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Heterotrophic respiration does not acclimate to continuous warming in a subtropical forest

Chuansheng Wu^{1,2,3}, Naishen Liang⁴, Liqing Sha^{1,2}, Xingliang Xu⁵, Yiping Zhang^{1,2}, Huazheng Lu^{1,3}, Liang Song¹, Qinghai Song¹ & Youneng Xie⁶

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As heterotrophic respiration (R_H) has great potential to increase atmospheric CO_2 concentrations, it is important to understand warming effects on R_H for a better prediction of carbon–climate feedbacks. However, it remains unclear how R_H responds to warming in subtropical forests. Here, we carried out trenching alone and trenching with warming treatments to test the climate warming effect on R_H in a subtropical forest in southwestern China. During the measurement period, warming increased annual soil temperature by 2.1 °C, and increased annual mean R_H by 22.9%. Warming effect on soil temperature (WE_T) showed very similar pattern with warming effect on R_H (WE_{RH}), decreasing yearly. Regression analyses suggest that WE_{RH} was controlled by WE_T and also regulated by the soil water content. These results showed that the decrease of WE_{RH} was not caused by acclimation to the warmer temperature, but was instead due to decrease of WE_T . We therefore suggest that global warming will accelerate soil carbon efflux to the atmosphere, regulated by the change in soil water content in subtropical forests.

Global soil CO_2 efflux was estimated to be 80.4 Pg C yr⁻¹ from 1980 to 1994¹, a value that had increased to 98.0 Pg C yr⁻¹ by 2008². Global soil organic carbon was estimated to be about 3300 Pg, four times the amount present in living plants globally^{3,4}. There is a positive relationship between global soil CO_2 efflux and temperature with an increase of 3.3 Pg C yr⁻¹ per °C¹, indicating that increased temperature has a great potential to affect soil CO_2 efflux and produce a positive carbon–climate feedback under global warming. Forests cover only about 30% of the terrestrial ecosystems, but contain about 45% of terrestrial carbon⁵. Therefore, forest soil organic carbon decomposition plays an important role in the regional and global carbon cycle and studies have increasingly been conducted to explore the effects of warming on forest soil organic carbon decomposition, aiming to improve prediction of carbon–climate feedbacks.

Many previous studies focused on the warming effects on soil respiration^{6–10}. However, soil respiration includes two main components: heterotrophic respiration (R_H) and autotrophic respiration (R_A). Responses of heterotrophic respiration (R_H) and autotrophic respiration (R_A) to warming are not always the same. For example, Schindlbacher, *et al.*¹¹ reported that R_H and R_A responded similarly to temperature increase; both increasing with warming. However, Zhou, *et al.*¹² reported a negative effect of warming on both R_H and R_A . Other studies showed that warming increased R_H , but decreased R_A ^{13,14}. Because only the R_H component of soil respiration has potential to increase atmospheric CO_2 ¹⁵, it is important to separate R_H from soil respiration and to examine how warming may affect R_H ^{16–18}.

Forest soil organic carbon decomposition plays an important role in the carbon cycle, but most previous studies about WE_{RH} were carried out in grassland ecosystems^{12–14,19–21}. A few studies have been conducted in boreal coniferous forest dominated by Norway spruce^{11,22,23} and in a temperate forest¹⁸. To our knowledge, no studies have been reported in subtropical forests.

¹Key laboratory of Tropical Forest Ecology, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Mengla, 666303, China. ²Ailaoshan Station for Subtropical Forest Ecosystem Studies, Jingdong, 676209, China. ³University of Chinese Academy of Sciences, Beijing, 100049, China. ⁴Global Carbon Cycle Research Section, Center for Global Environmental Research, National Institute for Environmental Studies, Tsukuba, 305-8506, Japan. ⁵Key laboratory of Ecosystem Network Observation and Modelling, Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, Beijing, 100101, China. ⁶Jingdong Bureau of National Nature Reserve, Jingdong, Yunnan 676209, China. Correspondence and requests for materials should be addressed to Y.Z. (email: yipingzh@xtbg.ac.cn)

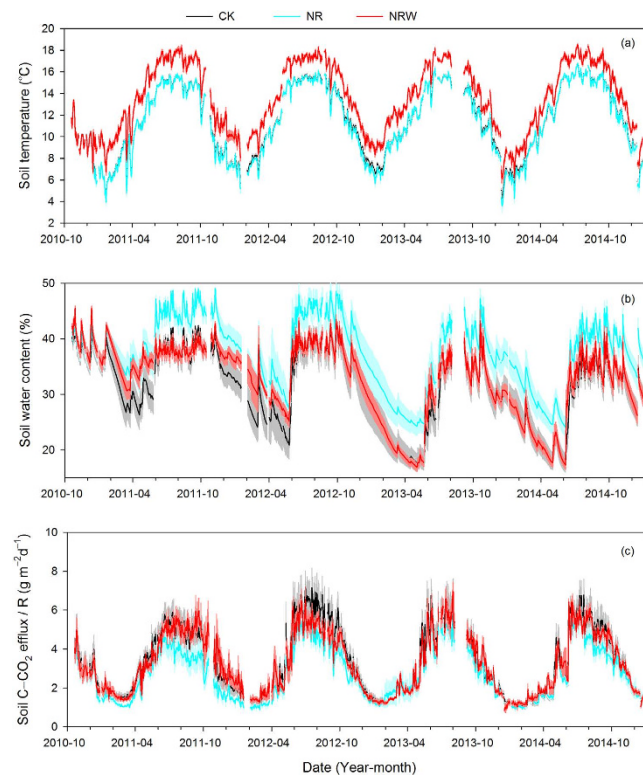


Figure 1. Seasonal variations of soil temperature (a), soil water content (b), and soil carbon efflux (c) after treatments began. Black lines with grey shadows represent the control treatment (CK), cyan lines with semi-transparent cyan shadows represent the trenching treatment (NR), and red lines with semi-transparent red shadows represent the trenching together with warming treatment (NRW) (Mean \pm SE).

The subtropical evergreen broad-leaved forest in the Ailao Mountains in Yunnan of southwestern China has been considered a carbon sink²⁴, but the carbon sink strength is likely to be weakened by warming²⁵. During the period from 1961 to 2004, the mean annual air temperature over the Yunnan Plateau (southwestern China) increased at a rate of 0.3 °C per decade²⁶. A recent study showed that the mean annual air temperature increased by 0.36 °C per decade from 1983 to 2010, leading to an increasing trend of 0.31 °C per decade from 1986 to 2010 in the top 10 cm of soil in the subtropical forest²⁷. Previous studies have shown that R_H exhibited seasonal variation and had a significant positive relationship with soil temperature^{28,29}. However, it remains unclear how soil warming affects R_H in this subtropical forest.

Previous studies have showed inconsistent results about WE_{RH} . A few studies suggested that warming decreased R_H ^{12,20}, while some demonstrated a positive effect on R_H which can be sustained for 5–10 years^{18,30,31}. Other studies argued acclimatization in terms of the warming effect declining over time^{8,22,32}, which may be ascribed to depletion of soil labile carbon^{16,19,33,34} or acclimatization of soil microbes^{35–38}. Other studies also argued acclimatization in terms of temperature sensitivity adaption^{11,17,21}. Based on mentioned studies above, we hypothesized that R_H acclimated to continuous warming in this forest. To test this hypothesis, we conducted ‘trenching alone’ and ‘trenching with warming’ treatments in this forest.

Results

Treatment effects on soil environmental factors and soil carbon efflux. Compared with the control treatment (CK), the trenching treatment (NR) did not change soil temperature (T , °C) (Fig. 1a), but increased soil water content (W , % (v/v)) (Fig. 1b). The trenching together with warming treatment (NRW) increased T (Fig. 1a), but did not change W , except for the first 1.5 years (Fig. 1b). Soil carbon efflux reduced in the NR treatment in the first year and changed little in the later years during the measurement period. In the NRW treatment, soil carbon efflux changed little (Fig. 1c).

Compared with the NR treatment, the NRW treatment increased T , but decreased W (by annual average of 5.1%, v/v) (Fig. 1a,b) and increased R_H especially in the first year (Fig. 1c).

There were similar seasonal variations in R , T , and W of the CK, NR, and NRW treatments (Fig. 1). The Pearson correlation analysis showed R more correlated to T (Table S1), and the two-factor regression model showed that R had a positive relationship with both T and W (Fig. 2).

Warming effects on R_H and T . To clarify the effect of warming on R_H , we focus on the affecting factor of changes to soil water content. Two-factor regression models (Fig. 2) were used for correction (for details see the data analysis section).

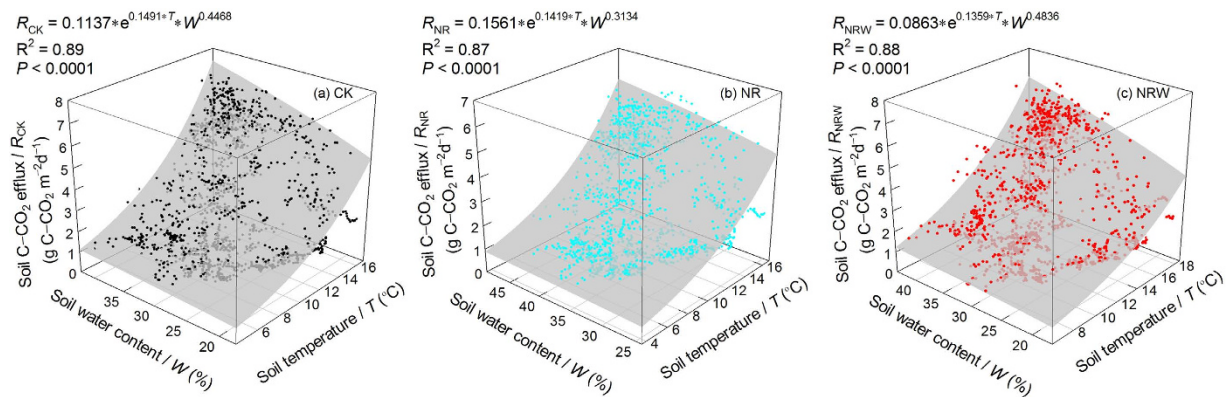


Figure 2. Two-factor regression of soil carbon efflux with soil temperature and soil water content.

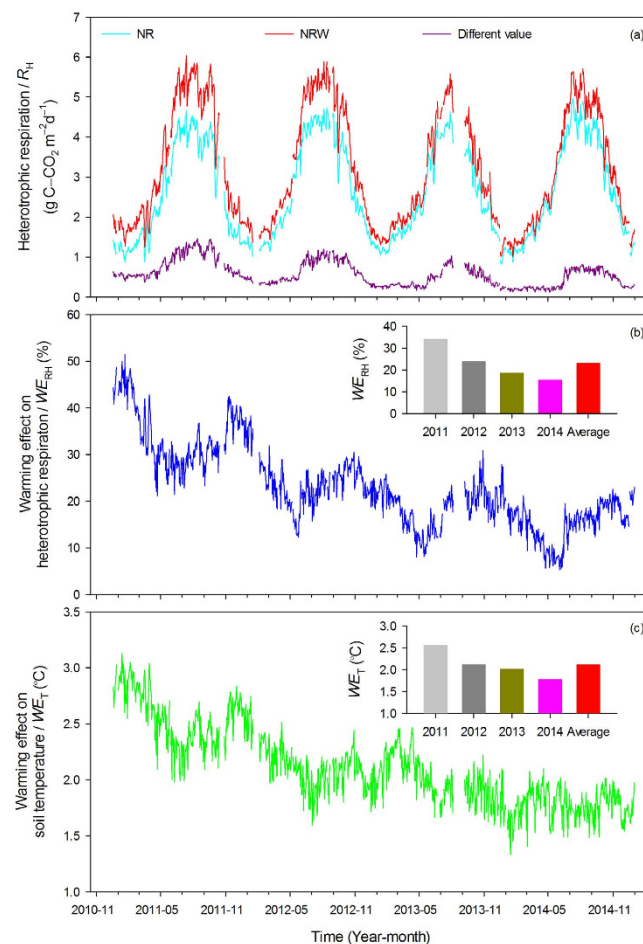


Figure 3. Variation of heterotrophic respiration in warmed (NRW) and unwarmed (NR) treatments and their difference ($R_{NRW} - R_{NR}$) (a), the variation of the warming effect on heterotrophic respiration (WE_{RH}) (b), and the variation of the warming effect on soil temperature (WE_T) (c).

Results show that R_H showed seasonal variation in both the NR and NRW treatments, with maximum values appearing in July or August and minimum values occurring in February. The difference value between NR and NRW ($R_{NRW} - R_{NR}$) also exhibited seasonal variation (Fig. 3a). WE_{RH} had similar seasonal variation to R_H with about 3 months delay; during the measurement period, WE_{RH} was 33.9, 23.8, 18.5, and 15.3% for 2011–2014, respectively (with an average of 22.9%), clearly decreasing year by year (Fig. 3b). Variation of the warming effect on T (WE_T) was very similar to that of WE_{RH} , and WE_T was 2.6, 2.1, 2.0, and 1.8 $^{\circ}C$ (with an average of 2.1 $^{\circ}C$) for 2011–2014, respectively (Fig. 3c).

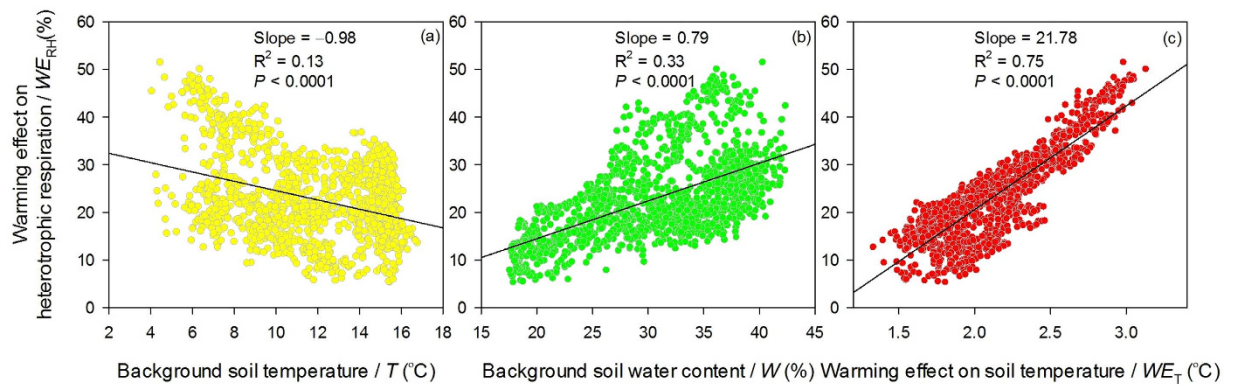


Figure 4. Relationships between WE_{RH} and T (a), W (b), and WE_T (c).

Factors affecting the warming effect on R_H . The factors T , W , and WE_T had a significant relationship with WE_{RH} ($p < 0.0001$), which meant WE_{RH} was affected by T , W , and WE_T . The factors T , W , and WE_T explained 13, 33, and 75% of WE_{RH} 's variation, respectively. A 1°C increase in T and a 1% decrease in W would reduce 0.98 and 0.79% of WE_{RH} , respectively; however, a 1°C increase in WE_T would cause a 21.78% increase in WE_{RH} (Fig. 4). This suggests that a range of increased soil temperature (WE_T) control WE_{RH} .

Discussion

Previous studies have shown that R_H acclimated to warmer temperature over the warming period^{8,22,32}. However, several long-term warming experiments have shown sustained WE_{RH} in ecosystems with high soil organic carbon^{18,30,31}. This indicates that such sustained stimulation may be attributed to high substrate availability¹⁸. In this study, soil organic carbon, soil labile organic carbon, and soil microbial carbon did not significantly ($p > 0.05$) decrease in NR and NRW comparing to CK during the 4-year measurement period, but they were larger in NRW than that in NR (Fig. S2). In this case, during the 4-year measurement period, substrate is unlikely to be a limiting factor^{28,39}. Our results show that WE_{RH} declined year by year. Without further analyses, this indicated “acclimatization” superficially (Fig. 3b). However, this decline probably was not caused by acclimation but was caused by the reduction in WE_T over the 4-year observation period (Figs 3c and 4c). The temperature sensitivity (Q_{10}) analysis showed that warming increased Q_{10} in the later period of this study which showed opposite result to previous studies^{11,17,21} and indicated the absence of “acclimatization” (Fig. S3). A current meta-analysis showed that WE_{RH} remained stable during warming period, which also challenged “acclimatization” of microbial activity to warmer temperature⁴⁰. Therefore, WE_T is likely to be the controlling factor in this study.

In this study, WE_{RH} (22.9%) was lower than that of Aguilos, *et al.*¹⁸, Schindlbacher, *et al.*¹¹ and Eliasson, *et al.*²² reported (82.0%, 42.0% and $> 30\%$, respectively). WE_T values in the experiments of Aguilos, *et al.*¹⁸, Schindlbacher, *et al.*¹¹ and Eliasson, *et al.*²² were 3, 4, and 5°C , respectively, all larger than ours (2.1°C). A review showed that warming by 2.0°C increased R_H by an average of 21.0%⁴⁰, similar to our result. Another 2-year soil monolith transplantation experiment at the same site demonstrated that soil organic carbon efflux increased by 70.5 and 62.6% because of an annual WE_T of 3.9 and 6.7°C (unpublished data, Fig. S4). These results suggest that a positive relationship between WE_{RH} and WE_T requires a specific range of WE_T , and when WE_T exceeds this range, WE_{RH} becomes restricted (Fig. S4). Therefore, WE_T controlled WE_{RH} in this subtropical forest.

Although soil temperature is more important than soil water content on affecting dynamics of soil carbon efflux (Fig. 1, Table S1), dynamics of soil water content may also affect WE_{RH} . For example, Liu, *et al.*²⁰ found that warming decreased soil water content, which resulted in a reduction in R_H . Similarly, a prolonged summer drought offsets soil warming effects⁹. Our results also suggest that soil water content affected WE_{RH} , mediating WE_{RH} under the warmer soil temperature conditions (Fig. 4b).

In this study, WE_T decreased yearly, perhaps owing to aging of warming lamps. WE_T also showed seasonal variation (Fig. 3c). These factors should be considered in future research when researchers are attempting to maintain a constant WE_T . Nevertheless, these results allowed us to analyse the WE_T effect on WE_{RH} . In this forest, R_H is $9.53 \text{ t C ha}^{-1} \text{ yr}^{-1}$ (Fig. 3a), and given an increase of 1°C in WE_T , WE_{RH} will increase by 21.78%, an additional soil carbon release to the atmosphere can be estimated to be about $2.65 \text{ t C ha}^{-1} \text{ yr}^{-1}$ from the subtropical forest.

Response of soil organic carbon to temperature depends on soil temperature⁴¹ and quality of soil organic carbon^{42,43}. Factors affecting soil carbon quality and decomposition processes can influence the temperature response and thus warming effect⁴⁴. As soil water also affects soil carbon efflux, its changes should be also considered to prediction of carbon–climate feedbacks^{40,45}. Further studies should focus on responses of WE_{RH} and interactions between factors.

Conclusions

In summary, our warming experiment increased soil temperature by 2.1°C and thereby increased heterotrophic respiration by 22.9% during the 4-year observation period. Continuous measurement allowed us to analyse variations of T , W , WE_T , and WE_{RH} and their inter-relationships. Our results show that the warming effect on R_H was controlled by a range of increased soil temperature and regulated by the variation of soil water content. This suggests that global warming will accelerate soil carbon efflux to the atmosphere, regulated by a change of soil

water content in response to warming and rainfall changes. Future warming in subtropical forests can accelerate release of soil organic carbon to the atmosphere.

Materials and Methods

Site description. This experiment was conducted at the Ailaoshan Station for Subtropical Forest Ecosystem Studies (24°32'N, 101°01'E; 2480 m above sea level) of the Chinese Ecological Research Network, which is located in Jingdong County, Yunnan Province, China. The annual mean air temperature was 11.3°C, with a minimum monthly mean temperature of 5.7°C in January and a maximum monthly mean temperature of 15.6°C in July. Average annual rainfall was 1778 mm, with 86.0% of this falling in the rainy season (May–October)²⁸. The dominant tree species in the forest are *Lithocarpus xylocarpus*, *Lithocarpus hancei*, and *Castanopsis wattii*. The soils are Alfisols with a pH of 4.5, soil organic carbon of 304 g kg⁻¹, and total nitrogen of 18 g kg⁻¹ in the humus horizon³⁹.

Data collection. A multichannel automated chamber system for continuous measurement of soil CO₂ effluxes was established in October 2010. The system comprised 20 automatic chambers (90 × 90 × 50 cm) and a control box (Fig. S1). On 17 December 2010, the 20 chambers were divided into four treatments (five chambers per treatment): control (CK), litter removal (NL), trenching (NR), and infrared light warming together with trenching (NRW). In this study, we only discuss the NR and NRW treatments and the control CK. The infrared light warming method has been frequently applied for soil warming in forest ecosystems^{3,34,46,47}, and is also used in this study. The key advantages of this method are that there is no disturbance to the soil structure and it involves the same process of warming as heating from the climatic warming effect (heating soil from the surface to a deeper depth). For trenching treatments, a 1 m × 1 m square trench (width 30 cm, depth 50 cm) was dug to form a cube of soil contained by PVC planks; soil was backfilled by its original layers with topsoil over subsoil. The main components of the control box are an infrared gas analyser (Li-820, Li-Cor Inc., Lincoln, NE, USA) and a datalogger (CR1000, Campbell Scientific Inc., Logan, UT, USA), as described in more detail in earlier studies^{48,49}.

Soil temperature (T , °C) at a 5-cm depth and air temperature (T_a , °C) were measured inside each chamber using self-made thermocouples⁴⁹. Soil water content (W , % (v/v)) at a 10-cm depth was monitored using time domain reflectometry (CS-616, Campbell Scientific Inc.)⁴⁹. Air pressure (P , hPa) at a 30-cm height in the centre of the plot was measured by a pressure transducer (PX2760, Omega Engineering, Inc., Stamford, CT, USA)⁴⁹.

Soil C–CO₂ efflux (R) was calculated as follows:

$$R = \frac{M}{V_0} \cdot \frac{P}{P_0} \cdot \frac{T_0}{T_a} \cdot H \cdot \frac{dc}{dt} \quad (1)$$

where R is soil C–CO₂ efflux (g C–CO₂ m⁻² d⁻¹); M is the C molar mass; V_0 , P_0 and T_0 are constants (22.4 L mol⁻¹, 1013.25 hPa, and 273.15 K, respectively); T_a and P are air temperature (K) and pressure (hPa), respectively; H is height of the respiration chamber (m); and dc/dt is the slope of CO₂ concentration variation with time over the measurement period (measurement for 3 min and the calculation ignores data from the first minute).

Data analysis. Every day, each chamber received 24 data points for R (hourly), and 48 data points for T and W (twice per hour), except during periods of electrical failure. For each chamber, available data were used to calculate daily average values. Previous analysis showed that R_H was unusual in the fifth group for both NR and NRW (Fig. S1); therefore, the fifth group was ignored, leaving four groups as the four repeats in this study.

The two-factor regression model was used to quantify the relationship of soil temperature (T) and soil water content (W) with soil carbon efflux (R) as follows⁵⁰:

$$R = a \cdot e^{bT} \cdot W^c \quad (2)$$

where a , b , and c are constants estimated from the regression model by the nonlinear regression dynamic fit wizard using Sigmaplot (Version 12.5, Systat. Software, Inc., Point Richmond, CA, USA) (for details see Fig. 2).

Figure 2 suggests that T and W had a positive effect on R , while treatments changed the soil microclimate (Fig. 1a,b). Compared to CK, NR increased soil water content; however compared to NR, NRW decreased soil water content (Fig. 1b). To eliminate the biases due to changed soil water content, WE_{RH} should be calculated under the same soil water content condition. Therefore, the background soil temperature and soil water content measured in the control treatment were used to correct the values of R_{NR} , using Equation (2). For NRW, the soil temperature of the NRW plots and the background soil water content of the control plots were used for the correction, so that the warming effect was due to the soil temperature increase without the effect of soil water content decrease (Fig. 3b). Parameters (a , b , and c) for NR and NRW were shown in Fig. 2b,c, and their estimation results were shown in Table S2.

Warming effects on R_H (WE_{RH} , %) and on soil temperature (WE_T , °C) were calculated using the following equations:

$$WE_{RH} = \frac{R_{NRW} - R_{NR}}{R_{NR}} \times 100\% \quad (3)$$

$$WE_T = T_{NRW} - T_{CK} \quad (4)$$

References

1. Raich, J. W., Potter, C. S. & Bhagawati, D. Interannual variability in global soil respiration, 1980–94. *Global Change Biology* **8**, 800–812 (2002).
2. Bond-Lamberty, B. & Thomson, A. Temperature-associated increases in the global soil respiration record. *Nature* **464**, 579–582, doi: 10.1038/nature08930 (2010).
3. Jobbagy, E. G. & Jackson, R. B. The vertical distribution of soil organic carbon and its relation to climate and vegetation. *Ecological Applications* **10**, 423–436, doi: 10.2307/2641104 (2000).
4. Tarnocai, C. *et al.* Soil organic carbon pools in the northern circumpolar permafrost region. *Global Biogeochemical Cycles* **23**, doi: 10.1029/2008gb003327 (2009).
5. Bonan, G. B. Forests and climate change: Forcings, feedbacks, and the climate benefits of forests. *Science* **320**, 1444–1449, doi: 10.1126/science.1155121 (2008).
6. Bronson, D. R., Gower, S. T., Tanner, M., Linder, S. & Van Herk, I. Response of soil surface CO₂ flux in a boreal forest to ecosystem warming. *Global Change Biology* **14**, 856–867, doi: 10.1111/j.1365-2486.2007.01508.x (2008).
7. Luo, Y. Q., Wan, S. Q., Hui, D. F. & Wallace, L. L. Acclimatization of soil respiration to warming in a tall grass prairie. *Nature* **413**, 622–625 (2001).
8. Melillo, J. M. *et al.* Soil warming and carbon-cycle feedbacks to the climate system. *Science* **298**, 2173–2176 (2002).
9. Schindlbacher, A. *et al.* Soil respiration under climate change: prolonged summer drought offsets soil warming effects. *Global Change Biology* **18**, 2270–2279, doi: 10.1111/j.1365-2486.2012.02696.x (2012).
10. Xia, J., Han, Y., Zhang, Z., Zhang, Z. & Wan, S. Effects of diurnal warming on soil respiration are not equal to the summed effects of day and night warming in a temperate steppe. *Biogeosciences* **6**, 1361–1370, doi: 10.5194/bg-6-1361-2009 (2009).
11. Schindlbacher, A., Zechmeister-Boltenstern, S. & Jandl, R. Carbon losses due to soil warming: Do autotrophic and heterotrophic soil respiration respond equally? *Global Change Biology* **15**, 901–913, doi: 10.1111/j.1365-2486.2008.01757.x (2009).
12. Zhou, X. *et al.* Concurrent and lagged impacts of an anomalously warm year on autotrophic and heterotrophic components of soil respiration: a deconvolution analysis. *New Phytologist* **187**, 184–198, doi: 10.1111/j.1469-8137.2010.03256.x (2010).
13. Verburg, P. S. J., Larsen, J., Johnson, D. W., Schorran, D. E. & Arnone, J. A. Impacts of an anomalously warm year on soil CO₂ efflux in experimentally manipulated tallgrass prairie ecosystems. *Global Change Biology* **11**, 1720–1732, doi: 10.1111/j.1365-2486.2005.01032.x (2005).
14. Li, D., Zhou, X., Wu, L., Zhou, J. & Luo, Y. Contrasting responses of heterotrophic and autotrophic respiration to experimental warming in a winter annual-dominated prairie. *Global Change Biology* **19**, 3553–3564, doi: 10.1111/gcb.12273 (2013).
15. Kuzyakov, Y. Sources of CO₂ efflux from soil and review of partitioning methods. *Soil Biology & Biochemistry* **38**, 425–448, doi: 10.1016/j.soilbio.2005.08.020 (2006).
16. Kirschbaum, M. U. F. Soil respiration under prolonged soil warming: are rate reductions caused by acclimation or substrate loss? *Global Change Biology* **10**, 1870–1877, doi: 10.1111/j.1365-2486.2004.00852.x (2004).
17. Zhou, X., Wan, S. Q. & Luo, Y. Q. Source components and interannual variability of soil CO₂ efflux under experimental warming and clipping in a grassland ecosystem. *Global Change Biology* **13**, 761–775, doi: 10.1111/j.1365-2486.2007.01333.x (2007).
18. Aguilos, M. *et al.* Sustained large stimulation of soil heterotrophic respiration rate and its temperature sensitivity by soil warming in a cool-temperate forested peatland. *Tellus Series B-Chemical and Physical Meteorology* **65**, doi: 10.3402/tellusb.v65i0.20792 (2013).
19. Hartley, I. P., Heinemeyer, A. & Ineson, P. Effects of three years of soil warming and shading on the rate of soil respiration: substrate availability and not thermal acclimation mediates observed response. *Global Change Biology* **13**, 1761–1770, doi: 10.1111/j.1365-2486.2007.01373.x (2007).
20. Liu, W. X., Zhang, Z. & Wan, S. Q. Predominant role of water in regulating soil and microbial respiration and their responses to climate change in a semiarid grassland. *Global Change Biology* **15**, 184–195, doi: 10.1111/j.1365-2486.2008.01728.x (2009).
21. Suseela, V., Conant, R. T., Wallenstein, M. D. & 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, doi: 10.1111/j.1365-2486.2011.02516.x (2012).
22. Eliasson, P. E. *et al.* The response of heterotrophic CO₂ flux to soil warming. *Global Change Biology* **11**, 167–181, doi: 10.1111/j.1365-2486.2004.00878.x (2005).
23. Schindlbacher, A., Zechmeister-Boltenstern, S., Kitzler, B. & Jandl, R. Experimental forest soil warming: response of autotrophic and heterotrophic soil respiration to a short-term 10 degrees C temperature rise. *Plant and Soil* **303**, 323–330, doi: 10.1007/s11104-007-9511-2 (2008).
24. Tan, Z.-H. *et al.* An old-growth subtropical Asian evergreen forest as a large carbon sink. *Atmospheric Environment* **45**, 1548–1554, doi: 10.1016/j.atmosenv.2010.12.041 (2011).
25. Tan, Z.-H. *et al.* An observational study of the carbon-sink strength of East Asian subtropical evergreen forests. *Environmental Research Letters* **7**, 10.1088/1748-9326/1087/1084/044017, doi: 10.1088/1748-9326/7/4/044017 (2012).
26. Fan, Z.-X., Braeuning, A., Thomas, A., Li, J.-B. & Cao, K.-F. Spatial and temporal temperature trends on the Yunnan Plateau (Southwest China) during 1961–2004. *Int J Climatol* **31**, 2078–2090, doi: 10.1002/joc.2214 (2011).
27. You, G. *et al.* Observed air/soil temperature trends in open land and understory of a subtropical mountain forest, SW China. *Int J Climatol* **33**, 1308–1316, doi: 10.1002/joc.3494 (2013).
28. Wu, C. *et al.* Influence of interactions between litter decomposition and rhizosphere activity on soil respiration and on the temperature sensitivity in a subtropical montane forest in SW China. *Plant and Soil* **381**, 215–224, doi: 10.1007/s11104-014-2106-9 (2014).
29. Schaefer, D. A., Feng, W. T. & Zou, X. M. Plant carbon inputs and environmental factors strongly affect soil respiration in a subtropical forest of southwestern China. *Soil Biology & Biochemistry* **41**, 1000–1007, doi: 10.1016/j.soilbio.2008.11.015 (2009).
30. Dorrepaal, E. *et al.* Carbon respiration from subsurface peat accelerated by climate warming in the subarctic. *Nature* **460**, 616–619, doi: 10.1038/nature08216 (2009).
31. Reth, S., Graf, W., Reichstein, M. & Munch, J. C. Sustained stimulation of soil respiration after 10 years of experimental warming. *Environmental Research Letters* **4**, 10.1088/1748-9326/1084/1082/024005, doi: 10.1088/1748-9326/4/2/024005 (2009).
32. Lin, G. H., Rygielwicz, P. T., Ehleringer, J. R., Johnson, M. G. & Tingey, D. T. Time-dependent responses of soil CO₂ efflux components to elevated atmospheric CO₂ and temperature in experimental forest mesocosms. *Plant and Soil* **229**, 259–270, doi: 10.1023/a:1004883221036 (2001).
33. Knorr, W., Prentice, I. C., House, J. I. & Holland, E. A. Long-term sensitivity of soil carbon turnover to warming. *Nature* **433**, 298–301, doi: 10.1038/nature03226 (2005).
34. Xu, X., Sherry, R. A., Niu, S., Zhou, J. & Luo, Y. Long-term experimental warming decreased labile soil organic carbon in a tallgrass prairie. *Plant and Soil* **361**, 307–315, doi: 10.1007/s11104-012-1265-9 (2012).
35. Bradford, M. A., Watts, B. W. & Davies, C. A. Thermal adaptation of heterotrophic soil respiration in laboratory microcosms. *Global Change Biology* **16**, 1576–1588, doi: 10.1111/j.1365-2486.2009.02040.x (2010).
36. Deslippe, J. R., Hartmann, M., Mohn, W. W. & Simard, S. W. Long-term experimental manipulation of climate alters the ectomycorrhizal community of *Betula nana* in Arctic tundra. *Global Change Biology* **17**, 1625–1636, doi: 10.1111/j.1365-2486.2010.02318.x (2011).
37. Rousk, J., Frey, S. D. & Baath, E. Temperature adaptation of bacterial communities in experimentally warmed forest soils. *Global Change Biology* **18**, 3252–3258, doi: 10.1111/j.1365-2486.2012.02764.x (2012).

38. Crowther, T. W. & Bradford, M. A. Thermal acclimation in widespread heterotrophic soil microbes. *Ecol Lett* **16**, 469–477, doi: 10.1111/ele.12069 (2013).
39. Chan, O. C. *et al.* 16S rRNA gene analyses of bacterial community structures in the soils of evergreen broad-leaved forests in south-west China. *Fems Microbiology Ecology* **58**, 247–259, doi: 10.1111/j.1574-6941.2006.00156.x (2006).
40. Wang, X. *et al.* Soil respiration under climate warming: differential response of heterotrophic and autotrophic respiration. *Global Change Biology* **20**, 3229–3237, doi: 10.1111/gcb.12620 (2014).
41. Hamdi, S., Moyano, F., Sall, S., Bernoux, M. & Chevallier, T. Synthesis analysis of the temperature sensitivity of soil respiration from laboratory studies in relation to incubation methods and soil conditions. *Soil Biology & Biochemistry* **58**, 115–126, doi: 10.1016/j.soilbio.2012.11.012 (2013).
42. Conant, R. T. *et al.* Sensitivity of organic matter decomposition to warming varies with its quality. *Global Change Biology* **14**, 868–877, doi: 10.1111/j.1365-2486.2008.01541.x (2008).
43. Davidson, E. A. & Janssens, I. A. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature* **440**, 165–173, doi: 10.1038/nature04514 (2006).
44. Conant, R. T. *et al.* Temperature and soil organic matter decomposition rates—synthesis of current knowledge and a way forward. *Global Change Biology* **17**, 3392–3404, doi: 10.1111/j.1365-2486.2011.02496.x (2011).
45. Moyano, F. E. *et al.* The moisture response of soil heterotrophic respiration: interaction with soil properties. *Biogeosciences* **9**, 1173–1182, doi: 10.5194/bg-9-1173-2012 (2012).
46. Moyes, A. B., Castanha, C., Germino, M. J. & Kueppers, L. M. Warming and the dependence of limber pine (*Pinus flexilis*) establishment on summer soil moisture within and above its current elevation range. *Oecologia* **171**, 271–282, doi: 10.1007/s00442-012-2410-0 (2013).
47. Yin, H. *et al.* Enhanced root exudation stimulates soil nitrogen transformations in a subalpine coniferous forest under experimental warming. *Global Change Biology* **19**, 2158–2167, doi: 10.1111/gcb.12161 (2013).
48. Liang, N. S., Inoue, G. & Fujinuma, Y. A multichannel automated chamber system for continuous measurement of forest soil CO₂ efflux. *Tree Physiology* **23**, 825–832 (2003).
49. Tan, Z. H. *et al.* Soil respiration in an old-growth subtropical forest: Patterns, components, and controls. *Journal of Geophysical Research-Atmospheres* **118**, 2981–2990, doi: 10.1002/jgrd.50300 (2013).
50. Xu, M. & Qi, Y. Soil-surface CO₂ efflux and its spatial and temporal variations in a young ponderosa pine plantation in northern California. *Global Change Biology* **7**, 667–677 (2001).

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Author Contributions

L.S., Y.Z., N.L., Q.S. and Y.X. designed the experiment. C.W. conducted the experiments, analysed the data, and wrote the manuscript. X.X., H.L. and L.S. assisted with the manuscript. All authors reviewed and commented on the manuscript.

Additional Information

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