currently no available time series stable isotope data available at HBEF. New stable isotope measurements of archived samples may be instructive, or new experiments testing various drivers of belowground C fluxes. The revised Discussion now states:

  Lines 395-397: Experiments or analysis of soil archives to track plant-derived C into soil pools using stable isotope approaches will also be an important direction to further evaluate the potential for a shift in the fate of plant-derived C belowground.

(4) Clarification of patterns in heterotrophic C mineralization (from laboratory mineralization assays), particularly across organic and mineral soils:

- Response: We appreciate the reviewer highlighting the interesting differences across soil organic and mineral soil horizons with respect to microbial C cycling metrics. Many of the observed differences were different across organic and mineral soils, so additional clarification of differences is outlined below to address points raised, particularly with respect to differences between organic and mineral soils.
  - Lack of increase in heterotrophic C mineralization (Fig. 4c) or microbial biomass C (Fig. 4a):
    - Here, this statement applies to **mineral soil** heterotrophic C mineralization and microbial biomass**,** neither of which change significantly in the post-breakpoint period (neither a significant decrease or increase). However, microbial mineralization rate and microbial biomass C increase significantly in the Oi/Oe horizons (i.e., **organic soil horizons**) (**Fig. 4c**). As the Oe horizon has a very high root density, this observation is an important finding that contributes to our suggestion of root-related drivers of overall increasing soil respiration.

- Are these observations consistent with a potential priming response, i.e. plant allocation to access
  - In this system, root density is very high in organic horizon soils. For example, soil profile descriptions that include root ratings across HBEF (Bailey et al., 2024) rated Oe horizons as "many" roots, the highest category. In addition, rates of heterotrophic mineralization (shown in **Fig. 4c**) and other nutrient turnover microbial processes (e.g., nitrification) are high in Oi/Oe horizons. We therefore suggest that the increase in microbial mineralization and microbial biomass in Oi/Oe horizons is consistent, and is a main justification for, our suggestion of root-related responses.
- Can these changes be tied to changes in litter layer C stocks?
  - While the suggestion regarding overlap with soil C stock changes is recognized and appreciated, we determined that the temporal overlap in measurements (every 5 years for C stocks) and the somewhat preliminary nature of the most recent set of measurements did not allow for more in-depth interpretation of soil respiration and C stock changes over time. However, we included forest floor C stock estimates as a reference for the potential magnitude of change (using averages over the past few measurement periods). We note that this is an important area for future exploration, as noted in the revised text in lines 371-376.

(5) The significance of the proportion of root respiration (termed soil autotrophic respiration) within soil respiration is particularly noteworthy, especially during the period of rapid growth. It is imperative to acknowledge the contribution of soil autotrophic respiration to overall respiration, despite the author's attribution of root respiration to belowground carbon allocation in the discussion (refer to lines 357-359). It should be noted that an increase in root respiration is distinct from an increase in belowground carbon allocation; these are two separate processes influencing soil carbon dynamics. The evidence presented in the manuscript suggests that enhanced root respiration is likely another critical factor contributing to the observed increase in total respiration.

- Response: We agree with the reviewer that more discussion in the manuscript is needed on autotrophic respiration, including clarification of how autotrophic respiration changes might be consistent with soil respiration patterns, especially temporal concentration in the summer months. We significantly expanded the discussion related to autotrophic respiration in the revised manuscript. We added a new paragraph in the Discussion, emphasizing that changes in summer respiration rates are consistent with increasing autotrophic respiration (line 378). We noted that changing environmental variables such as elevated CO_2 can also stimulate autotrophic respiration (lines 379-381). We also pointed out that soil respiration disproportionate to very high microbial biomass values leaves room for autotrophic respiration changes in addition to the heterotrophic respiration changes observed (lines 384-387). As noted for below-ground C fluxes above, we also emphasized the lack of long-term root/root respiration data that overlap with soil respiration measurements, an important area for further research (lines 389-397).

(6) Lastly, why is the trend of specific respiration in Fig. 4d different from that in Extended Data Fig. 5a. I am also keen to understand the relevance and role of this particular dataset (specific respiration or ln specific respiration) within the context of this study.

- Response: This is a great point, and something we noticed and double-checked as well. In this situation, the ln-transformation reduced the influence of very high points that drove an apparent positive slope in specific respiration over time in the Oa/A horizon. After ln-transformation, the very high point (in 2020) was less influential and the trend was negative (and non-significant). We think that this unusual behavior in slope fits underscores that the relationship is not very strong in the Oi/Oe and Oa/A horizons, and that the significant change in specific respiration was in mineral soils. In the revised manuscript, we revised **Fig. 4** to include all data points rather than only the mean value across elevations, which more clearly illustrates the minimal change in specific respiration Oi/Oe and Oa/A horizons relative to the mineral soil horizon.

Reviewer #3 (Remarks to the Author):

A.R. Possinger and colleagues present a long-term record of soil respiration data from Hubbard Brook. The dataset is highly interesting as it covers multiple sites in the catchment area and soil CO2 efflux jumps to a higher level in the range 2013-18. Supportive data include a time series of microbial biomass and efficiency measurements from organic layer and mineral soil samples and C stock data of the organic layer for a sub-sample (the Ca-treated part of the watershed). The data suggest that the increase in soil CO2 efflux is paralleled by increasing microbial biomass and mineralization rates. The results are very solid as they apply for all landscape positions and both treatments (control, Ca applied). Annual cumulative CO2 efflux patterns are not related to mean annual temperatures or precipitation patterns – hence another mechanism then simple temperature sensitivity must be the cause of the observed increase in soil respiration.

Authors argue (discussion) that increased below ground transfer of C likely is the cause of the soil CO2 efflux increase. Overall, this interpretation is plausible, or likely, but it remains highly speculative. The reason is that a lot of important information towards an improved process understanding is missing. It was not assessed how autotrophic respiration, an equally important soil CO2 source as heterotrophic respiration, had developed over time, nor were fine root biomasses or above ground litter inputs measured – but they all are key elements in the discussion. Soil C-stocks were assessed only of a subsample and only of the forest floor. It cannot simply be assumed that all the microbially respired CO2 arises only from the forest floor. Furthermore, it does not become clear in the text if there is already a decrease in forest floor carbon, or not, and if such a decrease is related to the jump in soil respiration rates. From supplementary table S2 it seems that there is a general C stock decrease in the forest floor over time, but not necessarily related to the increase in soil respiration from 2015 onwards. How does this fit together? Only increased belowground C allocation is provided as explanation for the increased soil CO2 efflux, but there might be alternative explanations as well. What about wintertime snow-cover and soil temperatures – was there any change? Other limiting factors or other than N nutrient availability might have changed and increased microbial biomass and respiration. Accordingly, the breakpoint in CO2 efflux indeed may be seen a symptom of a changing C cycling, but the causes remain open. The results might be discussed in view of other long-term datasets e.g from Harvard forest etc. Is there a similar trend visible?

There also are some methodological issues regarding the CO2 efflux data interpretation – please see below.

Response: We appreciate the reviewer's thorough and thoughtful review! We have made extensive revisions to provide a more balanced discussion of C allocation and other possible drivers of our observed increase in soil respiration:

(1) In the significantly revised Discussion, we provide better support for increases in below-ground C allocation as a driver of the observed increase in soil respiration, but also provide support for alternative drivers including changes in autotrophic respiration (lines 378-397), winter snow cover (lines 412-414), and soil temperature (lines 249-264 in Results, lines 329-332 in Discussion).

(2) We appreciate the suggestion regarding overlap with soil C stock changes. However, the temporal overlap in measurements (every 5 years for C stocks) make them useful as a reference for the potential magnitude of change but limit their value for interpretation of soil respiration and C stock changes over time. We note that this is an area for future exploration in the revised text in lines 371-376.

(2) We have improved the context of our observations relative to other published long-term measurements of soil respiration, including recently published work from Mann et al. (2024). In the revised manuscript, preliminary observations of an increase in ambient soil respiration since 2011 from Mann et al. (2024) were highlighted in the Discussion (lines 298-321). We emphasize that further work is needed to synthesize regional datasets on soil respiration in lines 314-321.

Detailed comments:

When I look at Figures 2 and 3, I wonder how they fit together. Fig 2 shows cumulative growing season CO2 fluxes - and here the fluxes during the three most recent three years were more than double as high than the previous ones. Fig 3 shows all CO2 flux rates – but here this pattern does not emerge, or at least by far not as strong as in Fig 1.

Response: Thanks for raising this point! **Fig. 2** presents cumulative growing season (May - October) fluxes, extrapolated from monthly rate measurements, while **Fig. 3** presents individual data points (each point represents a soil respiration measurement at one time point for a single replicate collar at a particular landscape position in a particular watershed). The values in **Fig. 2** integrate over many points and therefore show the long-term pattern much more clearly than the presentation of the individual points, which highlight point to point variation.

I also do not understand why Fig 3 does not show all the measurements together in one panel (a) and the solely July-August data in a second one (b). The current procedure showing one fraction (May-June + Sept-Oct) and the other fraction (Jul-Aug) in separate panels is confusing – one may ask why there is a breakpoint in panel a, if there is obviously no difference in fluxes throughout the time series.

Response: We appreciate the feedback about the presentation of these data. We agree that it is easier to interpret one panel with all rates together and one panel with July-August alone. In the revised version, we have updated **Fig. 3** to show two panels: (a) All respiration rates (the data used for breakpoint detection), (b) Respiration rates in July-August only (with breakpoints assigned based on the data in panel a).

How can fluxes become negative in panel a?

Response: There are two possible reasons for negative respiration rates. First, genuine CO_2 consumption by autotrophic microorganisms in the chamber could occur. Second, and more likely, is a low and variable rate that has a negative slope. We leave these low (and often statistically insignificant) rates like this in our dataset to avoid biasing results and analysis by either removing or substituting zeros for these rates.

Why are there some years (a) and one year (b) data missing in this figure – but cumulative sums are shown in Fig 1? Figure 2:

Response: We appreciate the comment about the representation of points in the cumulative figures. In calculating cumulative fluxes across the May-October period, we followed the approach of Taylor et al. (2021) with a simple rate extrapolation. Calculations using this approach underestimate fluxes in some years, namely 2016, given the likely importance of summer respiration rates. However, to capture the potential magnitude of the effects, we think that a simple extrapolation approach is simple, internally consistent and appropriate. We have noted in the text (lines 484-494) that this approach likely underestimates cumulative fluxes in some years and further note that this is a more conservative approach to the detection of increasing soil respiration 2015-present.

How can it be that there appear two dots in single years if the graph is supposed to show only a single cumulative flux per year? (panels reference/high elevation 2013-2017 and panel Ca-treated/high elevation 2016).

Response: We appreciate this question! This is a result of a plotting approach to simplify differences across higher and lower elevation groups. Since the detection of a breakpoint date was based on soil respiration rates at each landscape position, grouping the points by pre- or post-breakpoint phase results in some overlap in these categories over time. To address this issue, in the revised manuscript we have modified **Fig. 2** to include all data points for each individual landscape position, watershed, and replicate collar combination. The overall mean value for each year (with standard error) was then plotted over these raw data points. We feel that this plotting method both addressed the issue with overlap in breakpoint phase, and improve the clarity of the observed trend and breakpoint pattern.

Data analysis could be improved at one particular point. Currently, variations in soil CO2 effluxes are tested against variations in mean air or mean soil temperatures. However, due to the rather low frequency of CO2 measurements (1 monthly measurement during the growing season), it is not granted that the actual flux measurements took place at mean (monthly) air or soil temperature. It easily can happen that fluxes are measured during a cold or heat spell, and then for sure they do not relate to a mean (monthly) temperature. I therefore suggest testing the measured daily soil respiration rates exactly against the soil temperatures, that occurred exactly during the CO2 efflux measurement (and not against a monthly temperature mean). Even within single days, measurement time can play a role. Measurements early in the morning may produce much lower CO2 efflux rates than in late afternoon when soils are warmer. Only after such a more detailed analysis against real soil temperatures during CO2 efflux measurements is done, it can be guaranteed that there was no influence of temperature on the observed soil respiration trends.

Response: We appreciate the reviewer raising this point. Relationships with soil temperature were determined based on the actual mean soil temperature for the day of soil respiration measurement in the original manuscript. In the revised manuscript, we have clarified this methodological step, and further emphasized that soil temperature relationships were determined for the actual day of sampling in the methods (lines 540-556). In the revised manuscript, we have further paired soil temperature and soil respiration measurements by filtering soil temperatures to a reasonable daily sampling window (10am-4pm). Regarding potential time-of-day effects, we do not have exact measurement times for respiration measurements, but recognizing the potential impact of diurnal temperature variation in these measurements have expanded our assessment of potential Q_{10} effects based on the trend in minimum, maximum, and mean soil temperature within the measurement time window (10am-4pm) in July and August. Based on our new analysis of ranges in soil temperature within a given sampling day, we found a slightly higher average increase in minimum soil temperature (compared to mean soil temperature) and revised our estimation of potential sampling effects (2-5% based on mean; 4-12% based on minimum). This information is included in the text (lines 249-264) and new minimum and maximum figures are shown in the revised **Supplementary Fig. 7**. Note that our soil temperature measurements are not co-located with the soil respiration collars, and thus represent a potential maximum direct influence of soil temperature given the possible ranges at the site, which has now been clarified more strongly in the text (lines 253-257).

Growing season (May-Oct) total soil CO2 efflux estimates (Fig.2) are generally low with 1-2 tons C per ha. This may be a result of underestimated flux rates (Fig.3) due to the long chamber closure during CO2 flux measurements. As a result, quantitatively, the heterotrophic component of the annual flux estimates would be lower than soil C inputs implying increasing soil C-stocks over time. This might be mentioned somewhere.

Response: Thanks for raising this point! We agree that the static chamber method underestimates fluxes, and this has been addressed in the revision in lines 283-286 and in the Methods lines 484-494. Given that we are underestimating annual respiration C flux, we do not think it is justified to discuss mentioning that our low numbers might imply increasing soil C-stocks over time.

The method to assess mineralization rates is rough. Soil samples were left for 10 days at room temperature to accumulate CO2. Room temperature will greatly influence respiration rates. Was room temperature always stable? Already a few degrees change can have large effects on fluxes. How were samples stored and how was air sampled and CO2 measured?

Response: It is important to note that our 10-day laboratory incubations provide (admittedly rough) indices of the size of the microbial biomass (fumigated incubations) and microbial respiration rate and/or the size of the pool of readily labile carbon (non-fumigated incubations). They are not meant to be an index of *in-situ* microbial activity. However, they are quite useful as indices of changes in the nature and extent of carbon cycling over the long-term. The incubations were carried out in laboratories with excellent temperature control and have been done in the same room since 2000. In the revised manuscript, further methodological detail regarding incubation sampling and analysis was added in the Methods section (lines 514-517). In the revision, we clarified that the headspace of the incubation vessels was sampled by syringe. Air samples were stored in evacuated vials and then analyzed by gas chromatography.

Was there a general correlation between microbial biomass and respiration during all the years?

Response: We appreciate the reviewer raising this point. In the revised manuscript, we added an assessment of relationships between microbial biomass C (MBC) in Oi/Oe, Oa/A and mineral soil horizons and field soil respiration, focusing on measurements from July. There was an overall strongly positive relationship between microbial biomass C and soil respiration (Spearman Rank $\rho > 0.6$, $p < 0.001$ for all horizons). However, the relationship was stronger and less variable in the pre-2015 period ($\rho = 0.73$-0.79) compared to 2015-present ($\rho = 0.18$-0.60) (new **Supplementary Fig. 6**). In addition, *in-situ* soil respiration did not increase proportionally with very high microbial biomass C values (e.g., >4, 15, and 60 g MBC kg $soil^{-1}$ for mineral, Oa/A, and Oi/Oe horizons, respectively) (new **Supplementary Fig. 6**). These observations were integrated into the Results in lines 176-183, and used to supplement our discussion of possible drivers in the Discussion, lines 384-387.

DIC: Extended Data Fig. 4 and text. Was the break-point calculated separately for DIC, or was just the breakpoint for CO2 efflux used here? It would be interesting if the DIC data suggests a similar break-point as the CO2 data, or not. If there is no DIC break-point, then it does not make much sense splitting the DIC dataset exactly in 2015.

Response: For other time series data (e.g., soil pH), we used a more straightforward split between pre- and post-2015 measurements to explore general patterns. We agree that more sophisticated breakpoint analysis for DIC and other time series data would be an interesting direction. However, in this study, DIC is mainly a supporting dataset that is likely to be directly related to increased CO_2 concentrations in porewater. We have therefore opted to keep a simpler analysis for now, but will certainly explore these changes in further work.

Response Literature Cited:

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COMMSENV-24-2218A: Revision 2 Response to Review

Thank you to the reviewers for reviewing the revised version, and for their thorough and thoughtful feedback.

The most substantive aspect of the revision was in the way that we accounted for underestimation in our calculation of cumulative growing season fluxes from monthly measurements. Rather than calculating a cumulative "growing season" (May-October) flux, we calculated a gap-filled summer (June, July, and August) respiration flux in the revised manuscript, which resulted in identical conclusions regarding patterns in the increase from ~2015 to present across the landscape. We greatly appreciate the reviewer's feedback on this point and hope that this change strengthens the manuscript.

In addition, we integrated the reviewers' suggestions to (1) update Figure 6 to include potential changes in autotrophic respiration, (2) change the use of the terms "microbial carbon use efficiency" and "specific respiration" to "microbial substrate use efficiency" and "biomass specific respiration", respectively, and (3) clarify the hypothesized responses in the text.

Throughout the response, line numbers refer to the manuscript file without tracked changes.

Responses to specific comments from reviewers:

Reviewer #1 (Remarks to the Author):

The authors have done an excellent job responding to and addressing my previous concerns. The additional analyses, more conservative language, and reorganized discussion all make for a substantially improved manuscript--nice job. I'm pleased to recommend this for publication.

Author's response: Thank you to the reviewer for the positive feedback! We greatly appreciate the reviewer’s comments and suggestions on the first manuscript version. We are grateful for the time and thought that went into the review!

Reviewer #2 (Remarks to the Author):

After the previous round of revisions, the explanation regarding the sudden increase in soil respiration, which was my primary concern, has been significantly strengthened. Additionally, the scientific rigor of the methodology has been sufficiently improved. Therefore, I have no other major revision suggestions.However, some details regarding scientific accuracy need further enhancement by the authors.

Author's response: We are grateful for the reviewer’s detailed suggestions and questions, which helped us think in greater depth about potential explanations for our observations. We also thank the reviewer for raising the questions below. Please see below for our responses to the specific line comments.

For example:

Line 38-39: “have increased by more than 100% between 2015 and 2020” – I think this description somewhat unscientific because the increase in this context is a continuous process, and using a simple average value is not entirely accurate.

Author's response: We thank the reviewer for noting this. We agree that the wording of this is slightly oversimplified and would benefit from better phrasing. This value is also slightly lower (90% vs. 100%) in our revised calculation of summer (instead of "growing season") flux. In the revised version, we have clarified that we are comparing the summer respiration flux in 2020 with the average summer flux over the 2002-2015 period. In the revised version:

Lines 37-40: Using long-term (2002-2020) measurements at the Hubbard Brook Experimental Forest (New Hampshire, U.S.), we show that soil respiration rates have notably increased since ~2015. In 2020, cumulative summer respiration flux was approximately 90% higher than the average summer flux over the 2002-2015 period.

Line 67: “at HBEF has increased by 0.25 °C decade-1” – This seems inconsistent with Fig1 (a).

Author's response: We appreciate the reviewer catching this. We agree with the reviewer: the 0.012 degrees C per year increase from the dataset we used equates to 0.12 degrees C per decade, rather than 0.25 degrees C per decade. This difference was a result of integration of previouslyr published data (Campbell et al., 2007) from Hubbard Brook, which indicated a steeper increase. In the revised version, we have updated the value in our text to "at least 0.1 degrees C per decade" to align with the dataset used in Fig 1 (a) and other published datasets, which are now cited in the text (Hamburg et al., 2013; Campbell et al., 2010).

Line 348-349: “Increased root-to-rhizosphere C flux” – I believe this is the authors' hypothesis, and such wording may lead readers to assume it is an established fact.

Author's response: Good point: we have revised the wording to state that an increase in root-to-rhizosphere C flux "would be" consistent. The revised text reads:

Lines 290-291: Increased root-to-rhizosphere C flux would be consistent with the observed increase in microbial biomass C...

Reviewer #3 (Remarks to the Author):

Authors performed a thorough revision. Concerns regarding measurement errors could be ruled out and the revised manuscript reads exceptionally well. Limitations of the study are now clearly acknowledged. I suggest publication of this important study. Few last comments for fine-tuning, see below.

Author's response: We appreciate the thorough review of our manuscript that the reviewer has provided and for the positive feedback on the revision. In the current revision, we carefully considered your comment regarding "annual" fluxes and decided to take a more conservative and synthetic approach to represent these values. Please see the response to lines

841-845 below for more detail. Thanks to the reviewer for the thoughtful feedback, which was instrumental in improving the interpretations and conclusions in the manuscript!

L289: microbial carbon use efficiency is related to microbial growth, not to microbial biomass. You may use the term “microbial substrate use efficiency” instead. “specific respiration” is also a bit unclear (although defined once). Maybe better use “biomass specific respiration”. (The actual term respiration per biomass would be “metabolic quotient”)

Author's response: We appreciate this suggestion! We have changed "microbial carbon use efficiency" to "microbial substrate use efficiency" in line 289. We also changed "specific respiration" to "biomass specific respiration" throughout the manuscript; we agree that this term is clearer and more interpretable.

Figure 6: I would add an additional arrow from box “Belowground C allocation” that goes directly through the soil surface to “total soil respiration”. This arrow would mean the autotrophic soil respiration component (which also results from C below ground allocation) and could also have increased.

Author's response: Great suggestion: we have added a box for autotrophic respiration (with a hypothesized increase, consistent with our discussion in the text), and an additional arrow to total soil respiration. These changes are now in the updated Figure 6.

L841-845: if the annual respiration values are potentially massively underestimated due to the measurement procedure, it does not make sense to directly relate them to correctly estimates of C inputs. The few lines with direct C input – output comparison might therefore better be removed.

Author's response: This is an important point, and the reviewer’s suggestion led us to reconsider if we could address the concerns about underestimation more directly. In the revised manuscript, we calculated a summer (June, July, August) flux, rather than a "growing season" flux, and gap-filled any missing measurements by using the summer mean (in the revised **Figure 2**). We believe this change addresses several important reasons for the underestimation of a true "annual" flux: namely, potential missing observations in shoulder seasons and the lack of winter measurements. The inherent limitations of the closed chamber technique do still result in an underestimate, which is noted in the text (see changes to the text below). However, this method is unbiased, it captures the summer increase in respiration flux, and our new calculations allow for better contextualization with other parts of ecosystem C budget. **Importantly, the change in cumulative flux calculation did not result in different conclusions regarding the ubiquity of the increase across the landscape or the importance of the change relative to other C pools/fluxes (even though the absolute value of the "summer flux" increase is lower than the estimate of a "growing season" flux).**

In addition to improvement in the calculation of summer flux, we carefully reworded the sections where the change in summer flux over the 2015-2020 period is compared to other ecosystem C pools/fluxes. These changes helped us to clarify the goal of these comparisons, which was to provide context for the relative magnitude of changes, rather than direct comparisons among other pools/fluxes. These changes were made in the text as follows:

Lines 127-132: Across both Ca-treated and reference watersheds and elevations, cumulative summer (June, July, and August) soil respiration flux increased markedly after 2013-2018 (median 2015), in sharp contrast to minimal or negative trends prior to the time series breakpoint (**Fig. 2**). In 2020, the overall mean summer respiration flux (~170 g CO_2-C m^{-2} $season^{-1}$) across all watersheds and elevations was approximately 90% greater than the mean summer respiration flux prior to 2015 (~90 g CO_2-C m^{-2} $season^{-1}$).

Lines 183-187: However, there were weak or non-significant correlations between cumulative summer respiration flux and mean annual soil pH (Spearman Rank $\rho = 0.31$, $p = 0.23$) and soil solution Al^{3+} (Spearman Rank $\rho = -0.41$, $p = 0.09$) in the reference watershed across the available record (2002-2020, excluding 2016 due to lack of pH data) (**Supplementary Fig. 2**).

Lines 224-232: We estimated that cumulative summer (June, July, and August) respiration increased by about 11-15 g CO_2-C m^{-2} $season^{-1}$ since ~2015 (**Fig. 2**). We used this estimate to consider the magnitude of increasing soil respiration in the context of other C pools at HBEF. We estimated the forest floor C pool at HBEF to be ~2.6 kg m^{-2} using soil profile C inventories from 2014 and 2018 (conducted following methods described in Johnson et al.[30]) (see **Supplementary Methods**; **Supplementary Table 4**). Even as a conservative underestimate of actual flux (due to known limitations of the static chamber method)[48], the size of the summer soil respiration flux relative to the forest floor pool would increase from approximately 3.5% (pre-2015) to 20-30% by 2060.

Lines 306-311: In this study, the increase in soil respiration was mostly driven by summer months (**Fig. 3**), and we estimated cumulative summer respiration flux to be ~11-15 g CO_2-C m^{-2} $season^{-1}$ (**Fig. 2**). For a change in exudate C to match this increasing respiration C flux, annual root-rhizosphere flux would need to increase approximately 10-20% in summer, corresponding to a ~2-3% yearly increase in total belowground C allocation[25].

Lines 427-439: To provide an estimate of cumulative summer respiration flux, the monthly rate measurements (g CO_2 m^{-2} hr^{-1}) were extrapolated to 30 d increments (g CO_2 m^{-2} 30 d^{-1})[28] for June, July, and August. We used these months because they accounted for the most continuous record over the measurement period, and the strongest increase in respiration rates was found in summer (see **Fig. 3**). To account for missing combinations of elevations and sites in certain years, missing monthly measurements were gap-filled using the mean summer monthly flux. These values were summed to produce cumulative summer soil respiration fluxes. The static chamber method results in known underestimation of true soil respiration fluxes due to inherent feedbacks within the closed chamber[48]. Further, the 30-d extrapolation with gap-filling is a coarse estimate of cumulative fluxes, though represents internally-consistent changes over time[28]. However, we emphasize that these methodological considerations are more conservative with respect to the detection of trends in soil respiration and implications of changing fluxes for overall forest C balance.

Literature Cited in Response

Campbell, J. L., Ollinger, S. V., Flerchinger, G. N., Wicklein, H., Hayhoe, K., & Bailey, A. S. Past and projected future changes in snowpack and soil frost at the Hubbard Brook Experimental Forest, New Hampshire, USA. *Hydrol. Process.* **24**(17), 2465–2480 (2010).

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