

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 CO₂ flux measurements carried out, it seems that vegetation within the PVC collars was left intact. If this is the case your CO₂ 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 CO₂ 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 in 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 CO₂ 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 whole 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 Rs. 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 if 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 ($F_{2,33}=0.89$, $P=0.42$), nor a warming x removal interaction ($F_{2,33}=0.57$, $P=0.57$) 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 CO₂ 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)

Warming increases soil respiration in a carbon-rich soil without changing microbial respiratory potential

Marion Nyberg¹, Mark J. Hovenden¹

¹School of Natural Sciences, University of Tasmania, Hobart, 7001, Australia

5 *Correspondence to:* Marion Nyberg (current affiliation University of British Columbia) (mnybe1@mail.ubc.ca)

Abstract. Increases in global temperatures due to climate change threaten to tip the balance between carbon (C) fluxes, liberating large amounts of C from soils. Evidence of warming-induced increases in CO₂ efflux from soils has led to suggestions that this response of soil respiration (R_s) will trigger a positive land C–climate feedback cycle, ultimately warming the Earth further. Currently, there is little consensus about the mechanisms driving the warming-induced R_s response, and there are relatively few studies from ecosystems with large soil C stores. Here, we investigate the impacts of experimental warming on R_s in the C-rich soils of a Tasmanian grassy sedge-land, and whether alterations of plant community composition or differences in microbial respiratory potential could contribute to any effects. *In situ*, warming increased R_s on average by 28% and this effect was consistent over time and across plant community composition treatments. In contrast, warming had no impact on microbial respiration in incubation experiments. Plant community composition manipulations did not influence R_s or the R_s response to warming. Processes driving the R_s response in this experiment were, therefore, not due to plant community effects and are more likely due to increases in belowground autotrophic respiration and the supply of labile substrate through rhizodeposition and root exudates. CO₂ efflux from this high-C soil increased by more than a quarter in response to warming, suggesting inputs need to increase by at least this amount if soil C stocks are to be maintained. These results indicate the need for comprehensive investigations of both C inputs and losses from C-rich soils if efforts to model net ecosystem C exchange of these crucial, C-dense systems are to be successful.

25 1 Introduction

Globally, more carbon (C) is stored in soils than the amount of C in the atmosphere and in plants combined (Canadell et al., 2007). Simple physiology suggests that soil respiration (R_s) rates will increase as soil temperatures rise (Gillooly et al., 2001), stimulating CO₂ emissions from the soil – a response that has the potential to outweigh plant productivity responses to global warming and lead to a net loss of C from soils (Melillo et al., 2017). Recently, numerous studies have suggested that global warming is indeed disturbing the balance between ecosystem C inputs and outputs (Melillo et al., 2017). This suggests the possibility of a positive feedback whereby warming increases C efflux from soils, which accelerates climate change leading to further C losses (Bridgman et al., 2008; Melillo et al., 2017; Bond-Lamberty et al., 2018). **Importantly**, it is possible that warming-induced C losses increase with soil C content, as soils with large C stocks have greater susceptibility to warming since there is more substrate available for decomposition, and therefore soils storing the most C could shift from C sinks to C sources (Crowther et al., 2016).

Commented [MN1]: R1

"Delete .. "and so on" .."

Text altered as suggested

Commented [MN2]: R1: "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.

Increases in respiration of soil organic carbon (SOC) as an effect of experimental warming occur almost universally (Rustad et al., 2001) however, increasing soil temperatures stimulate not only soil microbes and enzyme activity, but also net primary productivity (NPP) and fresh C input from litterfall, and root exudations (Rustad et al., 2001), enhancing substrate availability for microbial respiration (Lu et al., 2013; Wang et al., 2017). Warming effects have also been demonstrated to drive microbial priming, whereby decomposition is enhanced through increased input of labile C compounds (van der Wal and de Boer, 2017). Despite this, greater above ground plant biomass is not directly linked to immediate or long-term increases in the storage of SOC and hence the mechanisms driving the response of Rs to warming are uncertain (Jackson et al., 2017).

45 The effects of temperature on environmental factors such as soil moisture, substrate availability and evapotranspiration also influence and mediate rates of decomposition of SOM, and efflux of CO₂ (Davidson et al., 2000; Eliasson et al., 2005; Lu et al., 2013). These effects include extension of growing seasons and shifts in species composition and community structure (Chen et al., 2016). Considering this, changes in plant community composition, and subsequent shifts in functional traits have the potential to influence the quantity and quality of organic matter in the soil, as well as the physical soil structure (Metcalf et al., 2011). For instance, an experiment implementing the CENTURY model revealed significant differences in SOC levels and the carbon to nitrogen ratio of the active SOM fraction between mixed grassland communities, and those comprised completely of C₃ or C₄ vegetation (Epstein et al., 1999; Parton et al., 1987). This suggests that there is potential for the response of soil C dynamics to warming to be partially or even wholly dependent upon changes to plant community composition (Jackson et al., 2017).

Commented [MN3]: R1

“Correct to: “The effects of temperature on..”
Text altered as suggested

Both experimental and global warming have impacts on soil water availability, which is itself a primary determinant of Rs (Schimel et al., 1994). Following a unimodal relationship, respiration is highest at an intermediate (35-50% by volume) soil water content (SWC), which stimulates microbial activity and enhances above and below ground labile C inputs (Chou et al., 2008; Zhou et al., 2010; Wang et al., 2017). Anaerobic conditions in wet and flooded soils suppress microbial activity, slowing decomposition of SOM (Davidson and Janssens, 2006). Similarly, low SWC can have a similar effect by reducing microbial activity, restricting soil respiration (Carey et al., 2016). As warming generally leads to lower soil water content (Zhang et al., 2013; Li et al., 2017), the impact on Rs depends upon the underlying soil water content, increasing respiration of wet soils but reducing respiration in drier soils (Almagro et al., 2009). Essentially, the effect of warming on SWC could either offset or exacerbate direct warming effects on soil respiration, potentially disturbing the entire global C balance.

Substrate availability is another factor that is affected by warming, and thus has the potential to shift the temperature sensitivity of SOM decomposition (Davidson and Janssens, 2006). Largely, increased temperatures lead to the loss of physical or chemical protection of SOM, and thus enhanced microbial respiration of soil organic carbon (SOC) (Davidson and Janssens, 2006). Partitioning SOC into pools as a function of recalcitrance and residence time assists with analysing effects of environmental manipulations on long-term C storage (Pendall et al., 2011). As C inputs to the soil and consequently into these various pools occur in response to the interplay between rates of NPP, decomposition, climatic conditions and soil characteristics (Ontl and Schulte, 2012), the fate of SOC is either transformation into highly recalcitrant humus, important for the stabilisation and long-term storage of SOC, or loss to the atmosphere as CO₂. Thus, factors such as oxygen availability, substrate quantity

Commented [MN4]: R2

“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).

and quality, nutrient limitation and activity of extracellular enzymes are key to the soil respiration response. Carefully controlled laboratory incubations are necessary to eliminate confounding factors and pinpoint the mechanisms driving responses observed in the field (Davidson and Janssens, 2006). Ultimately, distinguishing between the potential driving factors is vital for our ability to model future C fluxes and to extend the observations from field experiments more widely.

To understand the consequences of warming on soil C dynamics and particularly R_s , it is necessary to distinguish between warming-related increases in R_s that are simply due to an increase in the biochemical response of R_s to temperature, and potential alterations of the temperature sensitivity of R_s caused by climate warming. The increase in R_s with rising temperature has been widely documented (Luo et al., 2001; Rustad et al., 2001), however, the temperature sensitivity of R_s in soils that have undergone experimental warming is much more variable (Song et al., 2014; Carey et al., 2016). Shifts in the temperature sensitivity under warming are likely to be driven by both changes in microbial community composition and changes in the physical and chemical properties of the soil (Davidson and Janssens, 2006). Additionally, effects of warming such as soil drying affect various ecosystem processes and thus might shift the temperature response of R_s (Carey et al., 2016; Moinet et al., 2018). The effect of temperature on R_s is thus complex, and there are a number of biotic and abiotic factors influencing the response of SOM decomposition to warming. Until these various influences are characterised accurately, projecting future soil C emissions will remain problematic.

Although measurements of soil respiration *in situ* often demonstrate warming-related increases, the mechanisms behind this response cannot be revealed by simple field observations (Davidson and Janssens, 2006). In particular, it is difficult to distinguish changes in microbial community composition and functioning in response to warming from *in situ* measurements alone. These changes include acclimation (Luo et al., 2001) or adaptation (Bradford et al., 2008), encompassing both physiological and genetic changes within individuals and species, changes in community structure (Sheik et al., 2011) and a shift towards microbial use of slowly decomposing C (Bracho et al., 2016). Hence, a shift in temperature sensitivity of SOM decomposition is likely to be driven by warming through a change in microbial respiratory potential, expressed as the CO_2 mineralisation rate.

Here, we use a manipulative experiment to examine the potential influences of climate change, specifically warming and plant community composition, on soil C dynamics. We examine soil respiration responses both *in situ* and in laboratory incubation experiments to disentangle the mechanisms involved in the response of soil respiration to both warming and manipulation of the plant community. Specifically, we ask the following questions:

- 1.) Does warming increase soil respiration in a Tasmanian C-rich soil?
- 2.) If so, is this due to changes in microbial respiratory potential?
- 3.) Does altering plant community composition change the response of soil respiration to warming?

2 Materials and Methods

2.1 Study site

All field measurements and soil samples were taken at the Silver Plains warming experiment in the Tasmanian central plateau, Australia (42°09'S, 147°08'E; 890 m a.s.l.). The site is a natural grassy sedgeland with an average summer temperature of 16°C, average winter temperature of 6°C and average annual rainfall of 720 mm (BOM, 2018). Soil at the site is peaty, being an organosol containing on average 8 kg C m⁻² in the top 10 cm. The vegetation at the site is heavily grazed year-round by a range of native vertebrate herbivores, including wallabies, pademelon and wombats, as well as by feral fallow deer, resulting in an extremely low vegetation stature of a few cm, with the exception of inflorescences which can extend up to 30 cm above the ground.

120 2.2 Experimental design

The experiment was set up in the 2014 austral winter as a fully orthogonal, two-factor random block design, with warming and species removal as fixed factors across eight replicate blocks (Fig. 1). The warming chambers were installed in mid-2014 and have remained in place continuously up to the present day (mid-2020). The experiment consists of forty 2 x 2 metre plots, with 3 metres between each plot. Twenty 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. To investigate the impact of altering plant community composition, the dominant species, *Poa gunnii*, was removed by plucking in one warmed and one ambient plot (henceforth “dominant removal” plots) in each block. One warmed and one ambient plot in each block was left untouched (henceforth “no removal” plots). 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). In both dominant removal and random removal, plucked biomass was completely removed from plots, i.e. not replaced on the plot. Plant biomass was removed in the spring and summer of 2014/15 by gently removing small plants by hand and by repeatedly clipping larger plants to ground level until green shoots no longer emerged. The amount of biomass removed in each plot is presented in Table S1. After the initial removal treatment, all plots were left undisturbed until the following spring, at which time all plots were surveyed to determine whether removed plants had re-established. As removed plants had not re-established at this time, no further removal occurred. Plant biomass was not measured directly in the plots in order to reduce disturbance. However, measures of vegetation cover and height indicated that the vegetation in removed plots had recovered completely within two years and were very similar to untouched control plots by this time, except in terms of species composition.

Commented [MN5]: R1

“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.

Commented [MN6]: R2

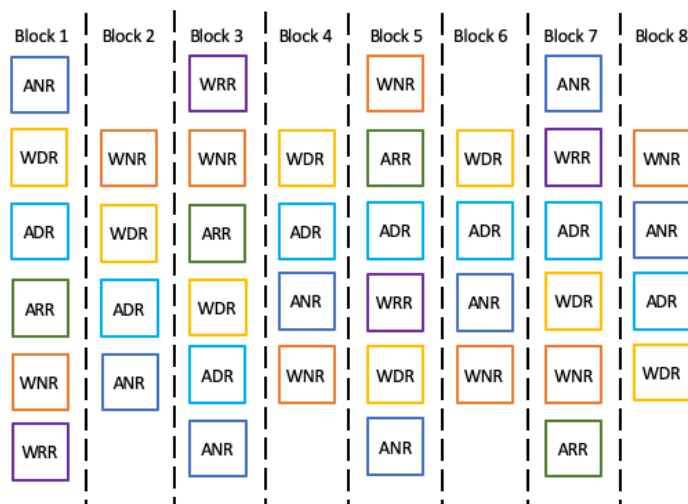
“this sentence is unclear: The experiment consists of forty 2 x 2 metre plots, with 3 metres between each plot, of which 20 were warmed using hexagonal polycarbonate 120 open-top chambers (OTC) with an internal diameter of 1.5 m, with the remainder of being unwarmed, ambient plots.”

Text altered for clarity (lines 123): “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.”

Commented [MN7]: R2

“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).



145 **Figure 1.** Conceptual diagram for the experimental design of the Silver Plains warming experiment. Each block contains a warmed and unwarmed plot with no species removed (WNR) and (ANR) respectively; a warmed and unwarmed plot with the dominant species removed (WDR) and (ADR) respectively; and in every second block, i.e. in four blocks, there is a warmed and unwarmed plot with random biomass removal (WRR) and (ARR) respectively.

150 Air temperature at 5 cm height and soil temperature at 5 cm depth in each plot was logged continuously with iButton dataloggers. Over the entire five year period, the warming treatment increased air temperature 5 cm above the soil surface by 1.56°C ($P < 0.004$) and soil temperature at 5 cm depth by 1.29°C ($P < 0.001$).

2.3 *In situ* methods

155 A 50 mm length of 100 mm diameter PVC pipe was inserted into the soil to a depth of 2 cm, extending 3 cm above ground height, within the centre 0.25 m² of each plot for soil respiration measurements. Soil respiration was measured with a CO₂/H₂O infrared gas analyser (IRGA) (Licor, model LI-6400) with attachment of a Licor 6400-09 soil chamber, which attached to PVC collars. Bare ground is practically non-existent in this ecosystem and the soil is extremely peaty. Therefore, vegetation in collars was not removed but was regularly clipped to just above ground height to minimise the influence of aboveground plant respiration. Our measurement of soil respiration thus did include small contributions of shoot respiration, but as soil at the site is peaty with extensive horizontal root growth, any respiration measurement from this site would include a substantial amount of respiration from belowground plant biomass. Respiration was measured *in situ* monthly from August 2017 to June 2018. On each occasion, three complete measurements of *in situ* soil respiration in each plot were averaged and used to define the CO₂ efflux rate. The average value of these three measurements was used in subsequent analyses. Soil temperature and moisture in each plot were measured at the exact same time as the soil respiration

Commented [MN8]: R1

"Based on your description of the CO₂ flux measurements carried out, it seems that vegetation within the PVC collars was left intact. If this is the case your CO₂ 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* CO₂ 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.

Commented [MN9]: R1

"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 CO₂ efflux rate and this single composite value was used in subsequent analyses.

165 measurements on each occasion. Soil temperature was measured with a soil thermocouple probe (LiCor 6000-
09TC) attached to the LI-6400. Volumetric soil water content (SWC) was estimated at 5 locations in each plot
using a hand held TDR probe at 0-5cm depth. Although the organic horizon in this soil is up to 1m in depth, the
5cm sampling depth is representative for the zone in which most microbial activity occurs in peaty soils (Fisk et
al., 2003). The 5 separate measurements of SWC were then averaged to obtain one SWC value per plot on each
170 measuring occasion.

Six randomly placed soil samples, amounting to a total of approximately 25-30 g fresh weight, were collected
from each plot using a 1.5 cm diameter hand corer to a depth of 5 cm below ground level, twice throughout the
year. Samples were collected on the 02/03/18, representing the end of summer, or growing season soil, and on the
25/06/18, representing winter soils.

175

2.4 Laboratory incubations

Soil cores collected *in situ* were immediately placed on ice for return to the laboratory, where they were
refrigerated (4°C) overnight. The following day, the samples were composited at the plot level and sieved through
a 4 mm sieve for one minute to remove leaves and large roots. A 10 g fresh-weight sub-sample was removed and
180 oven dried from each composite sample for the determination of total soil C. Each subsample was ground to a
powder in a Retsch Mixer Mill (MM200, Retsch GmbH, Haan) and then C content was analysed by combustion
in a Perkin Elmer 2400 Series II Elemental Analyser (Perkin Elmer Australia, Melbourne). The remaining soil
was used immediately for laboratory incubations to determine microbial respiration, as detailed below.

Microbial respiration as a function of temperature was determined by incubation using soils sampled in the Silver
185 Plains warming experiment at the end of summer and in mid-winter 2018. For each plot, three replicate samples
weighing four to eight grams from the composite sample were placed in 100 mL specimen jars, each of which
was incubated at a different temperature. Each sample was wetted to bring them to 90% of field capacity for
winter soils and 60% of field capacity for summer soils to represent prevailing soil moisture conditions in each
respective season. Once water was added to all soil samples, specimen jars were placed in 500 ml preserving jars
190 with tightly fitting lids containing a septum to allow gas headspace samples to be collected by syringe. Jars were
stored in dark incubation cabinets at temperatures at one of 10, 17 or 25°C, with one sample from each plot at
each temperature. Headspace gas of jars were sampled (20 ml) using a syringe on days 1, 2, 4, 5, 7, 9, 12, 15, 19,
23, 29, 35, 49, 56, 63. After extracting samples from each jar, headspace samples were analysed for CO₂
concentration, representing soil respiration, and microbial respiratory potential was thus defined as the rate of CO₂
195 release. To analyse headspace gas, samples were injected directly into an infrared gas analyser (LI-6262, Li-Cor,
Lincoln, NE). After measurements were taken and analysed, jars were ventilated for 20 minutes and headspace
gas equilibrated with atmospheric air. Following this, lids were replaced and headspace gas was sampled and
analysed again to obtain starting CO₂ concentration for each jar. C mineralisation over the sample period was
calculated from the increase in headspace CO₂ concentration.

200 Total C mineralisation over the entire incubation period was simply the sum of the amount of C mineralised over
each sample period. Daily C mineralization results (dC/dt) were analysed using non-linear curve fitting routines

Commented [MN10]: R1

"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 whole of the organic horizon?"
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 CO₂ efflux rate and this single composite value was used in subsequent analyses.

in R (version 3.4.3, R Core Team, 2017), with a single pool plus constant model (Pendall et al., 2011) to estimate the size of the labile C pool (C_a), the intrinsic decay constant of the labile pool (k), and the intrinsic decay constant of the stable C pool (Y_0):

$$\frac{dC}{dT} = C_a k e^{-kt} + Y_0 \quad (1)$$

2.5 Data analysis

Field soil respiration rates were analysed using a 2-factor repeated measures ANOVA with warming and removal as the fixed factors. Since soil temperature (T_s) and SWC are known controllers of R_s and varied substantially over the year, we also analysed field R_s with a 2-factor ANCOVA with T_s and SWC and the interaction between T_s and SWC as covariates. Treatment means were calculated as least-squares means using the `lsmeans` package to account for the influences of covariates (Russel V. Lenth, 2016). Treatment effects on SWC and T_s were analysed using 2-factor repeated measures ANOVA exactly as for R_s .

Because there was a significant influence of warming on R_s , we created a separate model of the influence of SWC and T_s on *in situ* R_s for warmed and unwarmed plots. Since the respiration temperature relationship is best described by an Arrhenius-type function (Fang & Moncrieff 2001), we used multiple regression techniques to fit an exponential relationship to R_s and SWC, T_s and the interaction between T_s and SWC. Such a non-linear relationship fitted the observed data far better than a linear model, as compared by the Akaike information criterion corrected for finite sample size.

Total cumulative CO_2 emitted in laboratory incubations, C_a , k , and Y_0 for each season were compared using three-factor analysis of variance ANOVA for both summer and winter soils with incubation temperature, warming and species removal as fixed factors, including all interactions. Seasonal differences were also analysed using four-factor ANOVA, with season also included as a fixed factor along with warming effect, removal and incubation temperature.

All statistical analyses were carried out in R (version 3.4.3). Data were checked for heteroscedasticity and normality and the required transformations were made using the Box Cox power and logarithmic transformations. Significant treatment effects were further analysed using Tukey's HSD *post hoc* comparisons.

3 Results

3.1 *In situ* soil respiration

3.1.1 CO_2 efflux

Experimental warming drove a significant increase in soil respiration over the course of the year ($F_{1,12}=58.48$, $P<0.001$; Table 1) but there was no significant influence of the species removal treatment, so neither the dominant nor random removal treatments were different to the untouched plots ($F_{2,12}=1.1$, $P=0.36$), nor was there a warming

x removal interaction effect on CO₂ efflux ($F_{2,12}=0.14$, $P=0.87$). As expected, time of year had a strong effect of CO₂ efflux ($F_{6,12}=11.84$, $P<0.001$), with the highest rates, $13.23 \pm 0.37 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, in summer, decreasing through to $1.4 \pm 0.06 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ in winter (Table 1). Despite the strong variation in C efflux rates across the year, there was no significant interaction between month and warming ($F_{5,12}=1.17$, $P=0.38$), indicating that the warming effect was consistent across the year.

Table 1. The impact of experimental warming on soil CO₂ efflux, soil temperature and soil water content in the Silver Plains Warming Experiment from August 2017 to June 2018. Values shown are means with standard errors in parentheses (n=20) Asterisks next to warmed means indicate significant differences ($p < 0.05$) between means of warmed and ambient plots within a month.

Month	Treatment	CO ₂ efflux	Soil temperature	SWC
		$\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$	$^{\circ}\text{C}$	%
August	Ambient	1.8 (0.1)	5.1 (0.1)	34.3 (0.6)
	Warmed	2.3 (0.1)*	5.3 (0.1)	27.4 (0.5)*
November	Ambient	8.0 (0.3)	16.0 (0.3)	19.8 (1.0)
	Warmed	12.2 (0.4)*	16.9 (0.3)	16.5 (0.7)*
January	Ambient	11.6 (0.3)	17.1 (0.2)	19.3 (0.4)
	Warmed	13.2 (0.4)*	17.8 (0.2)*	17.5 (0.6)*
February	Ambient	7.6 (0.2)	18.9 (0.1)	13.3 (0.2)
	Warmed	12.9 (0.5)*	18.8 (0.1)	12.6 (0.3)*
March	Ambient	6.1 (0.1)	13.3 (0.3)	20.6 (0.3)
	Warmed	9.0 (0.2)*	13.5 (0.2)	15.8 (0.3)*
April	Ambient	4.7 (0.1)	12.6 (0.2)	13.7 (0.3)
	Warmed	7.3 (0.1)*	13.2 (0.1)*	11.1 (0.3)*
May	Ambient	4.0 (0.1)	10.9 (0.3)	12.1 (0.3)
	Warmed	5.7 (0.1)*	12.3 (0.2)*	9.8 (0.2)*

Commented [MN11]: R2

"Table 1: indicate significant differences"

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

	Ambient	1.4 (0.1)	3.3 (0.2)	52.0 (1.7)
June	Warmed	1.7 (0.1)	4.1 (0.1)	45.3 (1.2)

245

3.1.2 Soil temperature

Time of year had a strong impact on soil temperature ($F_{6,12}=27.61$, $P<0.001$), which varied from 3.33 ± 0.18 °C to 18.89 ± 0.14 °C over the study period. Experimental warming had a significant impact on soil temperature increasing soil temperature at 5 cm depth by 0.55 °C on average ($F_{1,12}=7.31$, $P=0.02$). This impact was sustained over the year with no significant month x warming interaction ($F_{5,12}=0.88$, $P=0.52$), indicating that the warming chambers had a similar effect on soil temperature across the year. Neither removal treatment, i.e. neither dominant nor random biomass removal ($F_{2,12}=1.99$, $P=0.18$), nor warming x removal interactions ($F_{2,12}=0.45$, $P=0.65$) affected soil temperature. Thus, the warming treatment increased soil temperatures consistently over the year and across the species removal treatments.

255

3.1.3 Soil water content

Soil water content (SWC) also varied over the year ($F_{6,12}=6.21$, $P=0.003$) reflecting precipitation patterns at Silver Plains (BOM, 2018). Over the course of the year, SWC ranged from $9.83 \pm 0.17\%$ to $52 \pm 1.69\%$, with moisture levels decreasing from winter 2017 through to autumn 2018, and then steeply increasing again in winter 2018 (Table 1). Experimental warming significantly decreased SWC throughout the year by 3% on average ($P<0.001$), which is expected considering the drying effect of warming. However, the impact of warming on SWC depended upon the month, as indicated by a significant sampling month x warming effect ($F_{5,12}=6.09$, $P=0.005$). Warming had the greatest effect on SWC in August 2017 and June 2018, when SWC was highest and soil temperature was lowest. SWC in these winter months was substantially higher than during the rest of the year, with SWC on average 34 and 58% higher in August and June respectively, than the overall mean value ($21.3 \pm 0.5\%$). The proportional reduction in SWC due to warming in these months was nearly two-times the yearly average. Otherwise, the warming effect was similar between sampling months. There was no significant influence of the removal treatment, i.e. neither the dominant nor random removal treatments were different to the untouched plots ($F_{2,12}=0.23$, $P=0.8$), nor was there a warming x removal interaction effect on SWC ($F_{2,12}=0.52$, $P=0.61$), again indicating that plant species removal did not alter the influence of the warming treatment.

270

3.1.4 Relationships between environmental factors and CO₂ efflux

Both soil temperature ($F_{1,33}= 33.62$, $P<0.001$) and SWC ($F_{1,33}= 5.95$, $P=0.02$) were strong controllers of soil CO₂ efflux over the year at Silver Plains (Fig. 2). However, treatment effects on these abiotic factors alone were insufficient to explain the higher C efflux in warmed plots, as ANCOVA indicated that the warming treatment

275

still induced significant increases in CO₂ efflux when variation in soil T and SWC were accounted for ($F_{1,33}=44.83$, $P<0.001$). Thus, the warming treatment increased soil CO₂ efflux independently of its effects on soil temperature and SWC (Fig. 2). Across the whole year LS mean CO₂ efflux rates for ambient soils was 6.07 (C.I.=5.69,6.45) $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ but 8.48 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ (C.I.=8.09,8.86) for warmed soils, amounting to a warming-induced increase of 28% at a common soil temperature and SWC. As CO₂ efflux measurements spanned a large variation in both soil T and SWC, it was possible to discern a trend whereby the stimulation of C efflux by warming became more pronounced as soil temperature increased (Fig. 2). 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 CO₂ efflux, as indicated by ANCOVA.

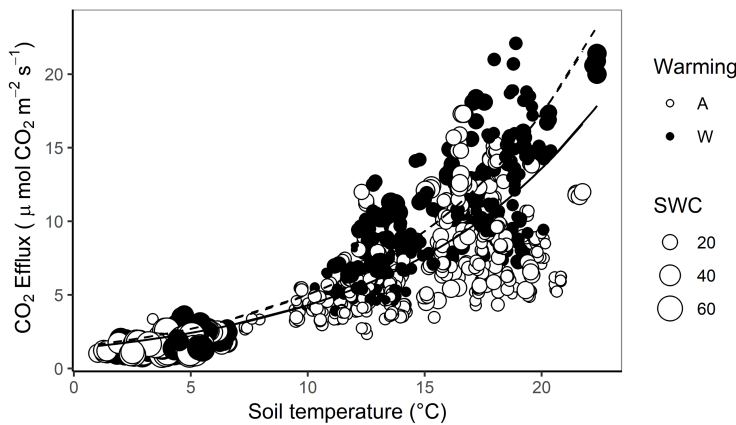


Figure 2. CO₂ efflux as a function of soil temperature and soil water content for warmed (W) and ambient (A) plots at Silver Plains from August 2017 to June 2018. Size of the point represents SWC %, with larger points corresponding to higher SWC. The regression lines indicate the relationship between CO₂ efflux and soil temperature at median SWC in ambient plots (solid line) and warmed plots (dashed line).

3.1.5 Models of CO₂ efflux

As ANCOVA indicated that soil CO₂ efflux at Silver Plains was significantly influenced by soil temperature, SWC and a strong warming effect, the relationship between these covariates and CO₂ efflux could be estimated separately for ambient and warmed treatments. First a general regression model of CO₂ efflux was fit and selected using model selection based on AICc. The most parsimonious and accurate model was one which included soil temperature (T_s), SWC, and a SWC x T_s interaction term (Int.term).

This model was then fit independently to ambient and warmed plots using the relative coefficient values, with 89% of the variance in CO₂ efflux explained in warmed plots Eq. (2) and 82% in ambient plots Eq. (3).

$$\text{CO}_2 \text{ efflux}_{\text{ambient}} = e^{(-0.8+0.359 \log(\text{SWC})+0.115 (T_s)+0.003(\text{Int.term}))}$$

Commented [MN12]: R2

“Neither removal, i.e. neither dominant nor random biomass removal ($F_{2,33}=0.89$, $P=0.42$), nor a warming x removal interaction ($F_{2,33}=0.57$, $P=0.57$) affected CO₂ efflux, as indicated by ANCOVA.”, is awkwardly worded. Please revise.”

This sentence 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 CO₂ efflux, as indicated by ANCOVA.”

Commented [MN13]: R2

“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.

$$R^2 = 0.82$$

(2)

$$\text{CO}_2 \text{ efflux}_{\text{warmed}} = e^{(-0.06 + 0.148 \log(\text{SWC}) + 0.124 (T_S) + 0.002(\text{Int.term}))}$$

$$R^2 = 0.89$$

305

(3)

Thus, it is possible to model CO₂ efflux across a range of soil temperature and SWC values in both ambient, unwarmed (Fig. 3A) and warmed conditions (Fig. 3B). From these plots, it is possible to determine that while the CO₂ efflux rate increases more steeply with rising temperature in warmed plots than in unwarmed plots, the way in which it does so is also dependent upon the SWC (Fig. 3A and B). Thus, the impact of experimental warming on soil CO₂ efflux was greatest in warm (T_s>15°C) relatively dry conditions (SWC<30%; Fig. 4).

310

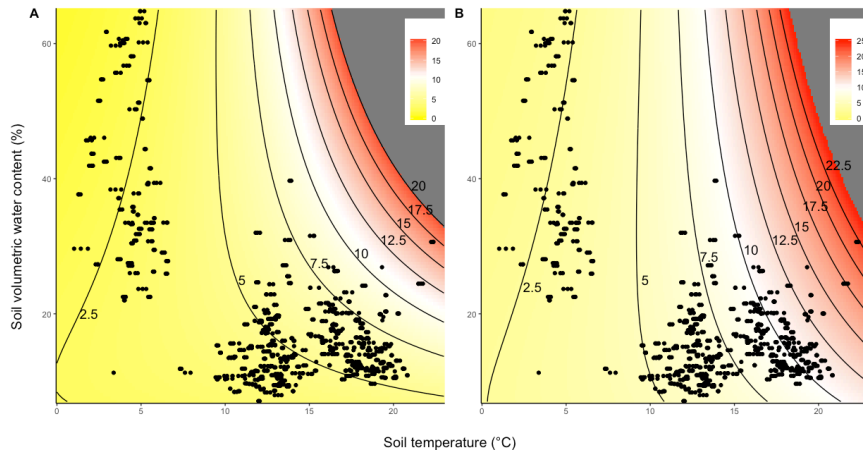
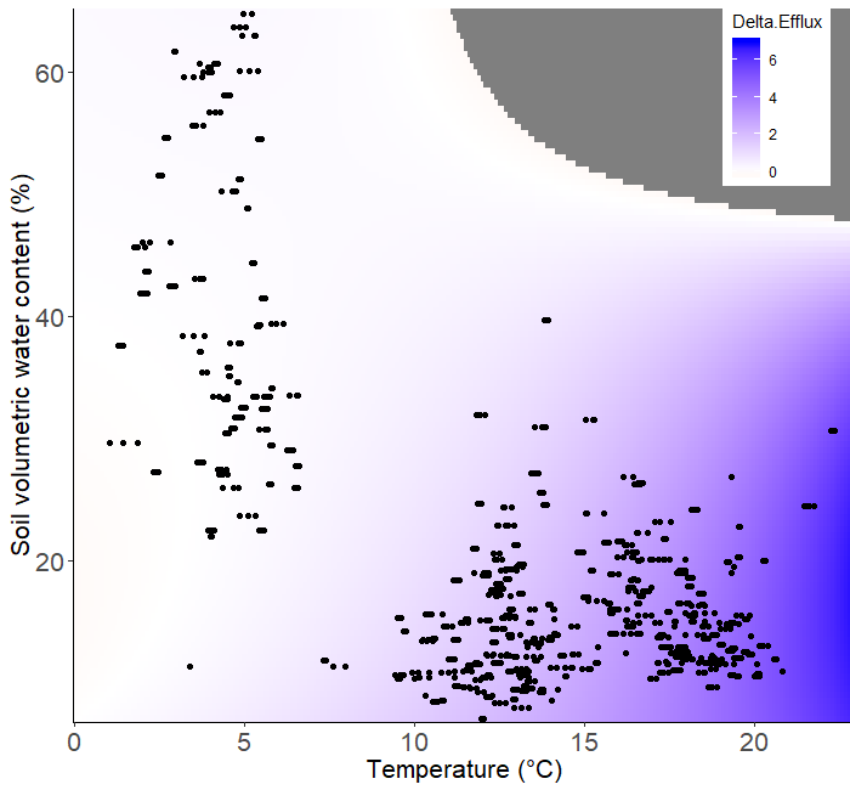


Figure 3. CO₂ efflux (μmol CO₂ m⁻² s⁻¹) modelled as a function of soil temperature and SWC in A.) ambient plots and B.) warmed plots. Colour indicates predicted CO₂ efflux values and field observations are shown as individual points. Regions beyond the observed range of CO₂ efflux rates are shown in grey.



315

Figure 4. Delta CO₂ efflux ($\mu \text{ mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$). The amount of extra CO₂ that is likely to be released due to warming as a function of soil temperature ($^{\circ}\text{C}$) and SWC (%). Data points represent actual measurements and colour indicates predicted CO₂ efflux. Points on the contour graphs are field observations and regions beyond field observations, and thus where CO₂ cannot be predicted, are greyed out.

320

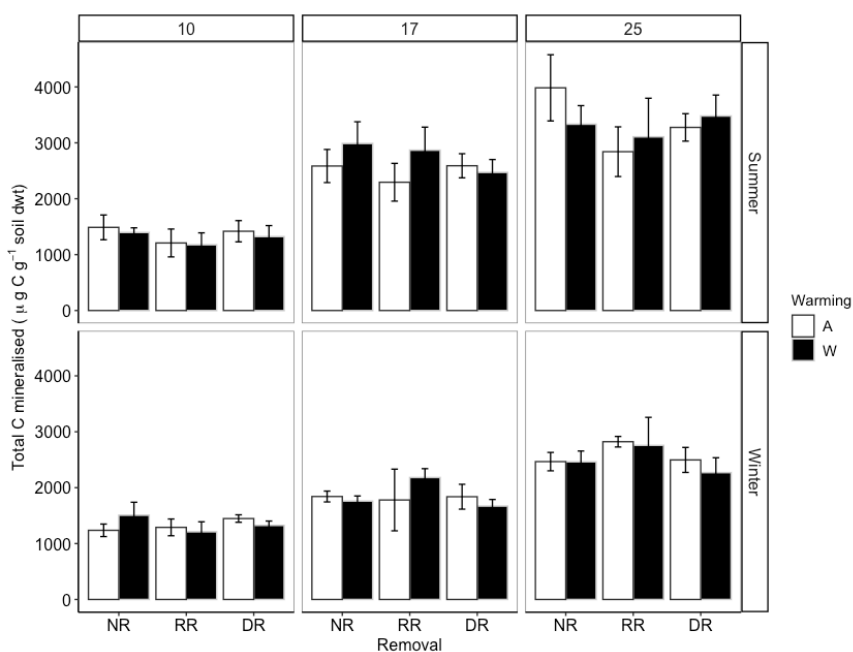
3.2 Laboratory incubations

3.2.1 Total C mineralisation

To determine whether experimental treatments altered potential microbial respiration, soil samples were collected in summer and winter for laboratory incubations. These incubations allowed the temperature sensitivity of soil respiration, the size of the labile C pool (C_a) and its decay constant (k) to be assessed, as well as estimating the decay constant of the more resilient stable C pool (Y_0) to be assessed in constant, optimal conditions. From soils collected in summer, the total amount of C mineralised increased substantially as an effect of incubation temperature, however there were no effects of either the warming or removal treatments. On average, soil incubated at 17°C for two months emitted 48% more C than at 10°C, and a further 22% at 25°C ($F_{2,82}=80.9$,

325

330 $P < 0.001$; Fig. 5). From soils collected in winter, total C mineralised again only increased significantly as an effect of incubation temperature, with on average a 26% increase in C emitted at 17°C from 10°C, and a further 27% increase at 25°C ($F_{2,112}=49.56$, $P < 0.001$; Fig. 5). Just as with soil collected in summer, there were no treatment effects on the total amount of C mineralised from winter soils ($F_{1,112}=0.04$, $P=0.84$). Between seasons, winter soils emitted on average 24% less C than summer soils ($F_{1,196}=33.66$, $P < 0.001$), most likely because of the higher SWC used for the winter soils, and neither removal treatment, i.e. neither dominant nor random biomass removal ($F_{2,196}=0.67$, $P=0.51$), nor warming significantly affected total C mineralised overall ($F_{1,196}=0.01$, $P=0.92$).



340 **Figure 5.** Total C mineralised in summer and winter from soils in no removal (NR), (random removal (RR), and dominant removal (DR) plots at incubation temperatures, 10, 17 and 25°C for warmed (W) and ambient (A) treatments.

3.2.2 Labile C

345 In summer soil, incubation temperature significantly increased the size of C_a on average by 50% from 10°C to 17°C, and by a further 18% at 25°C ($P < 0.001$) (Fig. 6A). There were no treatment effects on the size of C_a . Winter soil incubations reflect similar results to those for summer soils, with a 27% increase in C_a pool size from 10°C to 17°C, and a further 27% increase to 25°C ($P=0.001$). As with summer soil there were no treatment effects. Overall, season had no effect on C_a , however incubation temperature increased C_a across the two seasons of 36% from 10°C to 17°C and a further 24% at 25°C ($P < 0.001$).

350 The intrinsic decay constant of the labile pool (k) in summer soil was not affected by incubation temperature ($F_{2,82}=0.39$, $P=0.68$), the warming ($F_{1,82}=0.06$, $P=0.8$), or removal treatments, i.e. neither dominant nor random biomass removal ($F_{2,82}=0.31$, $P=0.73$), was significantly influenced by an interaction between warming and species removal ($F_{2,82}=3.14$, $P=0.05$) (Fig. 6C). In ambient plots, removing the dominant species tended to increase k , however, in warmed plots, the opposite occurred. 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. In winter, there were no treatment or incubation temperature effects on k , however k was on average 42% greater in summer ($F_{1,196}=201.09$, $P<0.001$).

Commented [MN14]: R1

"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

3.2.3 Intrinsic decay constant of the stable C pool

360 From summer soil, the size of the stable C pool (Y_0) also increased significantly ($F_{2,82}=78.01$, $P<2^{-16}$) as a function of incubation temperature with an average increase of 47% from 10°C to 17°C, and a further 20% at 25°C (Fig. 6C). There were no treatment effects on the Y_0 of summer soil. For winter soils, responses to treatments were similar to those of summer soils. There were no treatment effects, but incubation temperature increased Y_0 on average by 27% from 10°C to 17°C, and a further 28% at 25°C ($F_{2,112}=45.9$, $P<0$). Overall Y_0 was 39% higher in summer than in winter ($F_{1,196}=137.61$, $P<0.001$), and incubation temperature also significantly increased Y_0 overall, with on average a 38% increase from 10°C to 17°C, and a further 23% at 25 °C ($F_{1,196}=107.28$, $P<0.001$), however there were no treatment effects.

3.2.4 Proportion of total C that was labile

370 From summer soil, the proportion of total C that was from C_a was only affected by incubation temperature with on average a 49% increase from 10°C to 17°C, and a further 22% increase when incubated at 25°C ($F_{2,82}=77.73$, $P<0.001$; Fig. 6D). There were no treatment effects. Similarly, in winter, the proportion of total C that was C_a increased only as a function of increasing incubation temperature, with on average a 24% increase from 10°C to 17°C and a further 27% at 25°C ($F_{2,112}=22.19$, $P<0.001$). Overall, the proportion of total C that was C_a , increased substantially as a function of incubation temperature ($F_{2,196}=67.94$, $P<0.001$) with a 35% increase from 10°C to 17°C, and a further 25% increase at 25°C, however there were no overall treatment effects.

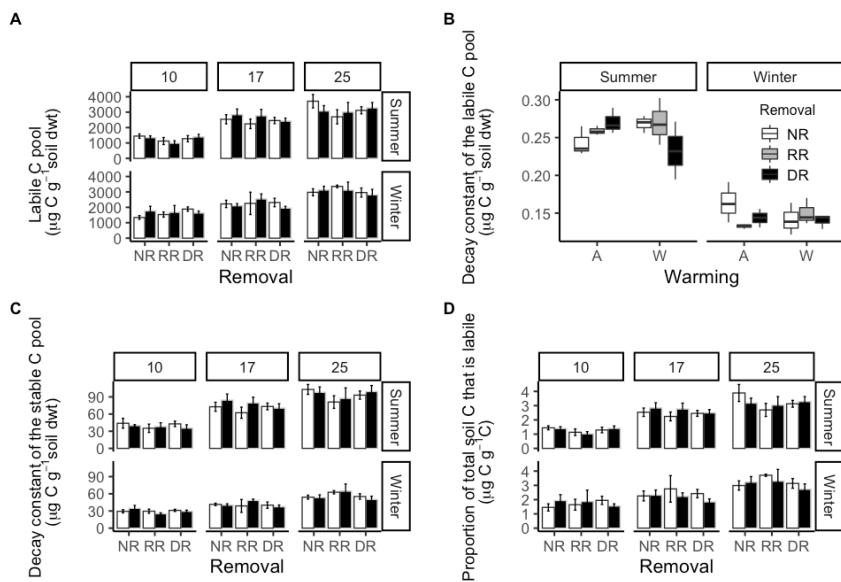


Figure 6. A.) Labile C pool size (C_a) in summer and winter soils in no removal (NR), random removal (RR) and dominant removal (DR) plots at incubation temperature, 10, 17 and 25°C for warmed (black) and ambient (white) treatments. B.) Intrinsic decay constant of the labile C pool (k) both summer and winter soils in no removal (NR), random removal (RR), and dominant removal (DR) plots at incubation temperature, 10, 17 and 25°C for warmed (black) and ambient (white) treatments. C.) The intrinsic decay constant of the stable C pool (Y_0) in summer and winter soils in no removal (NR), random removal (RR), and dominant removal (DR) plots at incubation temperature, 10, 17 and 25°C for warmed (black) and ambient (white) treatments. D.) Proportion of total C that is from the labile C pool (C_a) in both summer and winter soils in no removal (NR), random removal (RR), and dominant removal (DR) plots at incubation temperatures 10, 17 and 25°C for warmed (black) and ambient (white) treatments.

3.3 Total soil C content

Overall, irrespective of removal treatment, total soil C % averaged 19.2 ± 0.4 ($P < 0.001$). C % was 18.7 ± 0.7 and 19.7 ± 0.6 in ambient and warmed soils respectively, however there were no significant treatment effects.

All incubation results were also analysed per gram of soil C but results were essentially identical to those expressed per gram of soil dry weight, above.

4 Discussion

The main aim of this study was to investigate whether warming increases R_s *in situ*, and whether any observed treatment effects were due to an increased ability of the soil microbial community to mineralise SOC. Additionally, we investigated whether manipulating plant community composition affected the R_s response to warming. Results

demonstrated strong warming-related increases in R_s *in situ*, however there were no warming effects on microbial respiratory potential. Additionally, the warming treatment increased soil temperature and decreased soil water content significantly *in situ*, however the warming effect on R_s was greater than expected considering the impact it had on these abiotic factors. Thus, warming increased R_s more than simply by increasing soil temperature and reducing soil water content. Manipulating the plant community by removing the dominant species or removing biomass had no impact on R_s , nor did these treatments influence the impact of warming on R_s . This suggests that the warming-induced increase in R_s was independent of any influences on plant community composition. Similarly, removal treatments did not affect microbial respiratory potential, however there was a complex warming and removal interaction that influenced the decay constant of the soil labile C pool (k). Overall, the results from this study suggest that as there was no change in microbial respiratory potential, the observed increase in soil respiration *in situ* was largely an effect of altered plant activity in warmed plots.

4.1 Possible mechanisms leading to the warming-induced increase in soil respiration

Warming increased R_s *in situ* over the course of the sampling period from November 2017 to June 2018. This increase in CO_2 efflux observed from soils *in situ*, which encompasses the response of microbial (heterotrophic) respiration, as well as contributions from plant root and minor contributions from shoot (autotrophic) respiration, amounted to an average increase in soil C efflux of 28%. The observed increase of R_s in response to warming is in line with multiple other studies, although most of these focus on soils in the low- to mid-range of soil C stocks and in northern hemisphere locations (Lu et al., 2013; van Gestel et al., 2018). There are 5 possible mechanisms whereby R_s could have increased by warming: 1.) Increased temperature sensitivity of R_s ; 2.) Alteration of microbial community composition and function; 3.) Influence through change in plant community composition; 4.) Enhanced substrate supply through SOM and 5.) Plant induced alteration to soil microhabitat. The substantial R_s response to warming could be due to one or a combination of these processes and determining which were likely to be involved has significant ramifications for our ability to predict future soil C dynamics.

Commented [MN15]: R1
Further clarification of our use of soil respiration as opposed to ecosystem respiration

Commented [MN16]: R2
"Change to: There are 4 possible mechanisms whereby R_s could have increased (or similar)"
Text altered as suggested

4.1.1 Increased temperature sensitivity of R_s

One of the proposed mechanisms behind the increased R_s response to warming, and subsequent loss of soil C stores is an increase in the temperature sensitivity of R_s , i.e. increased decomposition of SOM (Kirschbaum, 1995). This response, mainly attributed to an increase in enzyme kinetics with temperature, is linked strongly to substrate availability (Davidson and Janssens, 2006). At Silver Plains, the overall significant increase in R_s rates from warmed plots *in situ* implied that the temperature sensitivity of R_s was higher under warming. The highest R_s rates were recorded during the growing season in spring and summer, suggesting primary productivity, microbial activity and environmental factors such as precipitation are likely to substantially influence respiration rates (Almagro et al., 2009). However, despite the strong dependence of R_s on soil water content and soil temperature, warmed plots had higher rates of C efflux from the soil under particular combinations of soil temperature and moisture (Section 3.1.5, Fig. 3). The restrictive effect of high soil water content and low soil temperature on R_s observed in this study is widely documented and due to the creation of anoxic conditions limiting microbial access to substrate (Schimel et al., 1994; Syed et al., 2006; Sierra et al., 2015). Hence the

observed effect of soil water content and soil temperature on R_s was anticipated, however the degree to which warming enhanced the response of R_s to temperature, was greater than expected. This observation could be explained by the greater effect of warming on air temperature than soil temperature at 5cm, thus considering 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)). Clearly, the influence of warming treatments on the soil temperature-depth profile is an area that requires further investigation. Interestingly, our results show that the degree of stimulation by warming increased as soil temperature increased, i.e., there was a greater warming-induced stimulation of C efflux when soil was warm than when it was cold (Fig. 2). This contrasts previous findings that indicate a greater warming effect on R_s at lower temperatures (Wang et al., 2014).

The large apparent increase in R_s observed *in situ* implied that warming possibly triggered an increase in microbial respiratory potential. Partitioning of R_s in incubation experiments allows the response of microbial respiration to warming to be observed under optimal conditions, controlling for soil water content and soil temperature. In stark contrast to the *in situ* observations, soil incubations revealed no differences in the temperature sensitivity of microbial respiration between warmed and ambient soil. There were no differences among treatments in the total C mineralisation rate measured under laboratory conditions, which would indicate that the ability of the soil microbial community to mineralise soil C was unchanged. This lack of any treatment effect was similar in winter and summer-collected soils, even though there was a strong seasonal effect on the CO_2 respiration rate in incubated soils. Winter soils emitted significantly less CO_2 than soils collected at the end of summer, a response attributed to decreased access to substrate as an effect of limited enzyme activity in cold temperatures (Suseela et al., 2012). Additionally, despite claims that the warming-induced increase in R_s is due to a strong, positive relationship between the average turnover time of labile C pools and mean annual air temperature (Trumbore et al., 1996), there was no difference in the size of the respired labile C pool (C_a) between warmed and ambient soils, or between seasons. Therefore, this suggests that the warming treatment did not increase the temperature sensitivity of labile C decomposition. Additionally, there were no warming or removal treatment effects on the decay constant of the stable C pool (Y_0), calculated from CO_2 emission rates late in the incubation period. This implies that stable C, which is chemically and physically protected (Schlesinger, 1997), was not sensitive to warming, a response that contrasts results obtained elsewhere (Leifeld and Fuhrer, 2005; Hartley and Ineson, 2008). Thus, lack of a warming effect indicates that warming-induced increases in labile, or stable C temperature sensitivity are not driving the R_s response to warming observed *in situ*.

Essentially, the incubation studies revealed that four years of experimental warming had not altered either the potential for microbial respiration or its inherent temperature response, as soils incubated at the same temperature respired more or less at the same rate, regardless of whether they were collected from warmed or ambient plots. These results indicate that the warming-induced stimulation of R_s *in situ* was not due to changes in the inherent temperature response of microbial respiration. Considering the soil incubation experiment decoupled microbial respiration from plant activity and particularly C inputs, the lack of a warming treatment effect on C emissions in the incubation experiments is evidence that plants play a large role in the respiration response.

Commented [MN17]: R1

"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)).

475

4.1.2 Alteration of microbial community composition and function

The role of microbial community composition and function in the respiration response to warming is complex as it encompasses multiple possible factors that could lead to changes in respiration rates (Bargett & Caruso 2020; Karhu et al., 2014). These factors include changes in individual microbial physiology, whereby temperature affects the rate at which microbes can take up and metabolise substrate (Hopkins et al., 2014), genetic changes within species, indicating possible adaptation to specific environmental conditions (Karhu et al., 2014), competition between species (Sheik et al., 2011), and changes in community composition to support taxa that thrive in warmer or drought prone conditions (Bardgett & Caruso 2020). From the R_s response observed *in situ*, a shift in microbial community composition and function seems to be a plausible driving factor. However, laboratory incubations of soil indicated the temperature response of respiratory potential in this study did not differ due to an increase in temperature in the warming treatment. If there were changes to the microbial community, they did not appear to have a role in altering the response of respiration to warming, implying the R_s response was not due to a shift in microbial community and function.

490

4.1.3 Influence through change in plant species community composition

Plant community compositional change drives ecosystem responses to global changes, particularly when it involves shifts in the dominance or abundance of plant functional types (Bret-Harte et al., 2008). This is particularly true with R_s and global warming, as warming-related changes in plant functional types, and hence the resources they input to the soil, are highly likely to occur (Saleska et al., 2002). By investigating the effects of dominant species removal, random biomass removal, and warming on the response of respiration, there is scope to gain insight into future ecosystem dynamics under a changing climate. Removal of a dominant species from an ecosystem has promoted species diversity and altered ecosystem function, implying dominants reduce the establishment of other species (Wardle et al., 1999). Metcalfe et al., (2011) highlight the significant role that functional traits of the dominant species hold on many soil processes, including decomposition and respiration and hence one would expect to observe these effects in this study. Plants modify local soil conditions through root exudations of hormones, sugars, phenolics and amino acids, essentially structuring the rhizosphere microbial community composition. This means that changes in plant community composition have the potential to affect R_s and thus ecosystem functioning (Van Nuland et al., 2016), and hence the motive to investigate how R_s responds to the combination of warming and manipulated plant community composition. Results from Silver Plains demonstrate neither removal of the dominant plant species nor random removal of biomass had any effect on R_s or the temperature response of R_s *in situ*, and very little effect on microbial respiratory potential. This suggests that, in line with previous studies, temperature had greater control on R_s than variation in plant community composition (Duval and Radu, 2018).

510 Multi-dimensional scaling analysis of plant community composition indicated that the removal of the dominant species did tend to shift community composition, but this change was not substantial in comparison to the natural

Commented [MN18]: R2

"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).

variation in community composition within the control plots (Fig. S1). The plant community composition in plots subjected to random biomass removal was similar to that of control plots. Furthermore, the removal of the dominant species did not appear to cause any functional shift within this species-rich community, suggesting it may have been replaced by a functionally-similar species, or there was a compensatory response by functionally-different species (Bret-Harte et al., 2008). Thus, the removal treatments had relatively modest influences on the plant community composition and function, potentially explaining why R_s was similarly unresponsive to the treatments. Nevertheless, the plant community composition did differ considerably among plots across the experiment (Fig. S1). Differences in plant chemistry, morphology and physiology affect the quantity and quality of root and leaf litter, leading to changes in SOM decomposition rates, shifts in microbial respiratory potential and community structure (Van Nuland et al., 2016). However, the consistent response of R_s *in situ* to the warming treatment indicates that warming effects were similar across the variety of plant community composition within this ecosystem.

Despite the absence of an effect on R_s *in situ* from manipulating species composition, microbial respiration dynamics indicated that warming and biomass removal (both random and dominant removal treatments) reduced the intrinsic decay constant of the labile C pool (k) in summer soil. Interestingly, biomass removal had the opposite effect in ambient plots. In a previous clipping experiment, which is representative of biomass removal, a decrease in R_s due to clipping was explained as relocation of assimilates to shoots, reduction in the supply of photosynthates to roots, and thus decreased root respiration (Zhou et al., 2010). Hence there is likely to be less available substrate under warming and biomass removal scenarios, and k is therefore lower. Considering this, the interactive effect of warming and biomass removal on k is complex and requires further investigation to explore the mechanistic basis behind the response. The absence of an influence on R_s through variation in plant community composition suggests this mechanism is not driving the warming-induced R_s response to warming.

4.1.4 Enhanced substrate supply

SOM

As SOM forms mainly from plant litter, warming related increases in both above and belowground primary productivity suggest supply of SOM will be greater under warming (Rustad et al., 2001; Lin et al., 2010; Wu et al., 2011), at least in systems that are not water limited. Additionally, experimental warming often increases leaf-drop, root-turnover and the subsequent decomposition of leaf and root litter (Lu et al., 2013), with the combined effects of warming and higher C inputs on respiration rates reported to be greater than the impact of either factor in isolation (Hopkins et al., 2014). Root and leaf litter have fast turnover times, implying they represent a major source of C for microbial decomposition. Therefore, 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 increasing formation of stable SOM through microbial decomposition products (Sokol et al., 2019). Considering this, an increase in substrate supply seems like a conceivable explanation for the increase in R_s observed *in situ*. However, incubation experiments indicated no influence of warming on the total amount of C between warmed and ambient plots, or on the size of the labile pool (C_o), or total C respired. This indicates that substrate supply and availability from plant biomass is similar in warmed and ambient plots. Previous

Commented [MN19]: R2

"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.

Commented [MN20]: R1

"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).

550 investigations suggest that despite warming-related increases in litter quantity, enhanced respiration due to
increased labile C concentration in soils is likely to offset additional C inputs (Lu et al., 2013), meaning changes
to both inputs and losses of soil C could balance each other. Interestingly neither *in situ* R_s , nor total soil C or Ca
was affected by plant community composition manipulations, suggesting substrate supply and availability was
similar regardless of warming and removal treatments. This result contrasts with those from previous clipping
555 experiments that demonstrated that biomass removal limits substrate supply (Wan and Luo, 2003; Xue et al.,
2016). Overall this suggests that increased substrate supply through SOM is not a driving mechanism behind the
warming-induced increase in R_s observed *in situ* although specific tests of this mechanism, such as through the
use of stable isotope tracing, would be required to be confident.

560 4.1.5 Plant induced alteration to soil microhabitat

The final mechanism that could be driving the warming-induced increases in R_s are plant induced alterations to
the soil microhabitat. In this study, as in most, soil for incubations was not analysed as intact soil cores, rather
being sieved and homogenised, altering the microhabitat conditions. This is potentially problematic, as it is
assuming that rhizosphere processes, including contributions from mycorrhizal fungi are not influencing the
565 overall R_s response. Previous studies have demonstrated the important role roots play in stabilising SOM
(Hinsinger et al., 2009), with disturbed soils having a lower capacity to protect SOM due to mechanical disruption
of macroaggregates, and hence C is more readily decomposed by microorganisms (Beare et al., 1994). Many, and
potentially all, of the plant species at the site have associations with arbuscular mycorrhizae, which are known to
increase SOM formation both directly as well as through their influence on soil aggregation (Rillig et al., 2001).
570 Thus, our incubations would have removed this important contribution, reducing SOM formation and potentially
increasing C mineralisation rates. Additionally, macrofauna such as earthworms and nematodes play an important
role in the early stages of SOM decomposition (Wardle et al., 2004), and therefore the absence of these species
from the incubations could also have influenced the rates of C efflux. Considering this, it is possible that through
homogenisation of soil in incubation studies, soil C dynamics and decomposition rates are confounded by
575 disturbances to the soil microhabitat.

4.2 Implications

580 Large C stocks within this type of peaty habitat are important for the global C cycle, thus understanding potential
losses are immensely important for the global C budget. Previous studies on the response of R_s to warming have
been largely centred around northern hemisphere sites, and with that there has been large unexplained variability
in the response (van Gestel et al., 2018). This implies that the mechanisms behind the R_s response to warming are
poorly characterised. Our results indicate that warming-related increases in CO_2 efflux from C-rich soils in grassy
peatlands are expected in the future, however microbial respiratory potential is not the driving factor, and thus
585 there is a strong link to plant activity and C inputs. Moreover, the results indicate that the impact of warming on
soil CO_2 efflux is strongly dependent upon both soil temperature and moisture conditions, improving the

Commented [MN21]: R1

"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 R_s , 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 if 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).

Commented [MN22]: R1

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

Text altered as suggested

confidence that current and future soil CO₂ efflux can be modelled from these variables. However, the lack of observations in certain combinations of soil temperature and moisture mean that predictions using the models presented here should be limited to the observed range. Future work should test the generality of these models in previously unobserved combinations of soil moisture and temperature.

Predictions regarding future climate conditions require a more comprehensive mechanistic understanding of temperature and decomposition relationships, especially considering the global variation in these relationships. Further investigation into the role of inputs is required, as warming could be driving increases in inputs, thereby balancing the accelerated C efflux and preventing net loss of C from soils. Alternatively, warming could lead to depletion of huge stores of C. This effect is no doubt subject to great variation depending on the ecosystem and hence the necessity to examine the response, accounting for heterogeneity in soil and vegetation types worldwide. Most importantly, this study revealed that C inputs through root exudates and root respiration were the two mechanisms most likely to be driving the R_s response to warming. Thus, more research into the influence of root exudates and root respiration on R_s, particularly under warming, will provide a more comprehensive insight to the R_s response. Ultimately, thorough investigations into the whole ecosystem C exchange is required to advance understanding into how warming will affect rates of inputs and outputs.

The increase in R_s in response to warming observed here is in line with previous experimental warming studies, although few have been conducted in C-rich soils. Thus, the results from this study contribute directly to a field of knowledge that is currently underrepresented. Despite a strong warming effect, there appear to be no significant effects of plant community manipulation, suggesting that warming exerts more of an influence on CO₂ efflux from soils than differences in plant communities. Additionally, results suggest that the microbial respiratory potential in this system is not altered by experimental warming and hence cannot be decoupled from plant activity if we are to enhance our ability to predict C cycling dynamics in a warmer climate. Current findings suggest warming is likely to trigger a positive feedback cycle whereby increases in global temperatures will enhance CO₂ efflux from soils, subsequently warming the earth further. As the huge C stocks in the soil have the potential to either amplify or attenuate global warming, the impacts of climate change on soil C dynamics require urgent investigation. A more comprehensive representation of ecosystem C exchange is needed, as well as the mechanisms involved, if we want to decrease CO₂ efflux from soils and ensure these huge C sinks are stabilised, or potentially even increased such that the biosphere can sequester more atmospheric CO₂ and help to stabilise the climate.

615

Author contribution: Marion Nyberg and Mark Hovenden were responsible for conceptualization and project administration. Marion Nyberg performed data acquisition and curation, formal analysis and writing. Mark Hovenden developed the methodology and provided supervision.

620 **Competing interest**

The authors declare that they have no conflict of interest

Acknowledgements

We would like to thank Meagan Porter and Rose Brinkhoff for help setting up the experiment and the Tasmanian Land Conservancy for permission to use the land for the experiment. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

- Almagro, M., López, J., Querejeta, J. I., and Martínez-Mena, M.: Temperature dependence of soil CO₂ efflux is strongly modulated by seasonal patterns of moisture availability in a Mediterranean ecosystem, *Soil Biology and Biochemistry*, 41, 594-605, <https://doi.org/10.1016/j.soilbio.2008.12.021>, 2009.
- Bardgett, R. D. and Caruso, T.: Soil microbial community responses to climate extremes: resistance, resilience and transitions to alternative states, *Philosophical Transactions of the Royal Society B: Biological Sciences*, 375(1794), 20190112, doi:[10.1098/rstb.2019.0112](https://doi.org/10.1098/rstb.2019.0112), 2020.
- Beare, M. H., Hendrix, P. F., Cabrera, M. L., and Coleman, D. C.: Aggregate-protected and unprotected organic matter pools in conventional- and no-tillage soils, *Soil Science Society of America Journal*, 58, 10.2136/sssaj1994.03615995005800030021x, 1994.
- Bengtson, P., Barker, J., and Grayston, S. J.: Evidence of a strong coupling between root exudation, C and N availability, and stimulated SOM decomposition caused by rhizosphere priming effects, *Ecology and Evolution*, 2, 1843-1852, 10.1002/ece3.311, 2012.
- Bond-Lamberty, B., Bailey, V. L., Chen, M., Gough, C. M., and Vargas, R.: Globally rising soil heterotrophic respiration over recent decades, *Nature*, 560, 80-83, 10.1038/s41586-018-0358-x, 2018.
- Bond-Lamberty, B., and Thomson, A.: Temperature-associated increases in the global soil respiration record, *Nature*, 464, 579-582, 10.1038/nature08930, 2010.
- Boone, R. D., Nadelhoffer, K. J., Canary, J. D., and Kaye, J. P.: Roots exert a strong influence on the temperature sensitivity of soil respiration, *Nature*, 396, 570-572, 10.1038/25119, 1998.
- Bracho, R., Natali, S., Pegoraro, E., Crummer, K. G., Schädel, C., Celis, G., Hale, L., Wu, L., Yin, H., Tiedje, J. M., Konstantinidis, K. T., Luo, Y., Zhou, J., and Schuur, E. A. G.: Temperature sensitivity of organic matter decomposition of permafrost-region soils during laboratory incubations, *Soil Biology and Biochemistry*, 97, 1-14, 10.1016/j.soilbio.2016.02.008, 2016.
- Bradford, M. A., Davies, C. A., Frey, S. D., Maddox, T. R., Melillo, J. M., Mohan, J. E., Reynolds, J. F., Treseder, K. K., and Wallenstein, M. D.: Thermal adaptation of soil microbial respiration to elevated temperature, *Ecology Letters*, 11, 1316-1327, 10.1111/j.1461-0248.2008.01251.x, 2008.
- Bret-Harte, M. S., Mack, M. C., Goldsmith, G. R., Sloan, D. B., Demarco, J., Shaver, G. R., Ray, P. M., Biesinger, Z., and Chapin, F. S.: Plant functional types do not predict biomass responses to removal and fertilization in Alaskan tussock tundra, *Journal of Ecology*, 96, 713-726, 10.1111/j.1365-2745.2008.01378.x, 2008.

- Bridgman, S. D., Pastor, J., Dewey, B., Weltzin, J. F., and Updegraff, K.: Rapid carbon response of peatlands to climate change, *Ecology*, 89, 3041-3048, 10.1890/08-0279.1, 2008.
- 660 Cai, T., Flanagan, L. B., and Syed, K. H.: Warmer and drier conditions stimulate respiration more than photosynthesis in a boreal peatland ecosystem: analysis of automatic chambers and eddy covariance measurements, *Plant, Cell & Environment*, 33, 394-407, 10.1111/j.1365-3040.2009.02089.x, 2010.
- Canadell, J. G., Kirschbaum, M. U. F., Kurz, W. A., Sanz, M.-J., Schlamadinger, B., and Yamagata, Y.: Factoring out natural and indirect human effects on terrestrial carbon sources and sinks, *Environmental Science & Policy*, 10, 370-384, <https://doi.org/10.1016/j.envsci.2007.01.009>, 2007.
- 665 Carey, J. C., Tang, J., Templer, P. H., Kroeger, K. D., Crowther, T. W., Burton, A. J., Dukes, J. S., Emmett, B., Frey, S. D., Heskel, M. A., Jiang, L., Machmuller, M. B., Mohan, J., Panetta, A. M., Reich, P. B., Reinsch, S., Wang, X., Allison, S. D., Bamminger, C., Bridgman, S., Collins, S. L., de Dato, G., Eddy, W. C., Enquist, B. J., Estiarte, M., Harte, J., Henderson, A., Johnson, B. R., Larsen, K. S., Luo, Y., Marhan, S., Melillo, J. M.,
- 670 Penuelas, J., Pfeifer-Meister, L., Poll, C., Rastetter, E., Reinmann, A. B., Reynolds, L. L., Schmidt, I. K., Shaver, G. R., Strong, A. L., Suseela, V., and Tietema, A.: Temperature response of soil respiration largely unaltered with experimental warming, *Proceedings of the National Academy of Science U S A*, 113, 13797-13802, 10.1073/pnas.1605365113, 2016.
- Chen, J., Luo, Y., Xia, J., Wilcox, K. R., Cao, J., Zhou, X., Jiang, L., Niu, S., Estera, K. Y., Huang, R., Wu, F.,
- 675 Hu, T., Liang, J., Shi, Z., Guo, J., and Wang, R.-W.: Warming effects on ecosystem carbon fluxes are modulated by plant functional types, *Ecosystems*, 20, 515-526, 10.1007/s10021-016-0035-6, 2016.
- Chou, W. W., Silver, W. L., Jackson, R. D., Thompson, A. W., and Allen-Diaz, B.: The sensitivity of annual grassland carbon cycling to the quantity and timing of rainfall, *Global Change Biology*, 14, 1382-1394, 10.1111/j.1365-2486.2008.01572.x, 2008.
- 680 Davidson, E. A., and Janssens, I. A.: Temperature sensitivity of soil carbon decomposition and feedbacks to climate change, *Nature*, 440, 165-173, 10.1038/nature04514, 2006.
- Davidson, E. A., Trumbore, S. E., and Amundson, R.: Soil warming and organic carbon content, *Nature*, 408, 789-790, 10.1038/35048672, 2000.
- Deyn, G. B. D., Cornelissen, J. H. C., and Bardgett, R. D.: Plant functional traits and soil carbon sequestration
- 685 in contrasting biomes, *Ecology Letters*, 11, 516-531, 10.1111/j.1461-0248.2008.01164.x, 2008.
- Duval, T. P., and Radu, D. D.: Effect of temperature and soil organic matter quality on greenhouse-gas production from temperate poor and rich fen soils, *Ecological Engineering*, 114, 66-75, 10.1016/j.ecoleng.2017.05.011, 2018.
- Eliasson, P. E., McMurtrie, Pepper, D. A., Stromgren, M., Linder, S., and Agren, G. I.: The response of
- 690 heterotrophic CO₂ flux to soil warming, *Global Change Biology*, 11, 167-181, 2005.
- Epstein, H. E., Burke, I. C. and Lauenroth, W. K.: Response of the shortgrass steppe to changes in rainfall seasonality, *Ecosystems*, 2(2), 139-150, 1999.

- Fischer, H., Eckhardt, K.-U., Meyer, A., Neumann, G., Leinweber, P., Fischer, K., and Kuzyakov, Y.: Rhizodeposition of maize: short-term carbon budget and composition, *Journal of Plant Nutrition and Soil Science*, 173, 67-79, 10.1002/jpln.200800293, 2010.
- 695 Fisk, M. C., Ruether, K. F. and Yavitt, J. B.: Microbial activity and functional composition among northern peatland ecosystems, *Soil Biology and Biochemistry*, 35(4), 591-602, doi:[10.1016/S0038-0717\(03\)00053-1](https://doi.org/10.1016/S0038-0717(03)00053-1), 2003.
- Fontaine, S., Bardoux, G., Abbadie, L., and Mariotti, A.: Carbon input to soil may decrease soil carbon content, *Ecology Letters*, 7, 314-320, 10.1111/j.1461-0248.2004.00579.x, 2004.
- 700 Gillooly, J. F., Brown, J. H., West, G. B., Savage, V. M., and Charnov, E. L.: Effects of size and temperature on metabolic rate, *Science*, 293, 2248, 2001.
- Hartley, I. P., and Ineson, P.: Substrate quality and the temperature sensitivity of soil organic matter decomposition, *Soil Biology and Biochemistry*, 40, 1567-1574, 10.1016/j.soilbio.2008.01.007, 2008.
- 705 Heimann, M., and Reichstein, M.: Terrestrial ecosystem carbon dynamics and climate feedbacks, *Nature*, 451, 289-292, 10.1038/nature06591, 2008.
- Hinsinger, P., Bengough, A. G., Vetterlein, D., and Young, I. M.: Rhizosphere: biophysics, biogeochemistry and ecological relevance, *Plant and Soil*, 321, 117-152, 10.1007/s11104-008-9885-9, 2009.
- Hogberg, P., and Read, D. J.: Towards a more plant physiological perspective on soil ecology, *Trends in Ecology and Evolution*, 21, 548-554, 2006.
- 710 Hollister, R. D., Webber, P. J., Nelson, F. E. and Tweedie, C. E.: Soil Thaw and Temperature Response to Air Warming Varies by Plant Community: Results from an Open-top Chamber Experiment in Northern Alaska, Arctic, Antarctic, and Alpine Research, 38(2), 206-215, doi:[10.1657/1523-0430\(2006\)38\[206:STATRT\]2.0.CO;2](https://doi.org/10.1657/1523-0430(2006)38[206:STATRT]2.0.CO;2), 2006.
- 715 Hopkins, F. M., Filley, T. R., Gleixner, G., Lange, M., Top, S. M., and Trumbore, S. E.: Increased belowground carbon inputs and warming promote loss of soil organic carbon through complementary microbial responses, *Soil Biology and Biochemistry*, 76, 57-69, 10.1016/j.soilbio.2014.04.028, 2014.
- Jackson, R. B., Lajtha, K., Crow, S. E., Hugelius, G., Kramer, M. G., and Piñeiro, G.: The ecology of soil carbon: pools, vulnerabilities, and biotic and abiotic controls, *Annual Review of Ecology, Evolution, and Systematics*, 48, 419-445, 10.1146/annurev-ecolsys-112414-054234, 2017.
- 720 Karhu, K., Auffret, M. D., Dungait, J. A., Hopkins, D. W., Prosser, J. I., Singh, B. K., Subke, J. A., Wookey, P. A., Agren, G. I., Sebastia, M. T., Gouriveau, F., Bergkvist, G., Meir, P., Nottingham, A. T., Salinas, N., and Hartley, I. P.: Temperature sensitivity of soil respiration rates enhanced by microbial community response, *Nature*, 513, 81-84, 10.1038/nature13604, 2014.
- 725 Kirschbaum, M. U. F.: The temperature dependence of soil organic matter decomposition, and the effect of global warming on soil organic C storage, *Soil Biology and Biochemistry*, 27, 753-760, [https://doi.org/10.1016/0038-0717\(94\)00242-S](https://doi.org/10.1016/0038-0717(94)00242-S), 1995.

- Leifeld, J., and Fuhrer, J.: The temperature response of CO₂ production from bulk soils and soil fractions is related to soil organic matter quality, *Biogeochemistry*, 75, 433-453, 10.1007/s10533-005-2237-4, 2005.
- 730 Li, G., Kim, S., Han, S., Chang, H., and Son, Y.: Effect of soil moisture on the response of soil respiration to open-field experimental warming and precipitation manipulation, *Forests*, 8, 56, 10.3390/f8030056, 2017.
- Lin, D., Xia, J., and Wan, S.: Climate warming and biomass accumulation of terrestrial plants: a meta-analysis, *New Phytologist*, 188, 187-198, 10.1111/j.1469-8137.2010.03347.x, 2010.
- 735 Lu, M., Zhou, X., Yang, Q., Li, H., Luo, Y., Fang, C., Chen, J., Yang, X., and Li, B.: Responses of ecosystem carbon cycle to experimental warming: a meta-analysis, *Ecology*, 94, 726-738, 10.1890/12-0279.1, 2013.
- Luo, Y.: Terrestrial carbon-cycle feedback to climate warming, *Annual Review of Ecology, Evolution, and Systematics*, 38, 683-712, 10.1146/annurev.ecolsys.38.091206.095808, 2007.
- Luo, Y., Wan, S. Q., Hui, D. F., and Wallace, L. L.: Acclimatization of soil respiration to warming in a tall grass prairie, *Nature*, 413, 622-625, 10.1038/35098065, 2001.
- 740 Melillo, J. M., Frey, S. D., DeAngelis, K. M., Werner, W. J., Bernard, M. J., Bowles, F. P., Pold, G., Knorr, M. A., and Grandy, A. S.: Long-term pattern and magnitude of soil carbon feedback to the climate system in a warming world, *Science*, 358, 101, 2017.
- Metcalfe, D. B., Fisher, R. A., and Wardle, D. A.: Plant communities as drivers of soil respiration: pathways, mechanisms, and significance for global change, *Biogeosciences*, 8, 2047-2061, 10.5194/bg-8-2047-2011, 2011.
- 745 Millar, D. J., Cooper, D. J., Dwire, K. A., Hubbard, R. M., and von Fischer, J.: Mountain peatlands range from CO₂ sinks at high elevations to sources at low elevations: Implications for a changing climate, *Ecosystems*, 20, 416-432, 10.1007/s10021-016-0034-7, 2016.
- Moinet, G. Y. K., Hunt, J. E., Kirschbaum, M. U. F., Morcom, C. P., Midwood, A. J., and Millard, P.: The temperature sensitivity of soil organic matter decomposition is constrained by microbial access to substrates, *Soil Biology and Biochemistry*, 116, 333-339, 10.1016/j.soilbio.2017.10.031, 2018.
- 750 Ontl, T. A., and Schulte, L. A.: Soil carbon storage *Nature Education Knowledge*, 3, 2012.
- Parton, W. J., Schimel, D. S., Cole, C. V. and Ojima, D. S.: Analysis of Factors Controlling Soil Organic Matter Levels in Great Plains Grasslands, *Soil Science Society of America Journal*, 51(5), 1173-1179, doi:[10.2136/sssaj1987.03615995005100050015x](https://doi.org/10.2136/sssaj1987.03615995005100050015x), 1987.
- 755 Pendall, E., Osanai, Y. U. I., Williams, A. L., and Hovenden, M. J.: Soil carbon storage under simulated climate change is mediated by plant functional type, *Global Change Biology*, 17, 505-514, 10.1111/j.1365-2486.2010.02296.x, 2011.
- Phillips, R. P., Finzi, A. C., and Bernhardt, E. S.: Enhanced root exudation induces microbial feedbacks to N cycling in a pine forest under long-term CO₂ fumigation, *Ecology Letters*, 14, 187-194, 10.1111/j.1461-0248.2010.01570.x, 2011.
- 760

- Rillig, M. C., Wright, S. F., Nichols, K. A., Schmidt, W. F. and Torn, M. S.: Large contribution of arbuscular mycorrhizal fungi to soil carbon pools in tropical forest soils, *Plant and Soil*, 233(2), 167–177, doi:[10.1023/A:1010364221169](https://doi.org/10.1023/A:1010364221169), 2001.
- 765 Rustad, L., Campbell, J., Marion, G., Norby, R., Mitchell, M., Hartley, A., Cornelissen, J., Gurevitch, J., and Gete, N.: A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming, *Oecologia*, 126, 543-562, 10.1007/s004420000544, 2001.
- Saleska, S. R., Shaw, M. R., Fischer, M. L., Dunne, J. A., Still, C. J., Holman, M. L., and Harte, J.: Plant community composition mediates both large transient decline and predicted long-term recovery of soil carbon under climate warming, *Global Biogeochemical Cycles*, 16, 2002.
- 770 Schimel, D. S., Braswell, B. H., Holland, E. A., McKeown, R., Ojima, D. S., Painter, T. H., Parton, W. J., and Townsend, A. R.: Climatic, edaphic, and biotic controls over storage and turnover of carbon in soils, *Global Biogeochemical Cycles*, 8, 279-293, 10.1029/94GB00993, 1994.
- Schlesinger, W. H.: *Biogeochemistry: an analysis of global change*, Second edition ed., Academic Press, California, 1997.
- 775 Schlesinger, W. H., and Andrews, J. A.: Soil respiration and the global carbon cycle, *Biogeochemistry*, 48, 7-20, 2000.
- Shahzad, T., Chenu, C., Genet, P., Barot, S., Perveen, N., Mougín, C., and Fontaine, S.: Contribution of exudates, arbuscular mycorrhizal fungi and litter depositions to the rhizosphere priming effect induced by grassland species, *Soil Biology and Biochemistry*, 80, 146-155, 10.1016/j.soilbio.2014.09.023, 2015.
- 780 Sheik, C. S., Beasley, W. H., Elshahed, M. S., Zhou, X., Luo, Y., and Krumholz, L. R.: Effect of warming and drought on grassland microbial communities, *The ISME Journal*, 5, 1692-1700, 10.1038/ismej.2011.32, 2011.
- Sierra, C. A., Trumbore, S. E., Davidson, E. A., Vicca, S., and Janssens, I.: Sensitivity of decomposition rates of soil organic matter with respect to simultaneous changes in temperature and moisture, *Journal of Advances in Modeling Earth Systems*, 7, 335-356, 10.1002/2014ms000358, 2015.
- 785 Song, B., Niu, S., Luo, R., Luo, Y., Chen, J., Yu, G., Olejnik, J., Wohlfahrt, G., Kiely, G., Noormets, A., Montagnani, L., Cescatti, A., Magliulo, V., Law, B. E., Lund, M., Varlagin, A., Raschi, A., Peichl, M., Nilsson, M. B., and Merbold, L.: Divergent apparent temperature sensitivity of terrestrial ecosystem respiration, *Journal of Plant Ecology*, 7, 419-428, 10.1093/jpe/rtu014, 2014.
- 790 Suseela, V., Conant, R. T., Wallenstein, M. D., and Dukes, J. S.: Effects of soil moisture on the temperature sensitivity of heterotrophic respiration vary seasonally in an old-field climate change experiment, *Global Change Biology*, 18, 336-348, 10.1111/j.1365-2486.2011.02516.x, 2012.
- Syed, K. H., Flanagan, L. B., Carlson, P. J., Glenn, A. J., and Van Gaalen, K. E.: Environmental control of net ecosystem CO₂ exchange in a treed, moderately rich fen in northern Alberta, *Agricultural and Forest Meteorology*, 140, 97-114, 10.1016/j.agrformet.2006.03.022, 2006.

- 795 Trumbore, S. E., Chadwick, O. A., and Amundson, R.: Rapid exchange between soil carbon and atmospheric carbon dioxide driven by temperature change, *Science*, 272, 393, 1996.
- van der Wal, A., and de Boer, W.: Dinner in the dark: Illuminating drivers of soil organic matter decomposition, *Soil Biology and Biochemistry*, 105, 45-48, 10.1016/j.soilbio.2016.11.006, 2017.
- van Gestel, N., Shi, Z., van Groenigen, K. J., Osenberg, C. W., Andresen, L. C., Dukes, J. S., Hovenden, M. J.,
800 Luo, Y., Michelsen, A., Pendall, E., Reich, P. B., Schuur, E. A. G., and Hungate, B. A.: Predicting soil carbon loss with warming, *Nature*, 554, E4-E5, 10.1038/nature25745, 2018.
- Van Nuland, M. E., Wooliver, R. C., Pfennigwerth, A. A., Read, Q. D., Ware, I. M., Mueller, L., Fordyce, J. A., Schweitzer, J. A., Bailey, J. K., and Fox, C.: Plant-soil feedbacks: connecting ecosystem ecology and evolution, *Functional Ecology*, 30, 1032-1042, 10.1111/1365-2435.12690, 2016.
- 805 Wan, S., Hui, D., Wallace, L., and Luo, Y.: Direct and indirect effects of experimental warming on ecosystem carbon processes in a tallgrass prairie, *Global Biogeochemical Cycles*, 19, n/a-n/a, 10.1029/2004gb002315, 2005.
- Wan, S., and Luo, Y.: Substrate regulation of soil respiration in a tallgrass prairie: results of a clipping and shading experiment, *Global Biogeochemical Cycles*, 17, 10.1029/2002GB001971, 2003.
- 810 Wang, J., Sun, J., Xia, J., He, N., Li, M., and Niu, S.: Soil and vegetation carbon turnover times from tropical to boreal forests, *Functional Ecology*, 32, 71-82, 10.1111/1365-2435.12914, 2017.
- Wang, X., Liu, L., Piao, S., Janssens, I. A., Tang, J., Liu, W., Chi, Y., Wang, J., and Xu, S.: Soil respiration under climate warming: differential response of heterotrophic and autotrophic respiration, *Global Change Biology*, 20, 3229-3237, 10.1111/gcb.12620, 2014.
- 815 Wardle, D. A., Bardgett, R. D., Klironomos, J. N., Setälä, H., van der Putten, W. H., and Wal, D. H.: Ecological linkages between aboveground and belowground biota, *Science*, 304, 1629-1633, 10.1126/science.1094875, 2004.
- Wardle, D. A., Bonner, K. I., Barker, G. M., Yeates, G. W., Nicholson, K. S., Bardgett, R. D., Watson, R. N., and Ghani, A.: Plant removals in perennial grassland: vegetation dynamics, decomposers, soil biodiversity, and
820 ecosystem properties, *Ecological Monographs*, 69, 535-568, 1999.
- Wu, Z., Dijkstra, P., Koch, G. W., Peñuelas, J., and Hungate, B. A.: Responses of terrestrial ecosystems to temperature and precipitation change: a meta-analysis of experimental manipulation, *Global Change Biology*, 17, 927-942, 10.1111/j.1365-2486.2010.02302.x, 2011.
- Xue, K., Yuan, M. M., Xie, J., Li, D., Qin, Y., Hale, L. E., Wu, L., Deng, Y., He, Z., Van Nostrand, J. D., Luo,
825 Y., Tiedje, J. M., and Zhou, J.: Annual removal of aboveground plant biomass alters soil microbial responses to warming, *MBio*, 7, 10.1128/mBio.00976-16, 2016.
- Yin, H., Li, Y., Xiao, J., Xu, Z., Cheng, X., and Liu, Q.: Enhanced root exudation stimulates soil nitrogen transformations in a subalpine coniferous forest under experimental warming, *Global Change Biology*, 19, 2158-2167, 10.1111/gcb.12161, 2013.

- 830 Zhang, N., Liu, W., Yang, H., Yu, X., Gutknecht, J. L., Zhang, Z., Wan, S., and Ma, K.: Soil microbial responses to warming and increased precipitation and their implications for ecosystem C cycling, *Oecologia*, 173, 1125-1142, 10.1007/s00442-013-2685-9, 2013.
- Zhou, X., Sherry, R. A., An, Y., Wallace, L. L., and Luo, Y.: Main and interactive effects of warming, clipping, and doubled precipitation on soil CO₂ efflux in a grassland ecosystem, *Global Biogeochemical Cycles*, 20, 10.1029/2005GB002526, 2006.
- 835 Zhou, Y., Li, M.-H., Cheng, X.-B., Wang, C.-G., Fan, A. N., Shi, L.-X., Wang, X.-X., and Han, S.: Soil respiration in relation to photosynthesis of *Quercus mongolica* trees at elevated CO₂, *PLOS ONE*, 5, e15134, 10.1371/journal.pone.0015134, 2010.
- Zhu, B., and Cheng, W.: Rhizosphere priming effect increases the temperature sensitivity of soil organic matter decomposition, *Global Change Biology*, 17, 2172-2183, 10.1111/j.1365-2486.2010.02354.x, 2011.
- 840

845

850

855

860

