

Dear reviewers, please note that all line and page numbers referenced below pertain to the updated clean version of the edited manuscript.

RC1: 'Comment on egosphere-2025-1204', Anonymous Referee #1, 02 Jun 2025

The topic and data are interesting. However, data analysis and presentation are poor. Therefore, substantial revisions throughout the manuscript are required. For example:

Thank you for your thorough review and valuable feedback. We appreciate the time and effort you took to provide your insights. We made improvements in our presentation of data and analysis.

1) Why did the authors consider only microbial respiration when they measured total respiration, including plant root respiration?

We documented and evaluated total respiration (L31-33, Page 1-2; L168-169, page 6), not just microbial respiration. In the introduction, we defined soil respiration and noted that plant root respiration influences soil respiration.

“Soil respiration, the process by which carbon dioxide (CO₂) is released from the soil surface to the atmosphere, is a critical component of the global carbon cycle. This process encompasses the microbial breakdown of organic material as well as the respiration of plant roots”.

To accurately measure in situ microbial respiration, it is necessary to isolate it from root and surface vegetation by removal. However, a prior study conducted by Vas et al. in 2023 (citation below) revealed that the removal of ground vegetation had a significant impact on the soil temperature and moisture conditions at the chamber site. Boreal forest ecosystems are very susceptible to disturbance, therefore, to reduce site disturbance and obtain a more accurate measurement of CO₂ site efflux, we chose to assess total soil respiration.

Vas D.A., Corriveau E.J., Gaimaro L.W., Barbato R.A. (2023) Challenges and Limitations of Using Autonomous Instrumentation for Measuring In Situ Soil Respiration in a Subarctic Boreal Forest in Alaska, USA. *ERDC/CRREL TR-23-18*.

2) Why did the authors evaluate only the linear relationships between soil CO₂ efflux and environmental variables, when they can be related non-linearly to each other? I think the authors should present and describe relations between soil CO₂ efflux and potentially important variables (i.e., temperature and VWC) in more detail before applying the simple analyses with linear correlations and random forest models.

Initially, we assessed linear relationships to determine the existence of distinct, linear trends between variables and CO₂ efflux across various sites, disturbances, and seasons L277-290,

Page 10). Such trends might remain unnoticed or become less apparent when utilizing the non-linear Random Forest analysis.

Subsequently, we presented the relative significance of the input variables derived from the *non-linear Random Forest model* (L291-306, Page 10-11) for predicting CO₂ efflux, as illustrated in Figure 10 (L476 page 19), along with the most significant variables listed in Table 1 (L482-484, page 19). This analysis provided a more thorough understanding of which variables were most influential in modeling CO₂ efflux across the different sites, disturbances, and seasons.

3) How did the authors determine the microbial activity from microbial abundances based on DNA amplicon analysis? The amplicon analysis just provided microbial abundances but never microbial activity. Presenting data of microbial species composition in the main text but not in the supplemental is required.

We did not determine microbial activity from abundance because they are measuring two different aspects of the soil microbial community. DNA sequencing offers insight into the major taxa that are present in the soil. Soil activity, as measured through soil respiration, measures active microorganisms and plant roots. Therefore, we separated these two approaches. We investigated the effect of disturbance on microbial community composition and diversity; we clarified this in the text (L366-L370, page 14). We included the microbial species composition in the main text as requested (Figures 6 and 7, L380-389, Page 14-15).

4) Why did the authors refer only to carbon dynamics studies on non-permafrost regions in their introduction (L49-L60)? Referring to those studies on permafrost regions is essential to clarify the position of the present study within the scientific context of this research field.

In the introduction, we also referred to carbon dynamics studies in permafrost regions. For example, Koster et al. (2017 and 2018), studied C dynamics in permafrost environments in Canada and Siberia, while the other studies were conducted in similar sites (boreal forest) in Canada (e.g. Halim et al., 2024; Amiro, 2001). We have made substantial modifications to the introduction to enhance its alignment with our research and have incorporated more studies conducted in permafrost regions (L45-101, page 2-4). Regrettably, there is a scarcity of literature addressing the impact of disturbances on soil respiration in high latitude areas. We are continuously seeking pertinent papers; therefore, we welcome any suggestions for relevant publications that you believe we should evaluate.

It is super challenging to summarize concisely all the issues of the manuscript. Therefore, substantial self-efforts by the authors are essential in thoroughly revising the manuscript.

Once again, thank you for your review and thoughtful comments. Your feedback is greatly appreciated and plays a crucial role in our continuous improvement efforts. We are committed

to making substantial revisions based on the changes suggested by the reviewers to enhance the quality of our work. We believe our substantial changes to the manuscript align with your summary of issues and of course welcome any additional comments at a later stage should they arise.

RC2: 'Comment on egosphere-2025-1204', Anonymous Referee #2, 12 Nov 2025

This study reports a comparison of soil respiration and associated edaphic and microbial community data between two boreal forest sites. It is well written in terms of readability and the data are well presented. The primary novelty is that such comparisons are rarely as detailed and comprehensive as this one which includes a plethora of soil physical, chemical and microbiological data.

Thank you for your detailed and constructive review of our manuscript. Your comments and suggestions are greatly appreciated, and we have made efforts to address them thoroughly.

My main concern with this study is that it is portrayed in a way that does not make sense to me. The sites are different – one has not been disturbed for multiple centuries and seems to be classic interior Alaskan Black Spruce forest with a moss-dominated understory. The other is described as ‘disturbed’ due to trail-building and firewood harvesting a century ago, and now has scattered birch and white spruce with a ground surface of grasses and sedges. The two sites are 10 m apart (line 89 p.3). The hypothesis is that soil respiratory carbon emissions are larger in the disturbed site compared to the undisturbed site in permafrost areas (lines 78-80, p.3). But the title is ‘Effects of Permafrost Thaw on Seasonal CO₂ efflux dynamics in a boreal forest site’. But both sites are on permafrost, the comparison is about the effect of disturbance, and there are two sites not one. This title seems like an extraordinary mismatch with the actual data. With respect to the permafrost thaw issue, yes the max active layer thaw is much deeper (~ 1m) in the disturbed site, but the explanatory variables that best explain the patterns of soil respiration are from relatively shallow depths – all within the ‘normal’ active layer depth range (as indicated by the undisturbed site). In other words, there’s no evidence anywhere in the manuscript that the deeper thaw in the disturbed site is the cause of the differences in seasonal CO₂ efflux dynamics. The title is way ‘off the mark’, and this mismatch creates a very bad initial impression that leads to niggling concerns about the ensuing manuscript.

Site Terminology: We acknowledge your point regarding the terminology used to describe our plots. Given the short distance between the undisturbed and disturbed plots, we initially considered them as one site. However, we agree that referring to them as two distinct sites will enhance clarity, and we made this change throughout the manuscript.

Title Revision: In response to your concern about the title, we revised it to better reflect the focus of our research. The new title is: “Effects of Disturbance on Seasonal CO₂ Dynamics in Two Boreal Forest Sites Underlain by Permafrost” (L1-2, Page 1).

Thaw Depth and Soil Respiration: We appreciate your insight regarding the relationship between thaw depth and soil respiration. While it is true that we did not collect soil temperature and moisture data at deeper depths in the disturbed site, our analyses, including

the linear correlation (L438-439, page 17) and the random forest model (L466-467, page 18), indicate a moderate correlation between thaw depth and soil respiration.

Hence, I have great concerns about the permafrost context in which the Conclusion opens. I do however understand and agree with the final conclusion that the study demonstrates that disturbed areas of the boreal forest such as this one, are fundamentally different in terms of CO₂ efflux and therefore warrant specific inclusion in forest C models. I recommend that the authors really step back and review the validity of the many, many references to permafrost. Yes it does provide some overall significance to the study, but only that (as far as I could tell). Hence, I recommend that they review and greatly restrain the linkages because the data simply don't warrant it.

We understand your concerns regarding the permafrost context in the conclusion. Our argument is that with increasing temperatures, the active layer will deepen due to thawing permafrost, leading to increased soil respiration. We adjusted the opening of the conclusion to emphasize disturbance and its effects on soil respiration.

By contrast, the authors do have a basis for comparing soil respiratory CO₂ release between the two sites. The problem for them is that there are significant differences between the two sites in daily rates only in summer (p. 13, line 345) (Fig 6). So in essence, they are left with a whole lot of potentially explanatory variables that they have used to predict the CO₂ release rates at the two sites, and to compare which variables are most effective between the sites and soil depths. The second problem is that the relationships between efflux and the explanatory variables do not differ significantly between the two sites – there's no site effect - line 365-367 page 14).

Seasonal Differences in Soil Respiration: We would like to clarify that significant differences in soil respiration between the two sites were observed not only during the summer (L411-413, page 16) but also in the winter (L417-420, page 16). We revised the paragraph to clarify this (L409-422, page 16).

Site Effects and Explanatory Variables: Regarding your observation about the relationships between efflux and explanatory variables, we respectfully disagree with your assessment. Our data demonstrate statistically significant differences in several variables between the two sites, including soil temperature during summer and autumn (L316-318, page 11), average maximum seasonal thaw depth (L332-334, page 12), and contrasting thermal regimes (L311-314, page 11). While we anticipated that the relationship between efflux and explanatory variables would be similar across sites, the magnitude of soil respiration (L410-413, page 16) and the magnitude of the differences in variables (L316-318, page 11; L332-334, page 12) highlight the distinct responses of each site.

Overall this data set may well contain useful information – I do not know for sure, but would assume there are many similar studies that have also done such comparisons... Therefore the particular novelty of this study that would warrant its publication is not at all clear. Perhaps the novelty is the unusually comprehensive data set of physical, chemical and biological variables.. . - if so the Introduction needs to highlight that. One other novelty aspect that strikes me is the inclusion of winter respiration data.. always difficult – especially in tundra. And therefore comparison of the seasons and in particular the proportion of total annual soil respiration that occurs in the non-growing season may be of interest.

Novelty of the Research: We believe the novelty of this research lies in demonstrating the significant effect of disturbance on increased soil respiration, as well as providing a comprehensive dataset of physical, chemical, and biological variables collected over an entire year in an extreme cold environment, despite the logistical challenges involved. We have significantly changed the manuscript to better highlight our findings. We have added text to emphasize the novelty of the comprehensive data set of physical, chemical and biological variables to the introduction.

Please find some specific comments and suggestions to the text below that I hope will assist the authors in improving the manuscript

Title: NEEDS complete revision. See comments above.

We changed the title to “Effects of Disturbance on Seasonal CO₂ Dynamics in Two Boreal Forest Sites Underlain by Permafrost” (Line 1-2, Page 1).

Abstract:

12-13... this study does not necessarily directly report carbon fluxes from thawed permafrost... see above.

We changed the focus to “the impact of surface disturbance on seasonal soil biological properties” and “the key environmental and geochemical factors influencing soil biology in the undisturbed and disturbed soils”. (L14-16, Page 1)

14 The type of disturbance needs to be described.

We expanded the disturbance description: “The disturbance was due to historical activities related to mining such as trail development and firewood harvest. These disturbances took place in the early 1920s, coinciding with the construction of a drainage ditch and an access trail to support mining operations in the area. During the trail and draining ditch development the groundcover vegetations and surface soils were disturbed, and the trees were harvested.

Currently, there is no active drainage at the research site, and the trail is seldom used” (L81-86, Page 3).

..suggesting a potential mechanism....But the variation (which needs to be fully explained in the Abstract) in CO2 efflux between sites seems to be minimal (as described above).

We rework the abstract (L10-29, Page 1) to better reflect the CO2 efflux variation. However, we believe that a 20% summer season, and a 14% mean annual increase in CO2 efflux (L17-L19, page 1) at the disturbed site as compared to the undisturbed one, is not minimal.

23.. in thawing permafrost.. see comment above.

We switched to “anthropogenically disturbed soils underlain by permafrost”. (L26, Page 1).

Page 2.

44 why would a decrease in net radiation lead to increased CO2 efflux... would more radiation reach the soil surface in disturbed sites, and therefore raise soil temperature as you found (and consequently stimulate microbial respiration)?

This is a very good observation; Amiro 2001 reported a 13% lower net radiation at the harvested site compared to the mature site which is the opposite of what would be expected due to reduce leaf area. The author noted in the Results and Discussion section that “Net radiation was slightly lower at the 1-y-old burn site.... but during some periods this was because the radiometer was shaded by some nearby trees” and that “Amiro et al. (1999b) found no difference in net radiation observed during aircraft flights over 1-y-old burns compared to older surfaces along a 500-km transect”. We extensively revised the introduction and removed this reference.

46substrates for soil microbial decomposition...

....flooding (Reference needed).

We comprehensively revised this paragraph and the introduction to better align with previous research in permafrost underlain boreal forests ecosystems (L45-101, Page 2-4).

Page 3

72 perhaps: ... mechanisms driving variation in soil respiration within and between these two ecosystems....

We changed to “To reveal the fundamental controls driving variation in soil respiration within and between the disturbed and undisturbed boreal forest sites, we examined seasonal soil CO2

efflux alongside various edaphic factors, including soil temperature, moisture content, soil organic matter (SOM), pH, and the composition of microbial communities.” (L90-93, Page 3).

... delete ‘permafrost areas’

We are of the opinion that the term 'permafrost underlain areas' most accurately characterizes the location where this research was conducted, and that the disturbance did have an impact on the depth of the active layer, which is typical for permafrost environments.

Are’nt they disturbed sites not just disturbed soils.. E.g. the vegetation is very different in composition and density.

We changed the relevant text to communicate that they are disturbed sites, thank you.

Do we know that the ‘disturbed site’ was similar/identical to the undisturbed prior to the trail building and wood harvesting.....Any evidence.. historical photos maybe. Presumably the sites are identical in terms of topography, parent material (i.e. the state factors) – say so in the manuscript.

To the best of our knowledge, there are no existing before images of this area. Nevertheless, the disturbed site is flanked on both sides by undisturbed boreal forest; we clarified this in the manuscript (L10-111, page 4). We also provided a site map (Figure 1, L115-119, Page 4-5) and photos (SI Figure 1) for clarification.

Is 10 m apart really sufficient? Are you confident that the two sites are independent of each other in terms of functioning. Any evidence. Otherwise there must be concerns about shading, leaf litter, and even root penetration across the ecotone/boundary. 10 m seems pretty close.

We are confident that the 10 m separation is adequate and that our two sites function independent of each other. The stark differences in active layer thickness, vegetation structure, and soil temperatures from that of the disturbed to undisturbed site clearly indicate the disturbed area has not impacted the adjacent, undisturbed forest, which maintains natural vegetation structure, thinner active layer, and cooler summer soil temperatures. Additionally, our sites are not located directly in the ecotone/transition zone, but are rather placed well within the representative soils of each site. There is no litter or root influence from the forested zone evident in the soils of the disturbed zone. Similarly, shading from the spruce is not an impact on the disturbed zone, as evidenced by the drastically thicker active layer and snow depths that occur there. The close proximity of these sites is advantageous for keeping soil state factors constant between the two sites as these can vary over distance, and in doing so we limit the soil ‘treatment’ to disturbance alone.

Page 4.

96-100. Reader really deserves to see photos of the two sites,..... to gauge the vegetation difference, the birch density. I suggest you add multiple photos to the supplementary files.. including soil profiles to see the overlying organic layer and the deeper mineral layer.. and where exactly is the permafrost – photos? And why is there so much more soils info given for the disturbed site.. seems unbalanced description.

We included an aerial image of the site (Figure 1, L115-119, Page 4-5) as well as photos of the sites (SI Figure 1) and vegetation to organic to mineral transitions (SI Figure S2). We have provided the equivalent soil information for both sites, please see SI Table S1 in the supplemental information document.

Section 2.2 and 2.3 Woefully inadequate description of this critical methodology

We fully revised sections 2.2 and 2.3 to enhance clarity and provide a more accurate description of the methodology (L138-202, Page 5-7).

How far apart were the collars – a map in the supplemental file would help... one that combines the disturbed and undisturbed sites.. are they really separate sites, or just zones??

As previously stated in the earlier comment, we incorporated an aerial image (Figure 1, L115-119, Page 4-5) of the study area along with a scale, which is intended to assist the reader in comprehending the layout more effectively. Also, we added more plot layout details in section 2.3; L172-173, Page 6).

Were the CO₂ flux plot measurements continuous, 3 per hour 3 per week... there's no information at all. For how long was each measurement period. Were there checks for leaks, and for adequate seal. The data presented in Fig 6 are grand cumulative totals per season... but the reader needs to know the details of how these totals were reached. Were there similar numbers of measurements per day week etc in winter as in summer.

The CO₂ flux was measured every 30 minutes (L140-143, page 5). We fully revised this section to better describe the measurement methodology. We check for proper chamber operation every time we went in the field to download the data, approximately once a week. We added the n number for the values used for our statistical analysis (L280-281, Page 10).

Page 5.

147-149. The temperature probes for topsoil and subsoil seem to be at different depths in the disturbed versus undisturbed... although the vegetation cover depth differences may account for this. Otherwise, this difference alone could explain the temperature differences observed in Fig.2

The variation in the topsoil sensor placement depth is due to the variation in the vegetation cover depth, we clarified this in the text (L152-156, Page 6). The subsoil sensors were placed at approximately the same depth at both sites (L156-160, page 6)

Page 6.

159-163 – What depths exactly were the soil samples taken... and did those sampling depths differ between winter and summer/spring? You need to at least give the range of depths at each sampling time. And depth from what exactly.. the top of the organic soil surface, or the vegetation.....

The soil samples were collected from the same depth throughout the seasons (L206-209, page 7-8), which is the depth of the soil moisture and temperature probes (L207-209, page 8). The depth of the probes as referenced from the top of surface vegetation were reported on page 6, L152-156.

All soil samples for microbiological analysis were placed in a freezer on collection. This is routine practice, but is there not a concern that the summer soil community will be impacted by freezing resulting in an altered microbial community composition? At least cite references to support this practice.

The deep freeze suspends microbial growth and enabled us to capture the microbial community structure at the time of sampling. We clarified this in the manuscript (L222-224, Page 8) and added the following supporting citations:

Baker, C.C., Barker, A.J., Douglas, T.A., Doherty, S.J. and Barbato, R.A., 2023. Seasonal variation in near-surface seasonally thawed active layer and permafrost soil microbial communities. *Environmental Research Letters*, 18(5), p.055001.

Doherty, S.J., Barbato, R.A., Grandy, A.S., Thomas, W.K., Monteux, S., Dorrepaal, E., Johansson, M. and Ernakovich, J.G., 2020. The transition from stochastic to deterministic bacterial community assembly during permafrost thaw succession. *Frontiers in Microbiology*, 11, p.596589.

Page 7.

184.. ...soil pH were each statistically...

Noted, thank you!

Page 9.

Presumably the winter soil temperatures were warmer in the undisturbed site because there was deeper snow accumulation there. Give info on snow depth comparison between sites... and here is a variable that might well be influenced by the very close proximity between the sites.

Both sites were snow denied (L143-146, Page 5; Figure 2b, L196-202, page 7). We acknowledge that this is a potential bias of the study, and we noted as such in the discussion (L557-559, Page 22). This was necessary to allow for proper chamber operation and the acquisition of CO₂ data during the winter.

267 and other graphs. How exactly were outliers determined?

The plots were made using Excel's built-in Box & Whisker chart. The plots outliers as individual points beyond the whiskers, following the standard statistical method where outliers are data points more than 1.5 times the Interquartile Range (IQR) away from the box.

Page 10

This is a comparison between sites. There was no 'treatment' enacted by the authors..and so I think this term should be removed entirely from all locations in the manuscript. Likewise, the statistical comparison is really between 'sites', and so the 5 uses of the word disturbance in Fig 4 should all be replaced with Site. And likewise for the 4 uses in Fig 5.

Noted, we corrected throughout the document.

283 and 287 and 290. The variable being reported in soil concentration of C or N, not content which would require multiplying the concentration by the bulk density. Please correct throughout.

Noted, we corrected throughout the document.

283-285 is duplicated at 290-292.

Noted, we removed the duplicate.

286 and elsewhere (not significantly different)

We changed it to (not significantly different)

Page 11

309 Perhaps: We compared microbial community composition and diversity between the disturbed and undisturbed sites.

We changed to the suggested phrasing (L66-367, Page 14).

Page 12.

327... there's no disturbance regime.. this is a site comparison

Noted, we revised the paragraph (L390-391, page 15).

Page 13.

Section 3.4. This text is written in a way that makes it very hard to determine whether respiration rates differed between the two sites... and exactly what the respiration variable is. But it seems only the summer daily rates were different (line 345). And are these comparisons only of the peak daily rates in each season.. it is all quite unclear. The final sentence (line 352) clearly indicates that there are overall whole season differences for the winter and for summer seasons.

We reworked the paragraph for enhanced clarity (L409-422, Page 16). The fluxes during summer (L410-413, page 16) and winter (L417-420, page 16) were found to be statistically different. We used mean daily respiration rates from each season for our comparison; we only mentioned that the peak seasonal soil respiration occurred in the summer and did not use it for comparison between seasons/sites. We hope that the reworded paragraph (L409-422, Page 16) will provide clarity.

One fundamental methodological issue concerning winter is that tables were used to cover over and keep the snow from building up on top of the CO₂ measurement enclosures. I understand the methodological necessity of doing this to allow the flaps to operate, but did it not result in confounding effects on soil temperature because there was no insulating snow cover there. Could this mean that the winter measures are likely underestimates of actual daily and seasonal rates?

As accurately pointed out by RC2, the denial of snow was essential for the proper functioning of the chamber. We concur that this may indicate that winter measurements were probably underestimated; we addressed this in the discussion (L557-559, Page 22).

Page 14.

Are these daily (ie. daytime only) or diel (full 24 hour) means??

They are full 24 hours means.

365-367. Perhaps: The relationships between soil efflux and the aforementioned variables did not differ significantly between the two sites (p...).

Noted, we revised the paragraph and removed the confusing sentence (L430-439, Page 17).

Page 17.

418 ...soil were markedly...

Noted, we revised to were.

That there were site differences in soil organic layer thickness was not apparent to me. In fact I don't think those depths were reported, and should be. And what is the topsoil layer.. does that include the OeOa organic layer right at the surface – directly beneath the mosses (at least in the undisturbed site). And what about in the disturbed site.. presumably it is thinner?

The top soil layer doesn't include the organic layer, for more clarity we changed the text to "The sampled soil material at the two sites were classified as mineral soil material (<20% organic matter [OM]; Soil Survey Staff, 2022) with a subdivision of more organic-rich fractions (10-18% OM) comprising a "topsoil" layer (akin to an A horizon) that starts below the organic layer (O horizon), and more mineral-rich material (<5% OM) comprising a "subsoil" layer. The topsoil textures ranged from loam to silt loam, reflecting a higher proportion of sand particles in the topsoil relative to the silt loam-textured subsoil (SI Table 1)." (L132-137, Page 5).

426-429. Okay if there's a thicker layer of organic soil right at the surface that might help. But surely the snow depth and density are both distinctive to the undisturbed site in terms of providing thermal insulation against severe air temperatures. Add the relevant data and maybe photos of the site through each of the seasons if available.

As mentioned in the comments above, both sites were denied the snow cover to ensure proper chamber operation. We added site photos in the Supplemental Information document (SI Figures S1 and S2).

again relating to snow cover depth and density may possibly explain why air temp was the best predictor variable... in the sense that only marked changes in air temp would influence the soil temp because of the snow insulation layer. One alternative explanation for the soil temperature being a better explanatory variable of flux from the disturbed site is that soil respiration there is primarily from deeper depths within the soil because the surface organic soil C is relatively low/depleted.

Please see above comment.

Page 18.

461-463. Is this novel and therefore the study has contributed new insights? If so, then the manuscript should be clearly structured around that conclusion.

We significantly restructured the manuscript, especially the abstract, introduction, and discussion sections, to better reflect the conclusion.

I sincerely hope these comments and suggestions are useful to the authors.

Thank you once again for your valuable feedback. We believe that these revisions will enhance the clarity and impact of our manuscript.

Citation: <https://doi.org/10.5194/egusphere-2025-1204-RC2>

Major comments for Authors:

There is often mention of microbial activity in the manuscript, but the authors are measuring both autotrophic and heterotrophic respiration. How does this link to microbial activity? How does it link to the measures of microbial community structure? There seem to be a lot of measurements that don't necessarily fit together.

Dear Reviewer,

Thank you for your thoughtful comments regarding our manuscript. We appreciate your concerns regarding the relationship between microbial activity, respiration measurements, and microbial community structure.

To clarify, our study focused on total soil respiration, which encompasses both autotrophic (plant root) and heterotrophic (microbial) respiration (L31-33, Page 1-2; L168-169, page 6). Soil respiration is a crucial component of the global carbon cycle and is influenced by various factors, including the microbial breakdown of organic material and the respiration of plant roots. While we did not isolate microbial respiration in our measurements, it is essential to recognize that microbial activity plays a significant role in the overall process.

In our methodology, we sought to reduce site disturbance, particularly within sensitive boreal forest ecosystems. A previous study by Vas et al. in 2023 indicated that the removal of ground vegetation significantly affected the soil temperature and moisture conditions at the chamber site. Boreal forest ecosystems are highly vulnerable to disturbance; thus, to minimize site disturbance and achieve a more precise measurement of CO₂ site efflux, we opted to evaluate total soil respiration.

Regarding the connection between microbial activity and community structure, while our study did not directly measure microbial respiration, the underlying microbial processes are inherently linked to the overall respiration rates observed. Changes in microbial community structure can influence the rates of organic matter decomposition and nutrient cycling, thereby affecting both autotrophic and heterotrophic respiration.

We acknowledge the complexity of integrating various measurements in our study. However, our approach was designed to provide a holistic view of soil respiration dynamics, considering the interplay between microbial processes, root respiration, and environmental factors. We hope this clarification addresses your concerns and enhances the manuscript's overall coherence.

Disturbance – needs to be a much more thorough discussion of the type of disturbance. How does it influence e.g. the location/distribution of the organic layer? How might this influence your measurements of carbon and organic matter distribution? Need to know more than it was

“thawed permafrost.” Is the permafrost continuous or discontinuous? Please rationalize the different sampling depths in disturbed vs. undisturbed.

To provide additional context, “The disturbance was due to historical activities related to mining such as trail development and firewood harvest. These disturbances took place in the early 1920s, coinciding with the construction of a drainage ditch and an access trail to support mining operations in the area. During the trail and draining ditch development the groundcover vegetations and surface soils were disturbed, and the trees were harvested.

Our study site was located in a discontinuous permafrost region, with underlying permafrost present. This discontinuity can affect the hydrology and thermal dynamics of the soil, leading to variations in the organic layer's development and distribution. In disturbed areas, we expect that the disruption of soil layers may have resulted in altered moisture retention and temperature profiles, which can influence microbial activity and organic matter decomposition rates.

The variation in the topsoil sensor placement depth is due to the variation in the vegetation cover depth, we clarified this in the text (L152-156, Page 6). The subsoil sensors were placed at approximately the same depth at both sites (L156-160, page 6). The soil samples were collected from the same depth throughout the seasons (L206-209, page 7-8), which is the depth of the soil moisture and temperature probes (L207-209, page 8). The depth of the probes as referenced from the top of surface vegetation were reported on page 6, L152-156.

How relevant is all the peatland literature that is cited? Are all these forests dominated by peat? Peatlands have issues with e.g. water table depth/movement that influence gas fluxes. Are those processes important here? .

We removed the references to peatland studies from the manuscript. While these studies provide important insights into carbon dynamics, we agree that they may not be directly relevant to the specific context of our research on soil respiration in permafrost areas. Additionally, we would like to highlight that there is a notable lack of research addressing the effects of disturbance on soil respiration specifically in permafrost ecosystems. This gap underscores the need for our study, as it aims to contribute to understanding how historical disturbances influence carbon dynamics in these unique environments.

The authors make a statement that their work “reveals the fundamental mechanisms of soil respiration.” I don’t see this. Maybe “fundamental controls”??

We changed to fundamental controls (L90, Page 3) thank you.

For discussion of carbon and SOM distribution, be careful about use of words like concentration and content. Content suggests amounts per unit area. To do this, you need bulk density data. I don't think you have those data based on what I see in the manuscript.

Noted, we changed to concentration.

How did you calculate microbial diversity? I saw data for richness. I see – Bray/Curtiss dissimilarities. Is that your estimate of diversity? Can you describe the diversity results directly (ie this was more diverse than that)?

We calculated alpha diversity using species richness, which provides a count of the number of different species present in our samples. This metric gives us an initial understanding of diversity within individual samples.

For beta diversity, we employed the Bray-Curtis dissimilarity metric, which quantifies the compositional differences between samples. This method allows us to compare the overall community structure and assess how similar or different the microbial communities are among the samples.

Random Forest shows an impact of disturbance on soil CO₂, but the regression analysis does not. So which do we believe?? Also, the use of respiration data to model disturbance (section 3.6) was not very clear and a bit hard to follow. You also state early in the Discussion that disturbance is key, yet your analyses do not seem to show much support for this.

Regarding the discrepancy between the Random Forest analysis and the regression analysis, we initially conducted linear regression to identify distinct trends between variables and CO₂ efflux across various sites, disturbances, and seasons. We reworked the paragraph for enhanced clarity (L430-439, Page 17). However, it is important to note that some relationships may not be linear and could be obscured in this analysis. The non-linear Random Forest model allows for a more flexible approach, capturing complex interactions that might be missed in a linear framework. Skewness and kurtosis values from the field data ranged from 0.04 - 1.24 and 1.61 - 4.56 at the disturbed site, and from 0.07 - 1.45 and 1.40 - 6.22 at the undisturbed site for all seasons, respectively. The ranges indicated that some instances contained a more normal distribution while other instances, especially winter, were notably skewed and tail-light and heavy. This is why we emphasized the results from the Random Forest analysis, as it provides a more nuanced understanding of the factors influencing CO₂ efflux.

In section 3.6, we do not use respiration data to model disturbance, rather, we are using the random forest model to describe the effects of disturbance on the variation in soil respiration (L450-452, page 18). To enhance clarity and readability, we rephrased the introductory paragraph to “The non-linear RF model effectively captured the impact of disturbance on the

variation in soil respiration at both locations, showing strong confidence levels with high R² and moderate to low mean absolute error (MAE) values.”

Our intention is to convey how disturbance impacts CO₂ efflux and to provide a comprehensive view of the variables that play significant roles in this process. For example, our findings indicate that the disturbed site exhibited significantly warmer soil temperatures during the summer (4.04 ± 0.19 °C; $p < 0.001$, ANOVA) and autumn (1.88 ± 0.23 °C; $p < 0.001$, ANOVA) (L317-320, page 11). Additionally, we observed a more than twice deeper seasonal thaw depth (L333-334, Page 12) and a significantly greater mean total carbon content in the subsoil (L344-348, page 12).

There is no connection of your results to previous literature. There needs to be a bit more discussion of how your results fit into the pre-existing literature and our understanding of the controls on these fluxes.

We respectfully disagree with your assessment that “there is no connection of your results to previous literature” as we had several citations in the old discussion section linking our research to previous literature. However, we agree that expanding our discussion to better integrate our findings with the pre-existing literature will enhance the manuscript. We fully revised the discussion section to provide a more comprehensive analysis of how our results fit within the broader context of current research and understanding of the controls on these fluxes (L485-581, Page 19-22).

Thank you again for your constructive feedback, and we look forward to improving the clarity and depth of our manuscript.