Soil respiration response to decade-long warming modulated by soil moisture in a boreal forest

🔴 Check for updates

Guopeng Liang ●[1] ✉, Artur Stefanski ●[1], William C. Eddy[2,3], Raimundo Bermudez ●[1], Rebecca A. Montgomery[1], Sarah E. Hobbie ●[2], Roy L. Rich ●[1,4] & Peter B. Reich ●[1,5,6] ✉

The effects of long-term climate warming on soil respiration and its drivers remain unclear in forests, which store approximately 40% of global soil carbon. Here we conducted a climate change experiment for 13 years in forest plots planted with tree juveniles at two southern boreal forest sites. Treatments included simultaneous above- and below-ground warming (ambient, +1.7 °C and +3.3 °C) under different rainfall scenarios (100% and 60% of summer rainfall) and contrasting overstory canopy openness (open and closed). Soil respiration increased by 7% and 17% under +1.7 °C and +3.3 °C warming, respectively, averaged across all sites, treatments and years. These increases in respiration were higher than impacts per degree warming of the only two prior long-term, but soil-only, forest warming experiments. Moreover, warming effects on soil respiration varied significantly over time. Under almost all conditions, moist soil exhibited a greater increase in respiration in response to warming than dry soil. Our results suggest that a realistic range of anticipated conditions, including both above- and below-ground temperature and moisture, should be accounted for when predicting warming effects on soil respiration.

Soils store more carbon (C) than plants and the atmosphere combined[1]. Therefore, a small increase in soil respiration caused by climate warming could substantially elevate the atmospheric CO_2 concentration and hence accelerate future global warming[2–5]. Although climate warming increases both above- and below-ground temperatures in the real world[6], no long-term (for example, >10 years) open-air studies have been conducted to determine the effects of simultaneous above- and below-ground warming on soil respiration and associated drivers in tree-dominated communities. The lack of relevant long-term results from ecologically realistic experiments could impact the accuracy of predictions of global C cycling under future climate change. Yet such predictions are necessary for improved future C cycle projections, given that forests store approximately 40% of global soil C[7]. Herein, we report on a field experiment conducted from 2009 to 2021 at two southern boreal forest sites in northern Minnesota, USA, that included combined manipulation of warming, canopy condition and rainfall amount.

Given that global warming can increase evapotranspiration more than precipitation[8] and that future summer rainfall may be reduced in boreal forests[8–10], soil water deficits in boreal forests are likely in the future[9–11]. Rainfall reduction can reduce soil microbial activity and plant

[1]Department of Forest Resources, University of Minnesota, St. Paul, MN, USA. [2]Department of Ecology, Evolution and Behavior, University of Minnesota, St. Paul, MN, USA. [3]Institute for Sustainability, Energy, and Environment, University of Illinois at Urbana-Champaign, Urbana, IL, USA. [4]Smithsonian Environmental Research Center, Edgewater, MD, USA. [5]Hawkesbury Institute for the Environment, Western Sydney University, Penrith, New South Wales, Australia. [6]Institute for Global Change Biology and School for Environment and Sustainability, University of Michigan, Ann Arbor, MI, USA. ✉e-mail: guopliang@gmail.com; preich@umn.edu
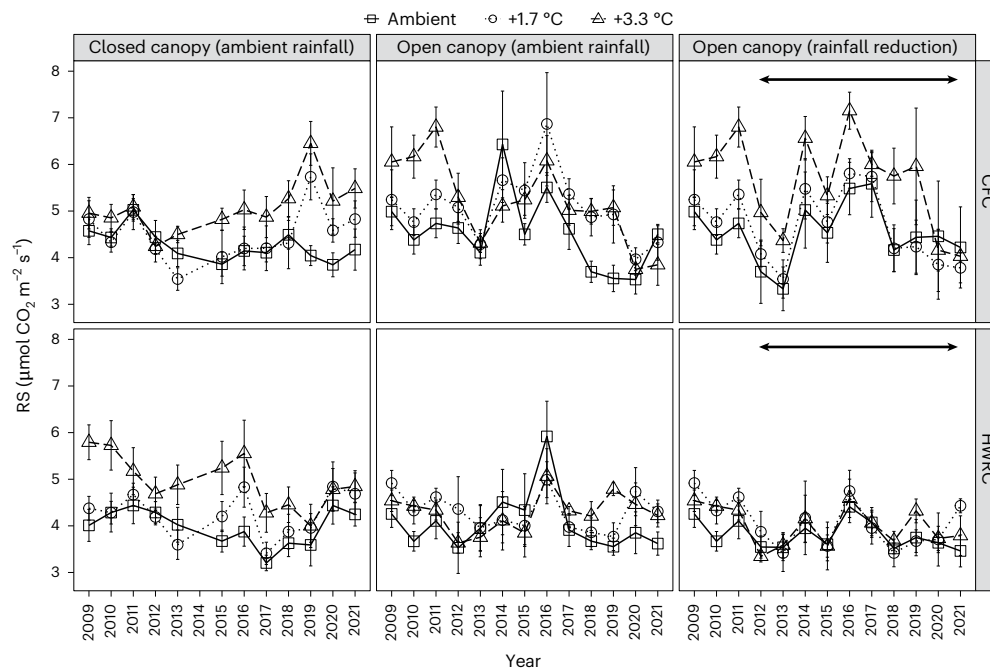
Fig. 1 | Annual average soil respiration (RS) under different treatments at two sites (CFC and HWRC) over 13 years. The 'Closed canopy (ambient rainfall)' and 'Open canopy (ambient rainfall)' contrast the results under different overstory canopy openness for the two sites, while the 'Open canopy (ambient rainfall)' and 'Open canopy (rainfall reduction)' contrast the results under different rainfall amounts for the two sites. The arrows represent the period that rainfall reduction treatment was applied. The error bars are s.e.m. for each treatment combination at each site. The results from all plots before the beginning of rainfall treatments (<2012) were used for both 'Open canopy (ambient rainfall)' and 'Open canopy (rainfall reduction)'; therefore, identical results before 2012 are shown for both treatments. The sample size was 6 for all treatments before 2012 and for all treatments under closed canopy across all years, and was 3 for all treatments under open canopy during 2012 and beyond.

growth (and thus plant inputs to soil) by decreasing soil moisture[12–14], and therefore potentially reduce warming effects on soil respiration[15,16]. Overstory canopy openness, which varies temporally and spatially in forests because of phenology, regeneration and natural and anthropogenic disturbances[17], can also affect microclimate in forests, including soil temperature and moisture[18], which are important in determining soil respiration[19]. Given the considerable effect of soil moisture on soil respiration, we hypothesized that when compared with ambient rainfall amount and closed canopy condition, the positive long-term effect of warming on soil respiration should be smaller under rainfall reduction and open canopy conditions, respectively (hypothesis 1).

Warming can affect soil respiration by altering the soil microbial activity[4,20] and community[21], as well as root biomass and function[22] and litterfall amounts[23]. Although warming generally increases soil respiration[22], ambient soil water conditions should regulate short-term fluctuations in warming effects on soil respiration. For example, we expected that warming would increase soil respiration whenever ambient soil moisture is high (hypothesis 2a). When ambient soil moisture is low, however, warming would further decrease soil moisture and then inhibit root growth and soil microbial activity, ultimately exacerbating drought effects. As a result, we expected to find insignificant or even negative effects of warming on soil respiration when ambient soil moisture is low (hypothesis 2b).

Long-term studies are crucial for understanding warming effects on soil C cycling because short-term results may not predict long-term effects well[24]. For example, a 26-year soil warming-only experiment at Harvard Forest found a significant temporally dynamic pattern of soil respiration[21]. As other long-term studies reporting soil respiration responses to warming in forests are extremely rare[25], it is unknown whether similar oscillating results would be found in other contexts[26]. Moreover, whether results at Harvard Forest would have differed if both soils and above-ground plants had been warmed is unknown. For example, the potentially greater stimulation of evapotranspiration

caused by both above- and below-ground warming in our study sites[18] may have resulted in larger warming-induced soil water deficits at our sites than at Harvard Forest[21]. Therefore, we hypothesized that intra- and interannual temporal patterns of warming effects on soil respiration in our study should be largely explained by the variation in annual precipitation and/or soil moisture[25] (hypothesis 3).

To test the three above-mentioned hypotheses, we measured soil respiration with standard shallow 'collars' under different warming levels (ambient, +1.7 °C and +3.3 °C), rainfall amounts (100% versus ~60% rainfall amount during summer), and overstory canopy openness (open (80% of full sunlight) and closed (8% of full sunlight)) from 2009 to 2021. We also partitioned total soil respiration (RS) into estimated heterotrophic (RH) and autotrophic (RA) components, using auxiliary deeper in situ soil cores that excluded live roots (Methods). As rainfall reduction only occurred in the open canopy condition, the study was an incomplete factorial of warming × site × canopy × rainfall, which included two complete factorial experiments (for example, warming and overstory canopy openness; warming and rainfall amount under open canopy). The large number of soil respiration measurements (22,386 for RS and 11,062 for RA and RH) across 72 plots at two sites and a wide range of ambient soil moisture conditions (0.13–0.27 m³ H_2O per m³ soil (10–90th percentile) from April to November) in our 13-year study provide an unparalleled opportunity to advance understanding of the role of temperature and soil moisture in modulating soil respiration and its temporal patterns in boreal forests.

Soil respiration under above- and below-ground warming

Warming generally increased long-term RS (Fig. 1, Table 1, Extended Data Fig. 1a and Supplementary Figs. 1 and 2). Specifically, on average across all observations in all years in both experiments and at both sites, RS increased by 6.5% and 17.2% under +1.7 °C and +3.3 °C, respectively (Extended Data Table 1). On average across all years, RS was reduced

Table 1 | The interactive effects of warming, canopy condition and rainfall reduction on soil respiration (RS) and stem biomass

	Effect	RS			Stem biomass		
		D.f.	F	P>F	D.f.	F	P>F
Warming and canopy experiment	Warming	1	159.2	**0.0000***	1	5.0	**0.0260**
	Canopy	1	0.9	0.3738	1	69.7	**0.0000**
	Year (yr)	12	9.9	**0.0000**	12	49.9	**0.0000**
	Site	1	4.1	0.0748	1	10.1	**0.0095**
	Warming×canopy	1	10.1	**0.0015**	1	0.5	0.4783
	Warming×yr	12	5.4	**0.0000**	12	1.0	0.4835
	Canopy×yr	11	8.4	**0.0000**	8	49.1	**0.0000**
	Warming×site	1	1.0	0.3182	1	0.1	0.7877
	Canopy×site	1	0.0	0.8766	1	0.7	0.4284
	Yr×site	12	3.3	**0.0000**	12	2.5	**0.0034**
	Warming×canopy×yr	11	4.0	**0.0000**	8	1.6	0.1230
	Warming×canopy×site	1	12.9	**0.0003**	1	2.7	0.1026
	Warming×yr×site	12	1.9	**0.0342**	12	0.7	0.7322
	Canopy×yr×site	11	1.0	0.4122	8	2.1	**0.0311**
	Warming×canopy×yr×site	11	6.0	**0.0000**	8	0.6	0.7738
Warming and rainfall experiment	Warming	1	40.7	**0.0000**	1	0.0	0.9644
	Rainfall	1	12.8	**0.0004**	1	1.5	0.2214
	Year	9	19.9	**0.0004**	9	55.4	**0.0000**
	Site	1	3.2	0.1425	1	3.6	0.1191
	Warming×rainfall	1	4.2	**0.0410**	1	4.3	**0.0386**
	Warming×yr	9	4.1	**0.0000**	9	1.9	0.0542
	Rainfall×yr	9	3.6	**0.0002**	9	0.2	0.9887
	Warming×site	1	16.4	**0.0000**	1	0.9	0.3514
	Rainfall×site	1	0.1	0.7190	1	0.3	0.5916
	Yr×site	9	2.9	**0.0019**	9	1.5	0.1387
	Warming×rainfall×yr	9	3.3	**0.0005**	9	2.6	**0.0072**
	Warming×rainfall×site	1	5.1	**0.0235**	1	0.7	0.4116
	Warming×yr×site	9	3.9	**0.0000**	9	0.1	0.9998
	Rainfall×yr×site	9	2.1	**0.0250**	9	0.2	0.9895
	Warming×rainfall×yr×site	9	0.9	0.5017	9	0.3	0.9735

Linear repeated-measures models were conducted. Warming: experimental warming level; canopy: overstory canopy openness; rainfall: experimental rainfall treatment; site: field site; F: F ratio. The measurements of RS and stem biomass from all campaigns were used in the analyses. The values of increased temperature were used to represent the experimental warming level in the analysis (0, 1.7 and 3.3 for ambient, +1.7 °C and +3.3 °C treatment, respectively); and year, canopy condition and rainfall amount were nominal factors in the mixed model. Significant effects ($P < 0.05$) are highlighted in bold.

by 4.7% in rainfall reduction compared with ambient rainfall treatment (Table 1 and Extended Data Table 1), but there was no significant effect of overstory canopy openness on RS.

Prior long-term studies of RS response to a greater magnitude of warming, but only below ground, reported much smaller effects per °C warming than found herein. For example, +5 °C warming increased RS on average by 8.5% in the 26-year warming experiment at the Harvard Forest[21] and by 2% in years 14–16 of the Flakaliden warming experiment in a spruce forest[27]. In contrast, we observed 6.5% and 17.2% increases in RS under +1.7 °C and +3.3 °C, respectively; several-fold to an order of magnitude greater increases per °C than in the prior studies.

Two potential explanations for the much greater effect per °C warming of combined above- and below-ground warming are as follows: first, when compared with below-ground warming alone, simultaneous above- and below-ground warming might further increase plant growth including root biomass[22], and thus enhance RA, an important component of soil respiration[28]. Second, combined

above- and below-ground warming could increase plant above-ground and below-ground litter and exudate inputs, increasing heterotrophic use of plant-derived C. Although the differences in tree species, warming level and experimental duration between our study and the other two aforementioned long-term studies make it difficult to attribute greater warming-induced RS in our study only to the different warming methods (for example, simultaneous above- and below-ground warming versus below-ground warming only), our findings provide cautionary advice that experiments increasing only above- or below-ground temperature may underestimate the increased soil respiration caused by future climate warming.

Different responses of respiration components to warming

Overall, estimated RH and RA showed different responses to warming (Extended Data Fig. 1b,c and Supplementary Figs. 3–6). For example, warming effects on RH were minor (Extended Data Table 1); however,
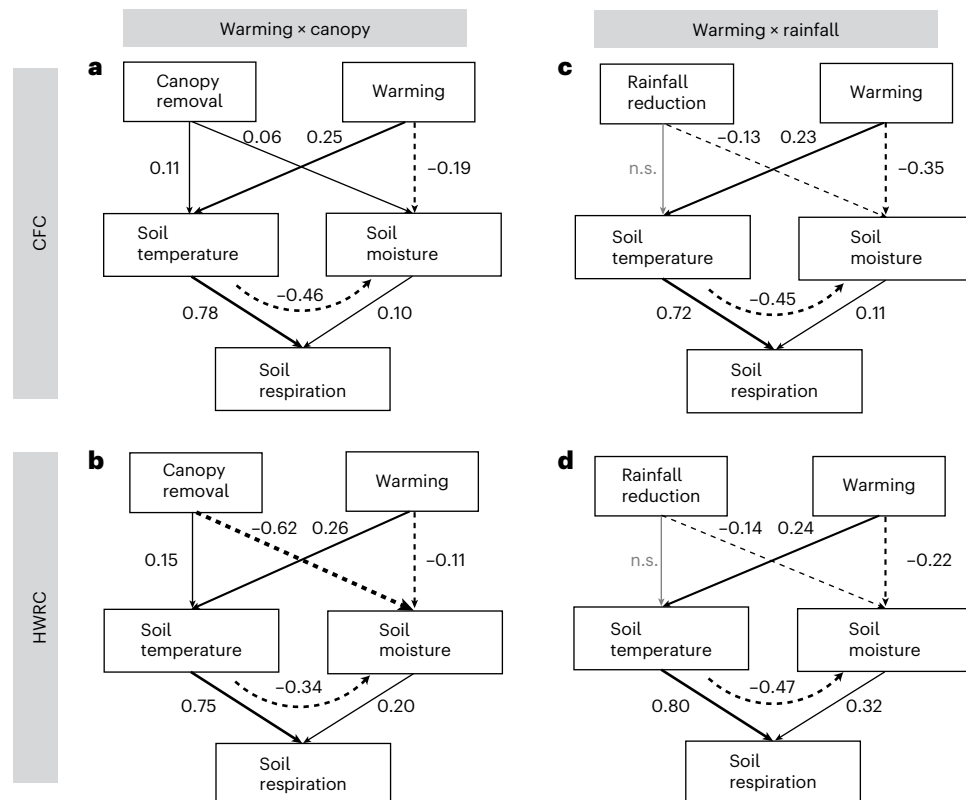
Fig. 2 | Structural equation model analyses of effects of experimental warming, canopy removal and rainfall reduction on soil respiration (RS) through altering soil temperature and moisture. Measurements of RS, temperature and moisture from all campaigns were used. **a**,**b**, The effects of warming and canopy removal for CFC (**a**) and HWRC (**b**). The results of the structural equation model are supported by the comparative fit index (CFI) of 1.0, the standardized root mean square residual (SRMR) of 0.04 and the root mean square error of approximation (RMSEA) of 0.01. **c**,**d**, The effects of warming and rainfall reduction for CFC (**c**) and HWRC (**d**). The results are supported by CFI of 1.0, SRMR of 0.04 and RMSEA of 0.01. The arrow thickness represents the estimated strength of the influences and the solid black, dashed black and grey arrows indicate the positive, negative and insignificant relationships, respectively. For the analyses, temperature increase above ambient was used to represent warming level (0, 1.7 and 3.3 for ambient, +1.7 °C and +3.3 °C, respectively).

higher RA was found under +1.7 °C (37% increase) and +3.3 °C (64% increase) than in the ambient temperature treatment. Across all treatments and years, RA contributed approximately 28% to RS.

No long-term (>10 years) studies in forests have continuously measured and reported RS and its components under warming, which inhibits understanding of how climate change alters RA and RH and thus influences RS. The lack of relevant information makes it difficult to modify the soil C cycling processes in Earth system models to increase their predictive abilities. Our study found contrasting RA and RH sensitivity to warming and rainfall reduction. For example, warming enhanced RA (probably via increasing root growth and/or direct thermal effects) but not RH and led to an increase in RS. By contrast, rainfall reduction mainly inhibited RH (probably due to reduced inputs associated with decreased growth[26] and/or decreased soil enzyme activity), ultimately decreasing RS. These findings suggest that different elements of climate change can have different effects on soil respiration components, which should be accounted for when predicting soil C cycling.

It should be noted, however, that although the trenching method (for example, inserting deep collars) has been widely used in situ to separate soil respiration components, it has some potential limitations (for example, increased soil moisture and decreased C substrate supply)[29,30]. This might result in an underestimation of the response of RH to long-term warming. Nonetheless, due to its cost effectiveness and ease of implementation, inserting deep collars is still commonly used to measure RH at field sites[31]. Given that our study mainly focused on the trend of warming effects on soil respiration and its components, our main findings and conclusions would probably not be greatly affected by the potential limitations of the trenching method.

Warming effects mediated by canopy openness and rainfall

We found interactive effects of warming and overstory canopy openness or rainfall amount on RS (Table 1), consistent with hypothesis 1. However, the underlying mechanisms were more complex than proposed. RS increased with warming level except in the open canopy condition at the Hubachek Wilderness Research Center (HWRC) site, which showed insignificant differences in soil respiration between +1.7 °C and +3.3 °C (Extended Data Fig. 2). This lack of temperature effect is probably because plots in open canopy condition at HWRC had significantly lower soil moisture (Extended Data Fig. 3a). Warming and open canopy condition together led to the lowest soil moisture under +3.3 °C and thus the negative effects of soil drying offset the positive effect of warming on RA (Extended Data Fig. 2), leading to the insignificant difference in RS between +1.7 °C and +3.3 °C.

A similar mechanism (for example, warming effects on soil respiration offset by rainfall reduction effects on soil moisture) existed for the 'warming and rainfall amount' experiment. For example, significant interactive effects of warming, rainfall reduction and site on RS were found (P < 0.05; Table 1). Specifically, when compared with Cloquet Forestry Center (CFC), warming had smaller effects on RS under rainfall reduction at HWRC (Extended Data Fig. 4). The absolute decline in soil moisture caused by rainfall reduction was similar between the two sites (Extended Data Fig. 3b). Since HWRC had lower ambient soil moisture overall than CFC, rainfall reduction led to much lower levels of soil moisture at HWRC, probably inhibiting RS response to warming more at that site. Overall, our findings suggest that overstory canopy openness and rainfall reduction can change warming effects on soil respiration by altering soil moisture.
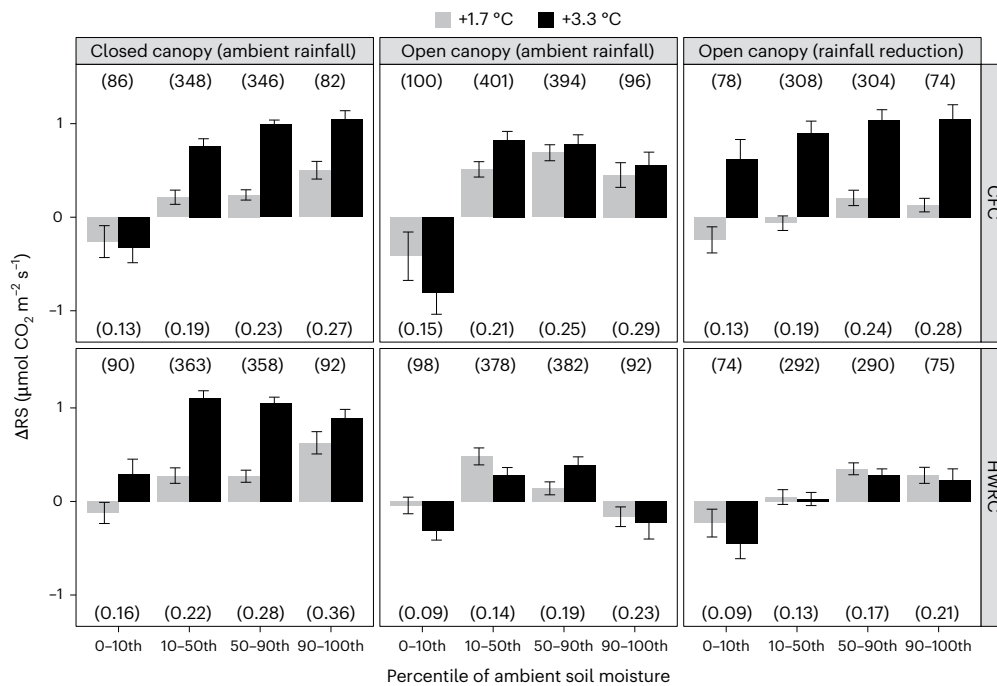
Fig. 3 | The change in soil respiration (RS) caused by experimental warming under different percentiles of ambient soil moisture. The 'Closed canopy (ambient rainfall)' and 'Open canopy (ambient rainfall)' show results under different overstory canopy openness for the two sites, while the 'Open canopy (ambient rainfall)' and 'Open canopy (rainfall reduction)' show results under different rainfall amounts for the two sites. Ambient soil moisture is the soil moisture under ambient warming treatment. The values in the parentheses on the bottom and top of each graph represent the mean value of soil moisture and the sample size under different percentiles for each treatment combination, respectively. The measurements of soil moisture and respiration from all campaigns over 13 years were used.

Warming effects on respiration dependent on soil moisture

Consistent with the interpretations above, our structural equation model indicated that warming and open canopy conditions increased soil temperature, ultimately increasing RS (Fig. 2a,b), whereas warming and rainfall reduction decreased soil moisture, ultimately decreasing RS (Fig. 2c,d). This is strong evidence that climate warming influences respiration in divergent ways through its impacts on soil temperature and soil moisture. As a technical aside, the effect of warming and rainfall reduction on soil moisture was in reality influenced by the warming of plants above ground as well as of soils—although only soil temperature was included in the structural equation model due to its connection to other soil processes, the strongly co-varying increase in plant temperature increased evapotranspiration and was probably the dominant driver of soil moisture. Additionally, despite mildly positive relationships among stem biomass and RS (Extended Data Fig. 5a), we found insignificant relationships between the changes in stem biomass and soil respiration caused by warming (Extended Data Fig. 5d–f), suggesting that treatments in our study affected soil respiration by influencing soil temperature and moisture but not inputs from aboveground (which stem biomass is a proxy for).

When soil water was low (for example 0–10th percentile ambient soil moisture), warming effects on RS were minor or even negative, but became positive when ambient soil moisture was higher (Fig. 3), supporting hypothesis 2. In addition, warming effects on RS were similar across a range of non-low soil moisture. Our estimates of RH and RA suggest that ambient soil moisture potentially regulated root growth and/or function (as inferred from RA), ultimately influencing the response of RS to warming (Extended Data Fig. 6). When soils are very dry, root growth and metabolism are probably inhibited[22] due to water limitation and decreased dissolution, diffusion, transport and uptake of nitrogen[13]. Our study suggests that overlooking the negative effect of warming on soil respiration in dry soils would lead to an overestimate of the positive effect of warming on soil respiration and thus the positive climate feedback, especially in regions that are projected to experience more drought events in the future.

Long-term temporal pattern of soil respiration under warming

We found significant interannual variability in RS response to warming for all treatments at both sites (Table 1, Fig. 4 and Extended Data Fig. 7). Overall, warming effects on RS exhibited either two- (decrease and then increase) or three-phase (decrease–increase–decrease) patterns (Fig. 4). For example, in closed canopy plots, the effects of both warming levels on RS decreased for the first 4 years at both sites, then increased in years 5 and 6, and then either increased continuously (at CFC) or showed another decline (at HWRC). Under open canopy condition and ambient rainfall at both sites, the effects of both warming levels were initially positive, generally peaked in year 2, then decreased to neutral or negative with a trough after 4–6 years, then increased to positive values again. For open canopy plots with rainfall reduction, warming effects started out positive and declined at CFC but were small and did not change a lot over time at HWRC.

The shifting pattern of RS response to warming over time in our study was not explained by the interannual variations of precipitation, soil moisture or temperature, which is inconsistent with hypothesis 3. Although long-term patterns of RS under warming were dynamic in both our study (especially in plots with ambient rainfall amount) and the Harvard Forest study[21] (for example, multiple phases including positive to zero or even negative to positive), the underlying mechanisms might differ. For example, changes in soil microbial variables (which should mainly contribute to RH) caused by warming were considered the driver of long-term patterns of RS in the Harvard Forest[21]. In contrast, our results suggest that the temporal pattern of warming effects on RS might have been driven by the interannual change in the response of roots[32] (which contribute to RA) to warming (Fig. 4 and
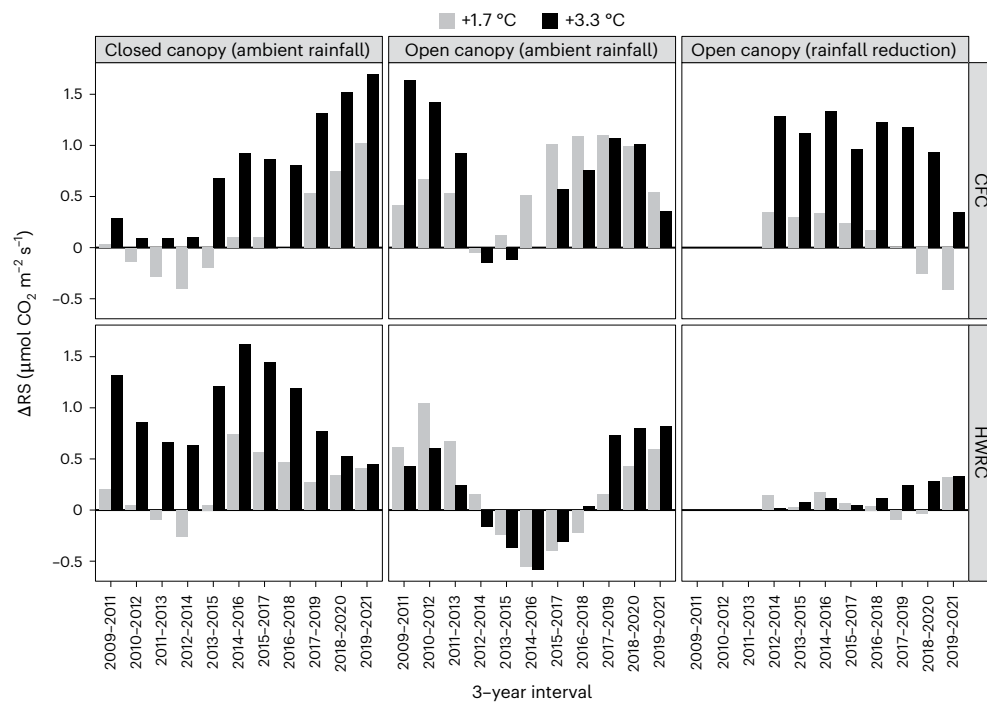
Fig. 4 | Three-year rolling mean change in annual soil respiration (RS) under experimental warming relative to ambient treatment. The 'Closed canopy (ambient rainfall)' and 'Open canopy (ambient rainfall)' show results under different overstory canopy openness for the two sites, while the 'Open canopy (ambient rainfall)' and 'Open canopy (rainfall reduction)' show results under different rainfall amounts for the two sites. No data are shown for 2009–2011, 2010–2012 and 2011–2013 under 'Open canopy (rainfall reduction)' treatment because rainfall reduction treatment was begun in 2012.

Extended Data Fig. 7b). Overall, the mechanisms underlying observed differences in temporal pattern of warming effects on soil respiration between sites and overstory canopy openness could be quite complex, which might be the result of inherent differences in soil moisture, stem biomass and root expansion of mature trees (whose stems were near our understory plots). However, such interpretations, including the discussion below, are highly uncertain.

The root biomass we measured before the experiment and stem biomass from each year could potentially help explain the different temporal patterns of warming effects on RS. For example, during the first years under the closed canopy, warming effects on RS were minor at the CFC site, but significantly positive at the HWRC site under +3.3 °C warming (Fig. 4). For the closed canopy, the warming-induced increase in stem biomass was similar between CFC and HWRC (Extended Data Fig. 7c), suggesting that warming-induced C inputs might be similar between sites. In contrast, the root density of mature trees under closed canopy in 2009 was higher at HWRC (1,357 g m^{-2} at 0–20 cm soil depth) than CFC (853 g m^{-2}); in closed canopy, a fraction of fine roots in our experimental plots probably were from mature trees outside but nearby our plots. As a result, the greater response of RA to +3.3 °C warming at HWRC than CFC (Extended Data Fig. 7b) might be in part related to this. Moreover, for the first years at CFC site, warming effects on RS were small under closed canopy, but were greatly positive under open canopy (Fig. 4). For the ambient rainfall amount treatment at CFC site, the warming-induced increase in stem biomass was also much greater under open than closed canopy during the first years (Extended Data Fig. 7c), and perhaps can help explain these differences.

Conclusions
Although global warming is projected to increase both above- and below-ground temperatures[6], no prior studies have reported long-term (for example, >10 years) effects of simultaneous above- and below-ground warming on soil respiration and its components in forests (or to our knowledge in any ecosystem). Results of our 13-year field experiment in southern boreal forests suggest that joint above- and below-ground warming may stimulate soil respiration more than manipulations that achieve either above- or below-ground warming. In addition, the magnitude or even direction of warming effects on soil respiration was mediated by ambient soil moisture, and soil respiration response to warming varied greatly in the long term for reasons we do not yet well understand. If we fail to account for such drivers, mechanisms and patterns, we may inaccurately predict global forest C cycling under future climate change.

Online content
Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41561-024-01512-3.

References
1. Scharlemann, J. P. W., Tanner, E. V. J., Hiederer, R. & Kapos, V. Global soil carbon: understanding and managing the largest terrestrial carbon pool. *Carbon Manag.* **5**, 81–91 (2014).
2. Crowther, T. W. et al. Quantifying global soil carbon losses in response to warming. *Nature* **540**, 104–108 (2016).
3. Bond-Lamberty, B. & Thomson, A. Temperature-associated increases in the global soil respiration record. *Nature* **464**, 579–582 (2010).
4. Davidson, E. A. & Janssens, I. A. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature* **440**, 165–173 (2006).
5. Schlesinger, W. & Andrews, J. Soil respiration and the global carbon cycle. *Biogeochemistry* **48**, 7–20 (2000).
6. Soong, J. L., Phillips, C. L., Ledna, C., Koven, C. D. & Torn, M. S. CMIP5 models predict rapid and deep soil warming over the 21st century. *J. Geophys. Res. Biogeosci.* **125**, e2019JG005266 (2020).

7. Pan, Y. et al. A large and persistent carbon sink in the world's forests. *Science* **333**, 988–993 (2011).

8. Sherwood, S. & Fu, Q. A drier future? *Science* **343**, 737–739 (2014).

9. Seager, R. et al. Dynamical and thermodynamical causes of large-scale changes in the hydrological cycle over North America in response to global warming. *J. Clim.* **27**, 7921–7948 (2014).

10. Wang, Y., Hogg, E. H., Price, D. T., Edwards, J. & Williamson, T. Past and projected future changes in moisture conditions in the Canadian boreal forest. *For. Chron.* **90**, 678–691 (2014).

11. Way, D. A., Crawley, C. & Sage, R. F. A hot and dry future: warming effects on boreal tree drought tolerance. *Tree Physiol.* **33**, 1003–1005 (2013).

12. Ma, Z. et al. Regional drought-induced reduction in the biomass carbon sink of Canada's boreal forests. *Proc. Natl Acad. Sci. USA* **109**, 2423–2427 (2012).

13. Schimel, J. P. Life in dry soils: effects of drought on soil microbial communities and processes. *Annu. Rev. Ecol. Evol. Syst.* **49**, 409–432 (2018).

14. Zhou, Z., Wang, C. & Luo, Y. Response of soil microbial communities to altered precipitation: a global synthesis. *Glob. Ecol. Biogeogr.* https://doi.org/10.1111/geb.12761 (2018).

15. Zhou, L. et al. Interactive effects of global change factors on soil respiration and its components: a meta-analysis. *Glob. Change Biol.* **22**, 3157–3169 (2016).

16. Schindlbacher, A. et al. Soil respiration under climate change: prolonged summer drought offsets soil warming effects. *Glob. Change Biol.* **18**, 2270–2279 (2012).

17. Frelich, L. E. & Reich, P. B. Spatial patterns and succession in a Minnesota southern-boreal forest. *Ecol. Monogr.* **65**, 325–346 (1995).

18. Rich, R. L. et al. Design and performance of combined infrared canopy and belowground warming in the B4WarmED (Boreal Forest Warming at an Ecotone in Danger) experiment. *Glob. Change Biol.* **21**, 2334–2348 (2015).

19. Hursh, A. et al. The sensitivity of soil respiration to soil temperature, moisture, and carbon supply at the global scale. *Glob. Change Biol.* **23**, 2090–2103 (2017).

20. Chen, J. et al. Differential responses of carbon-degrading enzyme activities to warming: implications for soil respiration. *Glob. Change Biol.* **24**, 4816–4826 (2018).

21. Melillo, J. M. et al. Long-term pattern and magnitude of soil carbon feedback to the climate system in a warming world. *Science* **358**, 101–105 (2017).

22. Song, J. et al. A meta-analysis of 1,119 manipulative experiments on terrestrial carbon-cycling responses to global change. *Nat. Ecol. Evol.* **3**, 1309–1320 (2019).

23. Giardina, C. P., Litton, C. M., Crow, S. E. & Asner, G. P. Warming-related increases in soil CO_2 efflux are explained by increased below-ground carbon flux. *Nat. Clim. Change* **4**, 822–827 (2014).

24. Leuzinger, S. et al. Do global change experiments overestimate impacts on terrestrial ecosystems? *Trends Ecol. Evol.* **26**, 236–241 (2011).

25. Carey, J. C. et al. Temperature response of soil respiration largely unaltered with experimental warming. *Proc. Natl Acad. Sci. USA* **113**, 13797–13802 (2016).

26. Reich, P. B. et al. Even modest climate change may lead to major transitions in boreal forests. *Nature* **608**, 540–545 (2022).

27. Lim, H. et al. Boreal forest biomass accumulation is not increased by two decades of soil warming. *Nat. Clim. Change* **9**, 49–52 (2019).

28. Bond-Lamberty, B., Wang, C. & Gower, S. T. A global relationship between the heterotrophic and autotrophic components of soil respiration? *Glob. Change Biol.* **10**, 1756–1766 (2004).

29. Kuzyakov, Y. & Larionova, A. A. Root and rhizomicrobial respiration: a review of approaches to estimate respiration by autotrophic and heterotrophic organisms in soil. *J. Plant Nutr. Soil Sci.* **168**, 503–520 (2005).

30. Yang, L. et al. Soil microbial respiration adapts to higher and longer warming experiments at the global scale. *Environ. Res. Lett.* **18**, 034044 (2023).

31. Bond-Lamberty, B. et al. Twenty years of progress, challenges, and opportunities in measuring and understanding soil respiration. *JGR Biogeosci.* **129**, e2023JG007637 (2024).

32. Kwatcho Kengdo, S. et al. Long-term soil warming alters fine root dynamics and morphology, and their ectomycorrhizal fungal community in a temperate forest soil. *Glob. Change Biol.* **28**, 3441–3458 (2022).

Methods

Site description and experimental design

The Boreal Forest Warming at an Ecotone in Danger (B4WarmED) experiment was conducted at two field stations of the University of Minnesota: the CFC, Cloquet, MN, USA (46° 40′ 46″ N, 92° 31′ 12″ W, 382 m a.s.l.) and approximately 150 km further north, the HWRC, Ely, MN, USA (47° 56′ 46″ N, 91° 45′ 29″ W, 415 m a.s.l.). Both sites are located in the ecotone that spans the transition from temperate to boreal biomes, and the dominant overstory species at the time of experimental establishment were 40–60-year-old aspen (*Populus tremuloides*), birch (*Betula papyrifera*) and fir (*Abies balsamea*) with intermixed individual old red and white pine (*Pinus resinosa* and *Pinus strobus*). The climatic and soil characteristics at these two field sites are presented in Supplementary Table 1. Plots were located in both closed canopy (8% of full sunlight) and open overstory canopy conditions (80% of full sunlight). In addition to the differences in soil temperature and moisture caused by the overstory canopy openness, the overstory mature trees root extension into the closed canopy plots might mediate warming effects on soil respiration in our study. Mature trees around the plots in the open canopy condition were cut before our experiment, and the density of overstory mature trees under closed canopy condition was approximately 57 trees per hectare. The experiment was an incomplete factorial of warming × site × canopy × rainfall, since rainfall reduction only occurred in the open canopy condition. Thus, we analysed our data as two distinct full factorial experiments as follows.

At both sites, simultaneous above- and below-ground warming treatments at three levels (ambient, +1.7 °C and +3.3 °C, with the latter two with respect to unwarmed plots in the same canopy and rainfall treatments) were accomplished through a chamberless infrastructure (approximately 1.6 m high infra-red lamp heaters and buried soil heating cables at a depth of 10 cm and spaced 20 cm apart; Supplementary Fig. 7a,b)[18,33]. Warming was implemented approximately from April to November between 2009 and 2021, except for the +3.3 °C treatment under open canopy (rainfall reduction) in 2012, closed canopy condition in 2014, open canopy condition in 2017, +1.7 °C treatment under closed canopy in 2018 and + 3.3 °C treatment under closed canopy in 2020 for a number of reasons (for example, burning and technical issues). Across all treatments (for example, canopy condition, rainfall reduction and sites) and years, above- and below-ground temperature was increased by 1.7 ± 0.2 °C and 3.3 ± 0.3 °C under +1.7 °C and +3.3 °C treatments, respectively, compared with the ambient temperature treatment. Initially, the experiment had a two site (CFC and HWRC) by two canopy conditions (open and closed) by three warming (ambient, +1.7 °C and +3.3 °C) factorial design, with three blocks each containing two replicate plots (7.1 m² circular with approximately 3 m diameter) of each treatment combination, for a total of 72 research plots until 2012. In 2012, rainout shelters were installed in half of the open canopy plots (18 plots), enabling two rainfall levels (100% rainfall (ambient) versus ~60% rainfall from June to September (rainfall reduction)). The simulated summer rainfall reduction was achieved by manually extending rainout shelters mounted 4 m above the plots before scheduled rain events (Supplementary Fig. 7c)[33,34]. Therefore, from 2012 onwards, we considered a second factorial experiment that contrasts two rainfall levels (for example, 2 sites × 3 warming levels × 2 rainfall amounts × 3 blocks for a total of 36 plots). For this experiment, to show the interannual patterns of soil respiration, soil moisture, soil temperature and the change in soil respiration caused by warming under different warming levels, the results from all plots before 2012 were used for both ambient rainfall and rainfall reduction treatments. Therefore, the results before 2012 were identical to each other between ambient rainfall and rainfall reduction treatments. Rainfall reduction treatment was not implemented in 2017 at either site.

Before initiating the warming treatments in 2008, blocks designated as open canopy condition were harvested, and stumps were cut to less than 30 cm height. In addition, all blocks were brush cut by hand to remove tree saplings and shrubs, and 11,616 juveniles of 11 species

including six native broadleaf (*Acer rubrum*, *Acer saccharum*, *Betula papyrifera*, *Populus tremuloides*, *Quercus macrocarpa* and *Quercus rubra*), four native needle-leaved (*Abies balsamea*, *Picea glauca*, *Pinus banksiana* and *Pinus strobus*) and one naturalized broadleaf (*Rhamnus cathartica*) species were planted. Over the years of the experiment, we have grown multiple cohorts of juveniles of 21 tree species in different mixtures (Supplementary Table 2). For each cohort, we sourced approximately 2–3-year-old bare root stock juveniles of all native species from the Minnesota Department of Natural Resources, while invasive species were sourced from the local Minnesota Department of Natural Resources nursery and/or parks and planted intermixed with the naturally occurring shrub, herb and fern vegetation in each plot.

Measurements and analyses of soil respiration, temperature and moisture

In 2008, one deep and two shallow polyvinyl chloride (PVC) collars (10.2 cm diameter) were randomly inserted approximately 50 cm and 2 cm into the soil in each plot to measure soil heterotrophic and total respiration, respectively (Supplementary Fig. 7d). From 2009, in situ soil respiration was measured approximately every 2 weeks when warming treatments were operational (from April to November, approximately) using a LI-COR 6400 with a soil chamber attached (LI-COR Biosciences Inc.). Before each measurement of soil respiration, living plants and litterfall inside the PVC collar were removed. For each measurement, we used the values from the shallow PVC collar (representing RS) minus the value from the deep collar (representing soil heterotrophic respiration) in each plot to calculate soil autotrophic respiration. Therefore, there were two, one and two replicates for soil total, heterotrophic and autotrophic respiration in each plot during each measurement time, respectively.

In each plot, two soil thermocouple probes were installed at 10 cm depth to continuously monitor soil temperature, and one time–domain reflectometer probe (CS-616, Campbell Scientific Inc.) was installed at depth 0–22.5 cm to continuously monitor volumetric soil moisture. We used soil temperature logged at 15 min intervals and soil moisture measured once per hour in all plots throughout the whole year. Soil respiration, temperature and moisture were not measured under closed canopy condition in 2014 because warming was not implemented. For statistical analyses, we matched the timing of the soil respiration measurements to the nearest interval for which soil moisture and temperature measurements were logged. To measure rainfall, a tipping bucket rain gauge (MET-One model 385AC, Campbell Scientific Inc.) was installed at each site.

Measurements of stem biomass

From 2008 to 2021, we harvested and collected 6,203 stem samples from planted juvenile tree species in all plots to measure the dry weight of stem biomass. Meanwhile, the diameter (5 cm above ground) and the height of the juveniles were measured before collecting stem samples. The dataset including measured stem biomass, tree diameter and tree height was then used to create equations to estimate the stem biomass of each juvenile that we did not directly measure in a given year. Briefly, for 15 out of 21 species that we planted in this study, their stem biomass was estimated by equation (1), which varied with species. For the other six species, due to the limited number of measured stem biomass, we used the equation that was created by all 6,203 stem samples without considering the species effects to estimate their stem biomass (equation (2)).

$$\text{Stem biomass} = -1.75 + 1.40 \times \log(\text{diameter}) + 0.90 \times \log(\text{height})$$
$$+ (\log(\text{diameter}) - 0.76)$$
$$\times (\log(\text{height}) - 1.65) \times 0.09 + a + b$$
$$\times (\log(\text{diameter}) - 0.76) + c \times (\log(\text{height}) - 1.65)$$
$$+ d \times (\log(\text{diameter}) - 0.76) \times (\log(\text{height}) - 1.65)$$

$$(1)$$

$$\text{Stem biomass} = -1.74 + 1.56 \times \log(\text{diameter}) + 0.78 \times \log(\text{height})$$
$$+ 0.21 \times (\log(\text{diameter}) - 0.76) \times (\log(\text{height}) - 1.65)$$
$$(2)$$

where the diameter is tree diameter (5 cm above ground); height is tree height; and a, b, c and d are constants that varied with species (Supplementary Table 3). The R^2 of the simple linear regression determining the correlation between estimated and measured stem biomass was 0.85 and 0.79 for the 15 and 6 species, respectively.

We measured the diameter and height of all juveniles in all plots after the summer season in all years. Then, the appropriate equations above were used to estimate the stem biomass of each juvenile in each plot. We summed up the stem biomass of each juvenile to calculate plot-level stem biomass. It should be noted that this community-level stem biomass considered juvenile mortality. For example, if the juvenile was dead, its stem biomass was zero when calculating plot-level stem biomass.

Statistical analysis

Repeated-measures linear mixed-effects models were used to test the responses of soil respiration and stem biomass to treatment combinations, year and site. Warming treatment was used as continuous variable, and block was added to each model as a random effect. More specifically, for the 'warming and canopy condition' experiment, measurements of soil respiration and stem biomass from 2009 to 2021 were included in the analysis (from 2012, only data in ambient rainfall plots were used). For the 'warming and rainfall amount' experiment, measurements of soil respiration and stem biomass in open canopy plots from 2012 to 2021 were included in the analysis. All measurements of soil respiration and its components, temperature and moisture from the growing season (for example, April to November) in each year were averaged to calculate the corresponding annual mean values. A structural equation model was performed to determine how warming, canopy removal and rainfall reduction affect soil respiration by altering soil temperature and moisture.

Data availability

All data used and source data for figures and tables in this study are archived in figshare (https://doi.org/10.6084/m9.figshare.26488219.v1)[35].

Code availability

The R scripts needed to reproduce the analysis are archived in figshare (https://doi.org/10.6084/m9.figshare.26488219.v1)[35].

References

33. Stefanski, A., Bermudez, R., Sendall, K. M., Montgomery, R. A. & Reich, P. B. Surprising lack of sensitivity of biochemical limitation of photosynthesis of nine tree species to open-air experimental warming and reduced rainfall in a southern boreal forest. *Glob. Change Biol.* **26**, 746–759 (2020).
34. Bermudez, R., Stefanski, A., Montgomery, R. A. & Reich, P. B. Short- and long-term responses of photosynthetic capacity to temperature in four boreal tree species in a free-air warming and rainfall manipulation experiment. *Tree Physiol.* **41**, 89–102 (2021).
35. Liang, G. et al. The dataset and R code for the paper "Soil respiration response to decade-long warming modulated by soil moisture in a boreal forest" in Nature Geoscience, 2024. *figshare* https://doi.org/10.6084/m9.figshare.26488219.v1 (2024).

Acknowledgements

Author contributions

P.B.R., R.A.M., R.L.R. and S.E.H. conceived and designed the original experiment and the experimental warming and rainfall manipulations. P.B.R., A.S., R.B. and R.L.R. managed the experiment over time. A.S., R.B. and R.L.R. collectively implemented the experiment. A.S., R.B. and W.C.E. supervised or performed the acquisition of all soil respiration data. A.S., R.B. and R.L.R. acquired all soil temperature and moisture and rainfall data, and curated all data. G.L. and P.B.R. developed the idea for this study. G.L. carried out all analyses with suggestions from P.B.R. and S.E.H., constructed the figures and tables and wrote the first draft. All authors contributed to the interpretation of the results and were involved in writing and editing subsequent drafts. P.B.R. and R.A.M. were responsible for acquiring the funding for the project, and P.B.R. was responsible for all project supervision and administration.

Competing interests

The authors declare no competing interests.

Additional information

Extended data is available for this paper at https://doi.org/10.1038/s41561-024-01512-3.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41561-024-01512-3.

Correspondence and requests for materials should be addressed to Guopeng Liang or Peter B. Reich.

Peer review information *Nature Geoscience* thanks Jennifer Watts and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. Primary Handling Editor: Xujia Jiang, in collaboration with the *Nature Geoscience* team.

Reprints and permissions information is available at www.nature.com/reprints.
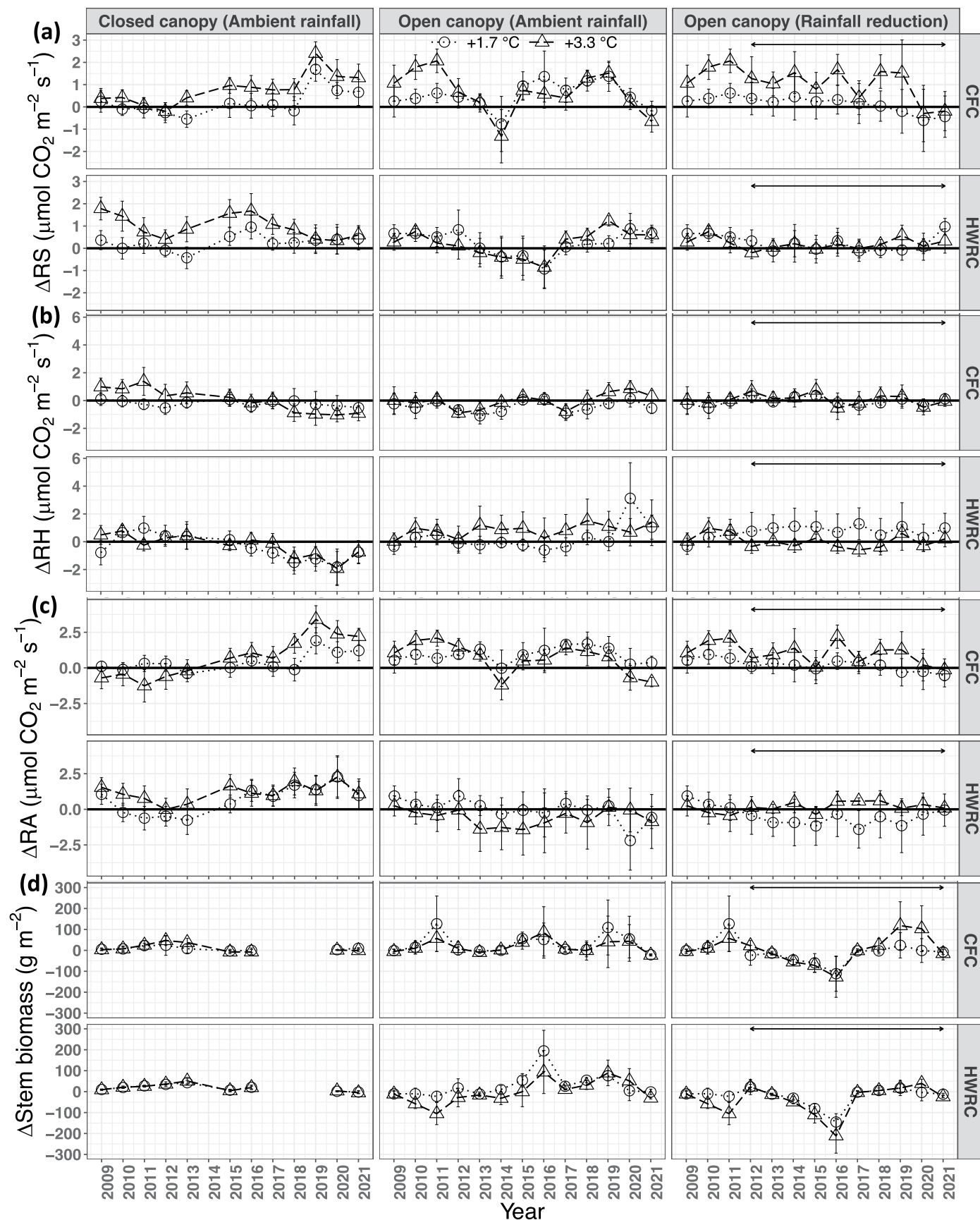
Extended Data Fig. 1 | See next page for caption.

Extended Data Fig. 1 | Change in annual soil respiration and stem biomass caused by warming under different treatments at two sites (CFC and HWRC) over 13 years. Panels under "Closed canopy (Ambient rainfall)" and "Open canopy (Ambient rainfall)" show results under different overstory canopy openness; panels under "Open canopy (Ambient rainfall)" and "Open canopy (Rainfall reduction)" show results under different rainfall amounts. Panels **a**, **b**, **c** and **d** show the results of the changes in RS, RH, RA and stem biomass caused by warming, respectively. Arrows represent the period that rainfall reduction treatment was applied. Error bars are standard errors of the mean for each treatment combination. The results from all plots before the beginning of rainfall treatments (<2012) were used for both "Open canopy (Ambient rainfall)" and "Open canopy (Rainfall reduction)"; therefore, identical results before 2012 were shown for both treatments. RS: soil total respiration; RH: soil heterotrophic respiration; RA: soil autotrophic respiration. The sample size was 3 for all treatments in each year at each site.
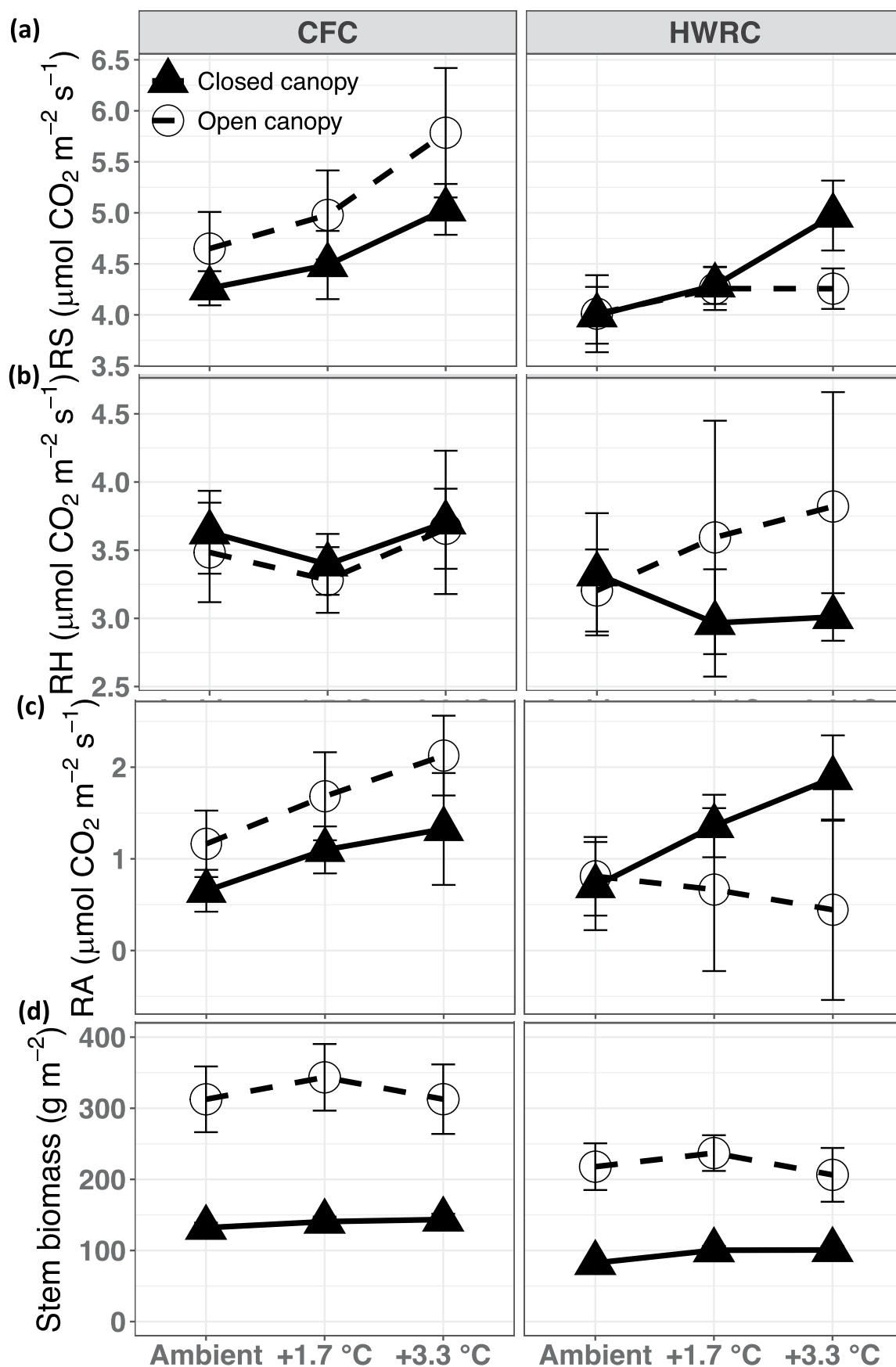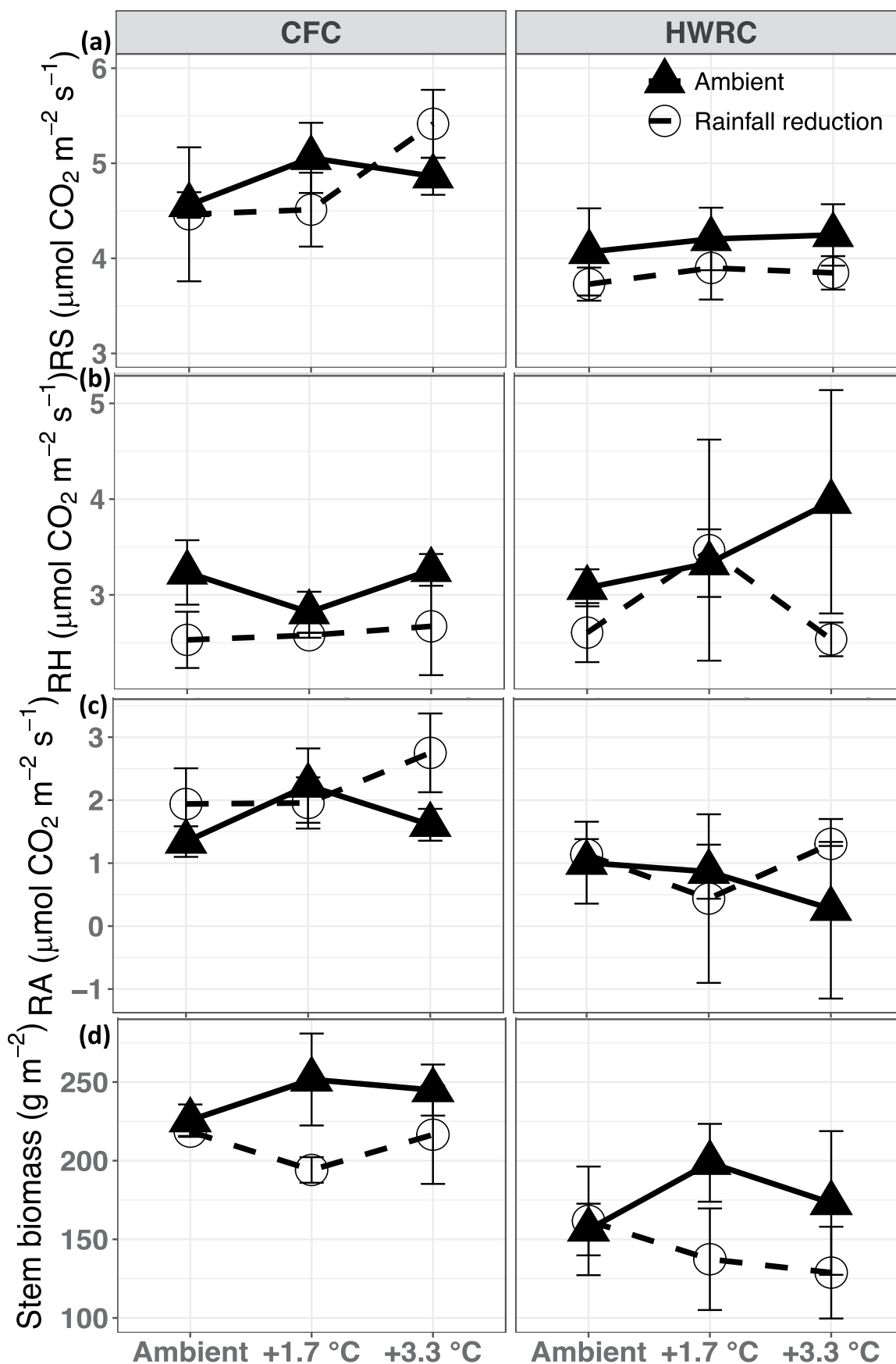
Extended Data Fig. 2 | Mean soil respiration and stem biomass averaged across all measurements at two sites (experimental warming × overstory canopy openness). Panels **a**, **b**, **c** and **d** show he results of RS, RH, RA and stem biomass, respectively. Error bars are standard errors of the mean for each treatment combination. RS: soil total respiration; RH: soil heterotrophic respiration; RA: soil autotrophic respiration. The sample size was 6 and 3 under closed and open canopy, respectively.
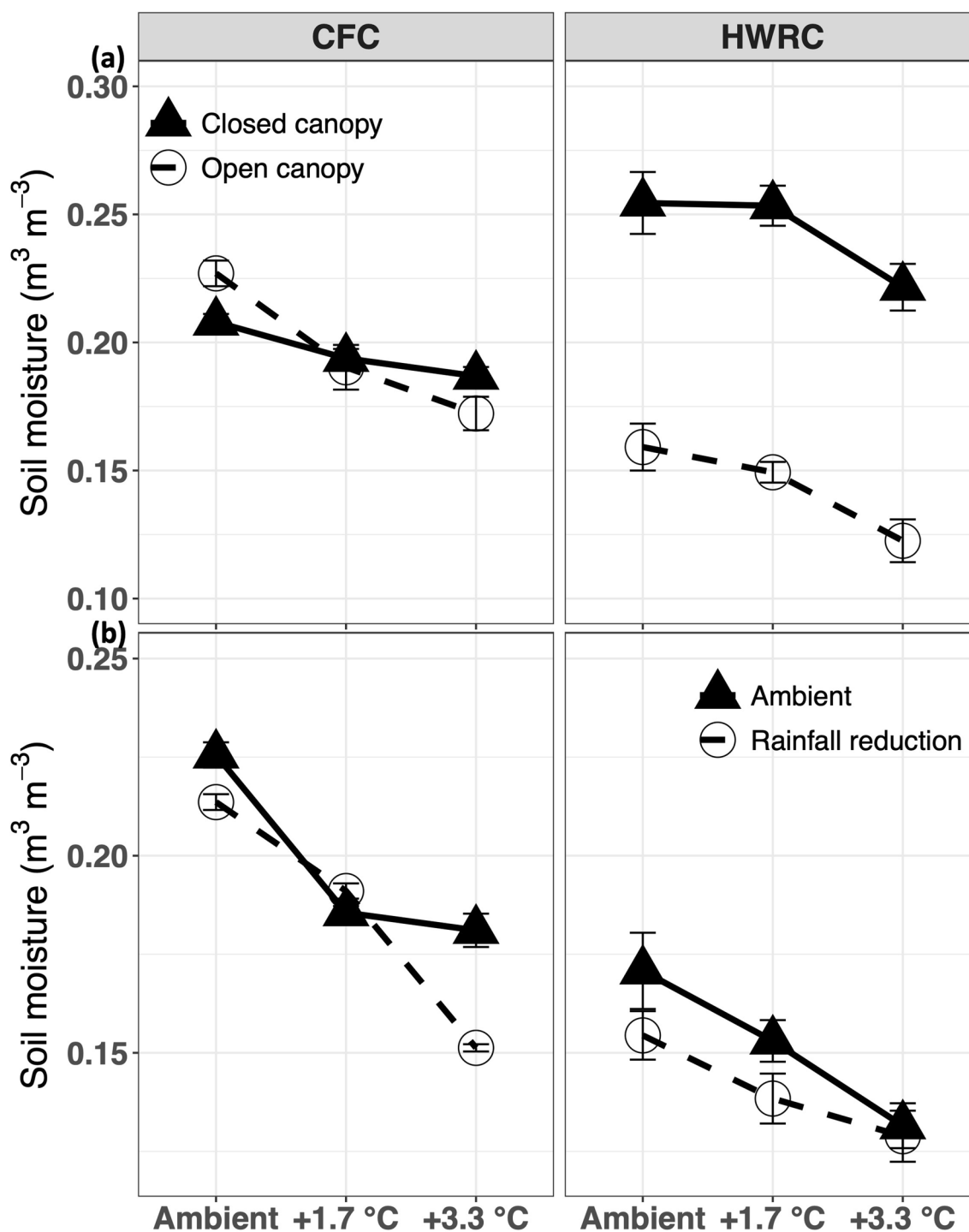
Extended Data Fig. 3 | Mean soil moisture averaged across all measurements at two sites. Error bars are standard errors of the mean for each treatment combination. For panel **a**, the sample size was 6 and 3 under closed and open canopy, respectively; for panel **b**, the sample size was 3 for all treatment combinations.
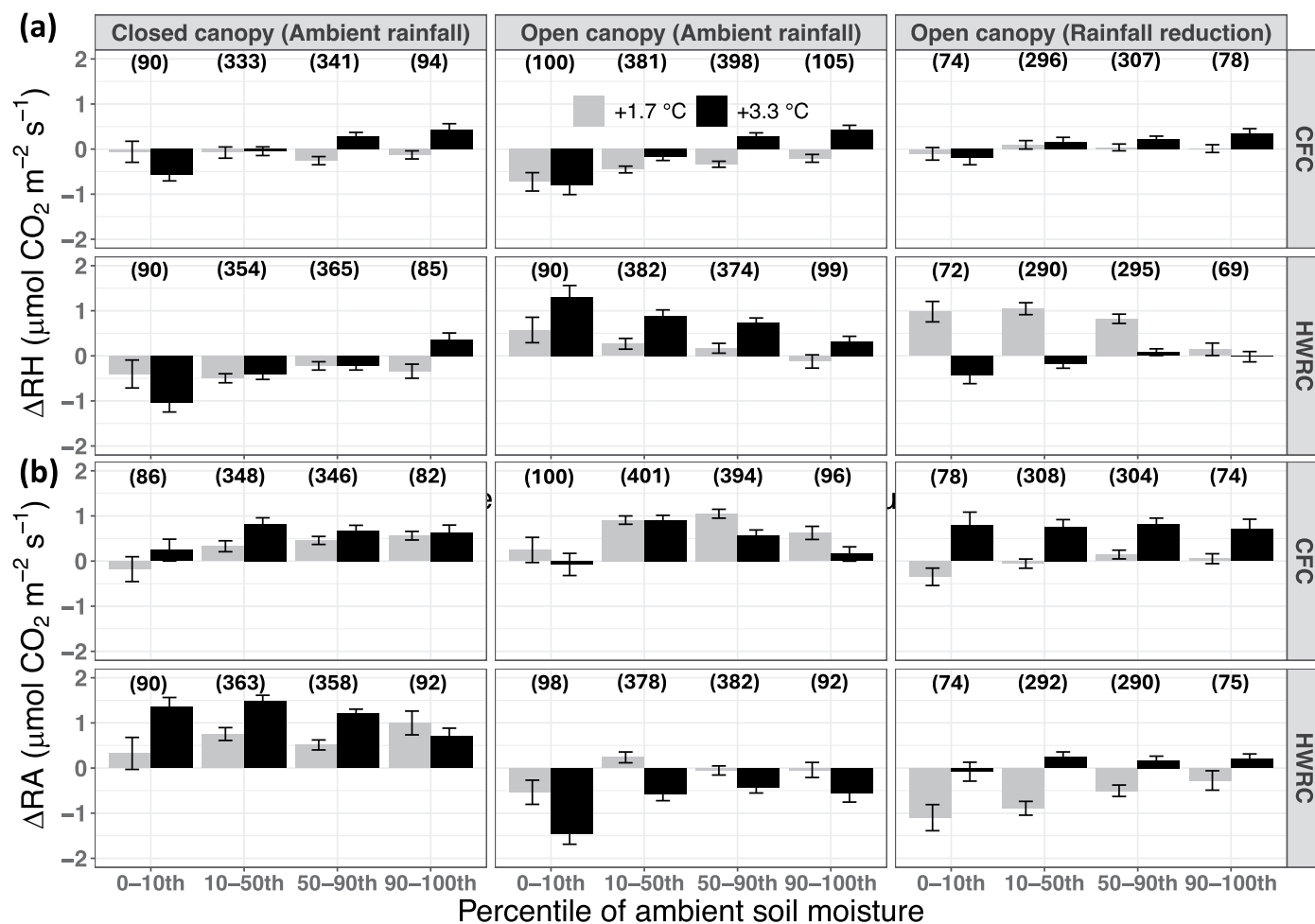
Extended Data Fig. 4 | Mean soil respiration and stem biomass averaged across all measurements at two sites (experimental warming × rainfall amount). Panels **a**, **b**, **c** and **d** show the results of RS, RH, RA and stem biomass, respectively. Error bars are standard errors of the mean for each treatment combination. RS: soil total respiration; RH: soil heterotrophic respiration; RA: soil autotrophic respiration. For RS and RA, the sample size was 6; for RH and stem biomass, the sample size was 3.
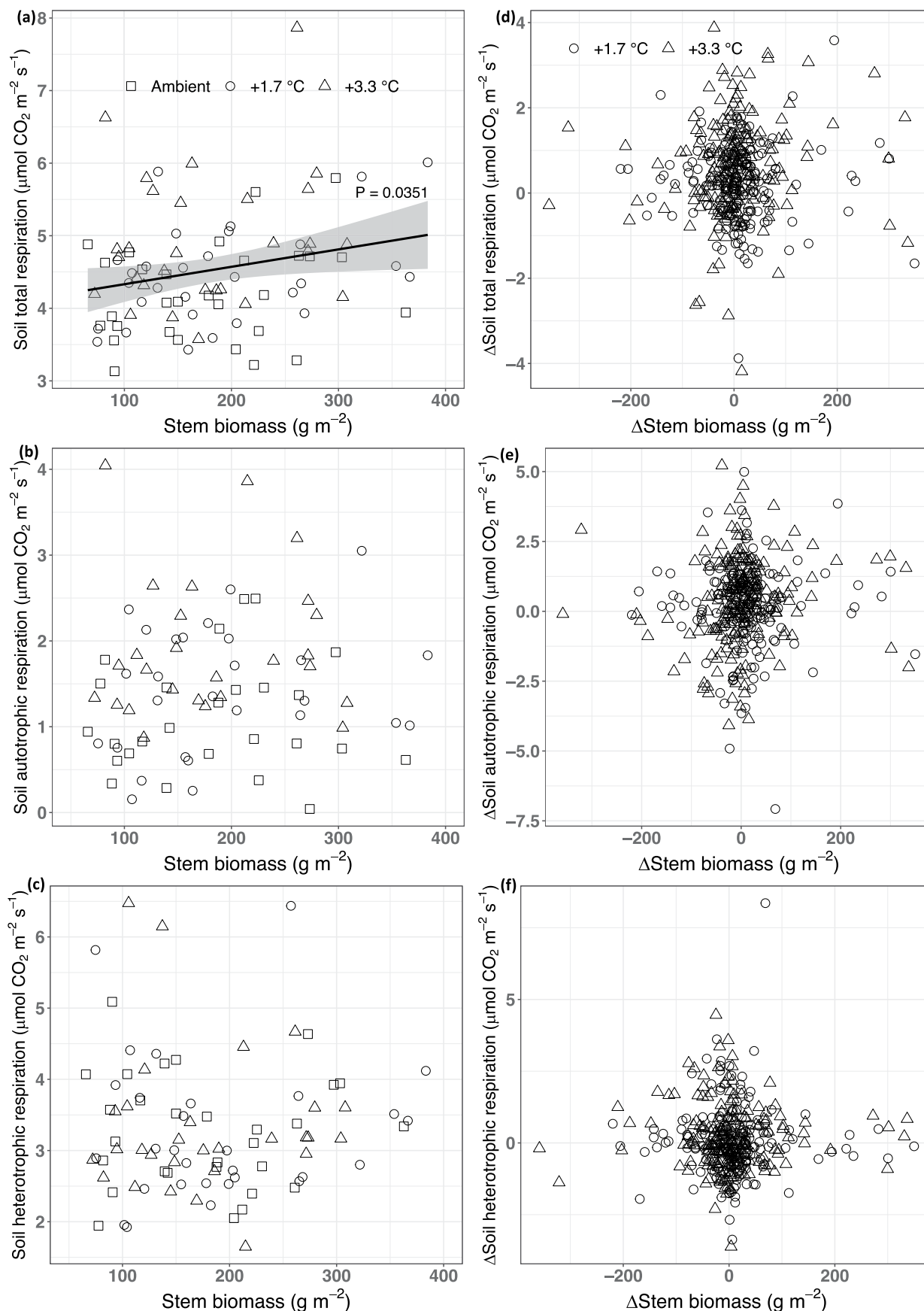
Extended Data Fig. 5 | The relationship between stem biomass and soil respiration (a-c) and between the changes in stem biomass and soil respiration caused by warming (d-f). Simple linear regressions were conducted, and P values were shown only when P < 0.1. The mean values across years from each plot were used to determine the relationships in panels **a-c**; the mean values of each block from each year were used to determine the relationships in panels **d-f**.

Extended Data Fig. 6 | The change in soil respiration caused by experimental warming under different percentiles of ambient soil moisture. Panels under "Closed canopy (Ambient rainfall)" and "Open canopy (Ambient rainfall)" show results under different overstory canopy openness; panels under "Open canopy (Ambient rainfall)" and "Open canopy (Rainfall reduction)" show results under different rainfall amounts. Ambient soil moisture is soil moisture under ambient warming treatment. Panels **a** and **b** show the results of the changes in RH and RA caused by warming, respectively. The measurements of soil moisture and respiration from all campaigns over 13 years were used. RH: soil heterotrophic respiration; RA: soil autotrophic respiration. Values in the parentheses represent the sample size under different percentiles for each treatment combination.
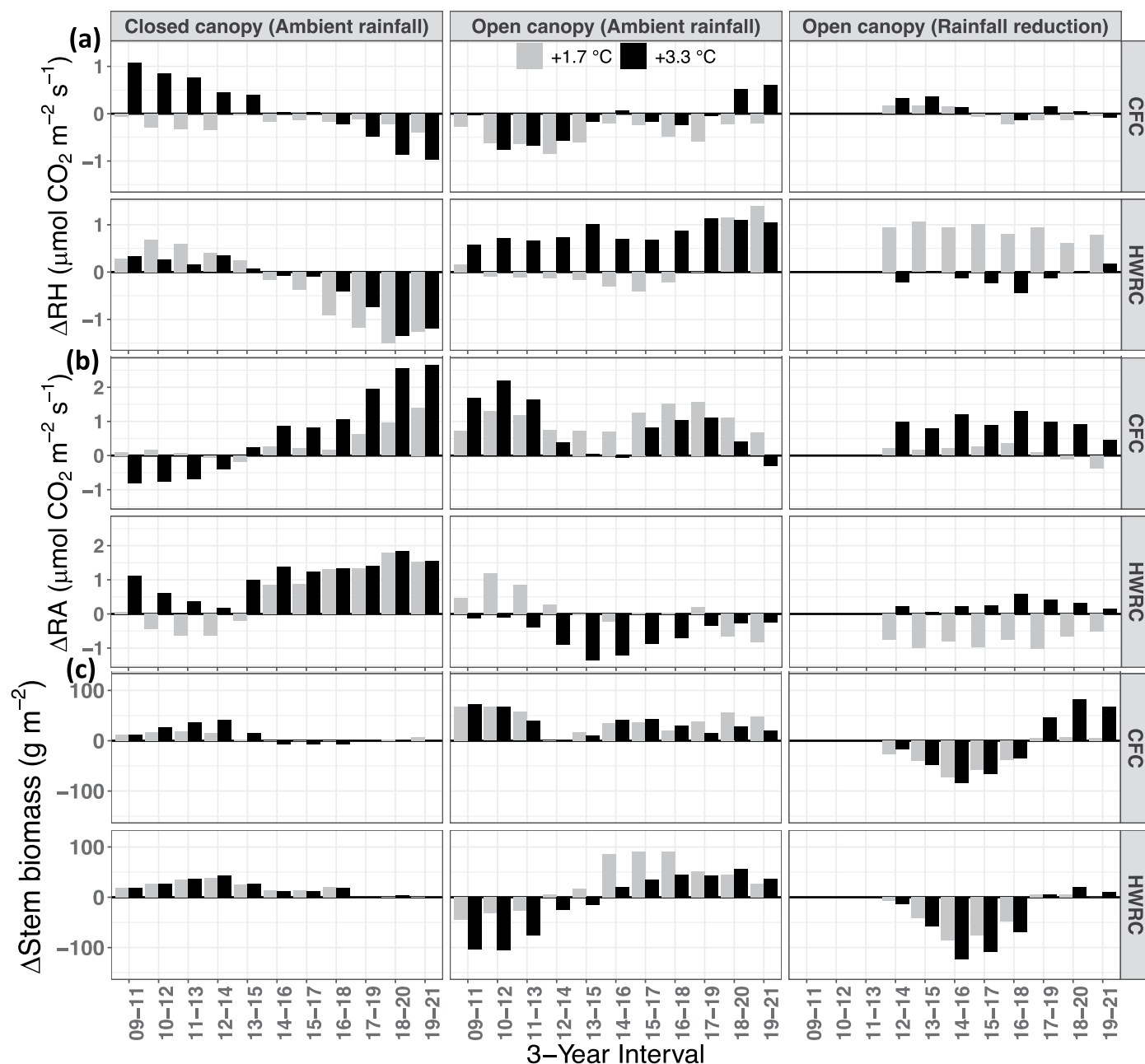
Extended Data Fig. 7 | Three-year rolling mean change in annual soil respiration and stem biomass under experimental warming relative to ambient treatment. Panels under "Closed canopy (Ambient rainfall)" and "Open canopy (Ambient rainfall)" show results under different overstory canopy openness; panels under "Open canopy (Ambient rainfall)" and "Open canopy (Rainfall reduction)" show results under different rainfall amounts. Panels **a**, **b** and **c** show the results of the changes in RH, RA and stem biomass caused by warming, respectively. No data were shown for "09-11", "10-12", and "11-13" under Open canopy (Rainfall reduction) treatment because rainfall reduction treatment was conducted in 2012. RH: soil heterotrophic respiration; RA: soil autotrophic respiration.

Extended Data Table 1 | Average values of soil respiration, temperature, moisture, and stem biomass under different experimental warming levels, overstory canopy openness, rainfall amounts, and field sites across the course of the experiment

Treatment	Site		RS	RH	RA	Soil T	Soil VWC	Stem
Warming	CFC	Ambient	4.41 (0.08)	3.39 (0.09)	1.04 (0.10)	13.8 (0.10)	0.22 (0.00)	204 (12)
		+ 1.7 °C	4.69 (0.06)	3.17 (0.08)	1.53 (0.10)	15.6 (0.10)	0.19 (0.00)	215 (15)
		+ 3.3 °C	5.25 (0.07)	3.43 (0.10)	1.83 (0.13)	17.1 (0.10)	0.18 (0.00)	216 (14)
	HWRC	Ambient	3.96 (0.09)	3.16 (0.10)	0.79 (0.11)	14.3 (0.12)	0.21 (0.00)	143 (10)
		+ 1.7 °C	4.21 (0.10)	3.22 (0.12)	0.99 (0.13)	16.1 (0.11)	0.20 (0.00)	152 (10)
		+ 3.3 °C	4.53 (0.08)	3.25 (0.11)	1.25 (0.14)	17.6 (0.12)	0.17 (0.00)	139 (9)
Canopy	CFC	Open	4.95 (0.08)	3.12 (0.07)	1.84 (0.08)	16.0 (0.12)	0.19 (0.00)	262 (13)
		Closed	4.60 (0.07)	3.56 (0.08)	1.05 (0.10)	14.9 (0.11)	0.20 (0.00)	139 (5)
	HWRC	Open	4.09 (0.05)	3.31 (0.09)	0.78 (0.10)	16.8 (0.12)	0.15 (0.00)	179 (9)
		Closed	4.39 (0.07)	3.10 (0.09)	1.26 (0.11)	15.2 (0.11)	0.24 (0.00)	95 (3)
Rainfall	CFC	Ambient	4.78 (0.06)	3.51 (0.06)	1.27 (0.07)	15.4 (0.10)	0.20 (0.00)	212 (9)
		Reduced	4.82 (0.14)	2.60 (0.08)	2.24 (0.13)	16.0 (0.19)	0.19 (0.00)	210 (15)
	HWRC	Ambient	4.33 (0.05)	3.29 (0.07)	1.03 (0.09)	15.8 (0.10)	0.21 (0.00)	145 (6)
		Reduced	3.84 (0.07)	2.90 (0.12)	0.95 (0.14)	16.7 (0.20)	0.14 (0.00)	143 (13)

RS: soil total respiration (μmol CO_2 m^{-2} s^{-1}); RH: soil heterotrophic respiration (μmol CO2 m^{-2} s^{-1}); RA: soil autotrophic respiration (μmol CO_2 m^{-2} s^{-1}); Soil T: soil temperature (°C); Soil VWC: soil moisture (m^3 m^{-3}); Stem: stem biomass (g m^{-2}). The mean annual values from each plot in each year were used, and the values in the parentheses were standard errors.