Response to reviews of the manuscript: Warming increases soil respiration in a carbon-rich soil without changing microbial respiratory potential

We thank the reviewers for their detailed comments and have thoroughly revised the manuscript accordingly. Below we provide a detailed response to each point, indicating how and where we have incorporated the suggestions and requests.

Response to reviewers' comments (responses provided in BLUE)

Reviewer 1:

General comments: I enjoyed reading this well written and well presented paper. The use of the orthogonal experimental design is well considered with very thorough statistical analyses. The authors find that warming increases soil respiration but plant community manipulations have no effect on soil respiration. These findings are well interpreted and their implications discussed. I have included some comments below which the authors may wish to consider.

L33-35: Can you briefly expand on, for the reader, why C loss increases with C content please? Is it because there's a higher potential for C loss or a greater proportion of unprotected C or some other mechanism?

We have revised the text in response to this worthwhile suggestion, expanding on this point (lines 33-35). Specifically, we note that soils with larger C stocks have greater susceptibility to warming, as there is increased substrate for decomposition, with the contention supported by reference to the literature.

L120: Please can you clarify how long the OTC's were in place for and were they in place year- round or during certain seasons only.

This point has been added to the MS (lines 122-123). OTCs have been in place continuously since the experiment was established in 2014 and are currently still operating.

L146-150: Based on your description of the CO_2 flux measurements carried out, it seems that vegetation within the PVC collars was left intact. If this is the case your CO_2 flux measurements will have included root, shoot and soil respiration which amounts to ecosystem respiration rather than soil respiration as described. Given that the focus of the paper is on soil respiration I think the contribution of plant respiration to the in situ CO_2 flux measurements should be addressed.

This is definitely an important suggestion by the reviewer and it is very worthwhile making the situation crystal clear. We have added a more detailed explanation in the methods section to clarify this point (lines 156-161). Further, we addressed this fair point in the discussion section as well (line 413). The reviewer is correct in that our measurements of soil respiration also included a contribution by plant biomass. However, we decided not to completely remove plants from the collars because bare ground is practically non-existent in this ecosystem, partly because of the peaty soil and continuous plant cover. Therefore, we were concerned that removing all plants would create a highly unrepresentative sample of the ecosystem.

We did clip the vegetation within the collars to just above ground height in order to reduce the contribution of aboveground plant parts, but the peaty soils contain a continuous root mat that we were not keen to disturb. Our main concern was that removing all plant biomass would cause the peat to dry out unreasonably quickly, creating cracking and further drying. Therefore, we have maintained our use of the term soil respiration the manuscript, rather than changing to ecosystem respiration, but have pointed out in the methods and discussion that our field measurements include a contribution from roots and a minor contribution from plant shoots.

L150-151: It is not entirely clear what you did here. Did you measure the efflux rate three separate times and take an average of that or did you measure the CO2 concentration at three separate time points and use this to calculate the efflux rate? Please can you clarify this.

The text has been altered to clarify this (lines 162-163). On each occasion, three complete measurements of *in situ* soil respiration, each lasting several minutes, were made in each plot. The results of these three estimations were averaged and used to define the CO_2 efflux rate and this single composite value was used in subsequent analyses.

L157-159: How deep is the organic horizon in these soils? Does 5cm depth cover the whole organic horizon? If not, can you include some details on how representative the top 5 cm of soil might be of the hole of the organic horizon?

This has been clarified in the MS (lines 167-168). The organic horizon at the site is deep, up to 1m in depth. However, the 5cm sampling depth is representative of the zone in which most microbial activity occurs in peaty soils (Fisk et al., 2003). Further, the soil profile is fairly consistent for the top ~20 cm and the top 5 cm is indeed representative of this upper layer of soil.

L421-424: Please can you clarify what you mean by “surface soil layers”. I assume from the context you mean soil <5cm deep, but it is not entirely clear from the way it currently reads. Would it be possible to speculate on the variation in temperature between the soil surface and at 5cm depth from literature? This would be useful information to have here, if it exists.

This section has been expanded to clarify the situation (lines 437-442). While we do not have measurements of the soil depth-temperature profile, and such measurements appear to be rare in the literature, we added some speculation on the variation in soil temperature along the profile as the reviewer suggested. Specifically, as most soil microbial activity occurs in the uppermost few centimetres, it is possible the most biologically-active soil layer was warmed more than the amount measured, partially accounting for the large increase in R_S. However, the warming-depth profile at Silver Plains is unknown and also largely unreported from other warming experiments, except at greater depths (e.g. 0-5 cm versus 5-15 cm (Hollister et al., 2006)).

L501-502: I think it is worth considering here (or at another appropriate place within this paragraph) that increased C input not only stimulates microbial C mineralisation and C efflux but also increases stable SOM formation through microbial decomposition products. I appreciate that SOM formation is not the focus of this work but I think for balance it is worth highlighting the multiple fates of soil C inputs.

This is a valid point and the MS has been altered to include this (lines 543-545). Specifically, we now state that an increase in the input of easily degradable C would both promote microbial activity (Wan et al., 2005; Hogberg and Read, 2006), potentially stimulating soil C efflux, as well as increase formation of stable SOM through microbial decomposition products (Sokol et al., 2019).

L518: In this paragraph you rightly discuss the limitations of your (and most) soil incubations. You mention roots and macrofauna as being absent from the incubated soils. It strikes me that mycorrhizal fungi, which play an important role in soil C dynamics and indeed Rs, are absent from your discussions here. I am not familiar with the plant species at your field sites and they may not be mycorrhizal in which case their omission makes sense, however it the plant communities in question are mycorrhizal it would be worth acknowledging the potential consequences of this in your incubation experiments.

This is a very valid point and we have added specific statements regarding the importance of mycorrhizal fungi to SOM formation as well as how removing their influence is likely to have altered our results (lines 568-659).

Technical corrections:

L32: Delete .. “and so on”..

Text altered as suggested

L44: Correct to: “The effects of temperature on.. “

Text altered as suggested

L147: correct “m2” to include the “2” as superscript. The use of sub- script rather than superscript occurs a few times throughout (e.g. L223 & 224). This may be a formatting error in the conversion to pdf or the authors personal preference, just check that it is as you want it and aligns with journal specifications.

Text altered as suggested

L317: Delete ...”the situation”...

Text altered as suggested

L338-340: For clarity and flow I suggest re-writing this sentence to: “Post hoc analysis revealed the greatest differences in k were observed between; i) warmed x no removal and warmed x dominant removal plots, and ii) warmed x dominant removal and ambient x dominant removal plots.”

Text altered as suggested

L401: I think the word “warming” is missing from this sentence. I assume it should read: “There are 4 possible mechanisms whereby warming could have increased Rs: “

Text altered as suggested

L532: Consider re-writing to: “Large C stocks within this type of peaty habitat are important for the global C cycle, ...”

Text altered as suggested

Reviewer 2:

The study presents results from an established warming experiment in carbon-rich soils located in Tasmania. It investigates the mechanisms that drive warming-induced responses in soil respiration, and whether changes in the plant community or changes in microbial soil respiration potential are important drivers. The researchers used both field manipulation and laboratory incubation experiments. There was a consistent effect of warming over time and across all plant community treatments in the field experiment, suggesting that plant community composition manipulations do not influence soil respiration responses to warming. Warming also did not affect microbial respiration in incubation experiments. They conclude that the warming response is most likely due to increased autotrophic respiration and more labile substrate availability to the rhizosphere.

Overall the study presents novel results, is well-written and represents obvious effort and contribution to the field. There are few studies that investigate the mechanisms by which soil respiration will increase as a result of warming in carbon-rich soils. Yet, these soils are most likely to contribute to CO2 efflux when warmed. I have a few suggestions for the authors to consider that may enhance their message:

I found it a bit strange that plant community composition data was not presented. In the Discussion (line 475) they state that removing the dominant species did not appear to cause any functional shifts in the community, and that the dominant species may have been replaced by a functionally-similar species. If the authors are able to present community composition data after 1 year, that would help clarify whether the overall plant community changed in some way after removal of the dominant species vs. random removal vs. the control. It would answer the question of whether the plant community was really altered enough to expect possible changes, or whether the plant community treatment just wasn’t strong enough to elicit changes.

This is a valid point. We have included a supplementary figure (Fig. S1) showing plant community composition responses to the treatments at the end of the first growing season after treatments began. In addition, we have also added a relevant section to the discussion (lines 510-519). However, we do not present detailed plant community composition data as a full analysis is beyond the scope of this manuscript and will be published elsewhere.

It’s unclear in the Methods whether the removed plant biomass is replaced on the plots as litter or just completely removed. Please clarify this point.

Text altered as suggested to clarify that removed plant biomass was completely removed from plots and not replaced as litter (lines 133-134).

The models presented in Fig 3 and Fig 4 came across as an off-shoot from the main story. If developed further, perhaps including data from similar studies conducted in other soil types, I thought that those two figures could be expanded into a different manuscript that is more broad-reaching. I suggest you remove that information from this paper and just focus in on the experimental results.

It would be interesting indeed to create a separate manuscript as the reviewer suggests but, as they acknowledge, this would require more results from different sites. We will definitely keep act upon this suggestion. However, we believe that the data presented in Figs 3 and 4 is key to understanding the field observations because they help to demonstrate the patterns we discovered more clearly. These figures allow readers to easily identify the relative influences of soil temperature and SWC on C efflux rate and the specific conditions that led to the largest influence of experimental warming on C efflux. Therefore, while we do appreciate the reviewers point and helpfulness, we feel that these figures make an important contribution to this manuscript and would prefer to include them here.

I was surprised that there wasn’t more of a mention of the effect of warming on soil microbial community composition in the discussion. There is a broad base of literature on this topic, and it is likely that warming/drying not only alters microbial physiology but also community structure. I suggest you expand the background literature and Discussion to address this point. For example:
https://doi.org/10.1098/rstb.2019.0112

This is a valid point, and a new section ‘4.1.2 Alteration of microbial community composition and function’, has been added to expand on this (line 476).

Line 11: capitalize Earth

Text altered as suggested

Line 17: due to plant community

Text altered as suggested

Line 34: it has been suggested

Text altered for clarity

Line 53: This would be more effective if you specifically mention Century model examples

This is a valid point and a CENTURY model example has been included in the text (lines 50-53).

Line 120, this sentence is unclear: The experiment consists of forty 2 x 2 metre plots, with 3 metres between each plot, of which 20 were warmed using hexagonal polycarbonate 120 open-top chambers (OTC) with an internal diameter of 1.5 m, with the remainder of being unwarmed, ambient plots.

Text altered for clarity (lines 123-126): "The experiment consists of forty 2 x 2 metre plots, with 3 metres between each plot. 20 of the plots were warmed year-round using hexagonal polycarbonate open-top chambers (OTC) with an internal diameter of 1.5 m, and the remainder were unwarmed, ambient plots."

Line 123, sentence starting with: "To control for possible effects of removing..." is run-on and difficult to understand. Revise.

We have revised the text to make two shorter sentences. The text now reads (lines 129-133): "To control for possible effects of removing biomass during the dominant species removal treatment, we removed biomass from one additional warmed and unwarmed plot in every second block. We removed the same amount of biomass as from the "dominant removal" plots in the same block, however, biomass was removed randomly from across the plot, rather than from a single species (henceforth termed "random removal" plots)."

Table 1: indicate significant differences

Significant differences within months between warming treatments have been indicated and the table heading updated to explain the addition.

Line 268-270: "Neither removal, i.e. neither dominant nor random biomass removal (F2,33=0.89, P=0.42), nor a warming x removal interaction (F2,33=0.57, 270 P=0.57) affected CO2 efflux, as indicated by ANCOVA.", is awkwardly worded. Please revise.

This sentence (lines 283-285) has been revised to now read: "Neither removal treatment, ($F_{2,33}$=0.89, P=0.42), nor a warming x removal interaction ($F_{2,33}$=0.57, P=0.57) affected CO_2 efflux, as indicated by ANCOVA."

Line 401: Change to: There are 4 possible mechanisms whereby Rs could have increased (or similar)

Text altered as suggested (lines 416-417)