Effects of *in situ* warming and glucose addition on soil respiration in a temperate forest

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(Received March 8, 2023; revised April 13, 2023; accepted May 5, 2023)

ABSTRACT

As studies on priming effect (PE) under field conditions are scarce, the carbon sequestration potential of PE on soil organic carbon (SOC) turnover remains uncertain. Therefore, the present study assessed the direct effects of glucose addition and *in situ* warming on the extent of PE under field conditions in a temperate forest. A simple and novel technique based on Keeling plots was applied. Glucose addition significantly enhanced native SOC decomposition and induced strong PE. However, the effect of *in-situ* warming on the extent of PE was not significant. The present study confirms the importance of PE in regulating SOC turnover under field conditions.

Key Words: ^{13}C-glucose, global warming, priming effect, phospholipid fatty acids (PLFAs), SOM mineralisation, soil enzymes

Citation: Dong X H, Fang Y T, Liu D W, Guan X, Huang K, Liu Y Q, Wang Q K, Wang S L, Zhang W D. 2023. Effects of *in situ* warming and glucose addition on soil respiration in a temperate forest. *Pedosphere*. **33**.

INTRODUCTION

Atmospheric temperature has increased by over 1 °C since the 1900s and has been predicted to increase by another 2.7 °C by 2100 (IPCC, 2021). In the face of ongoing climate change, the effects of warming on

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soil carbon and nutrient cycling (Zhang *et al.*, 2005; Allison *et al.*, 2010; Butenschoen *et al.*, 2011; Conant *et al.*, 2011; Xu *et al.*, 2016) have been extensively studied. As such, many studies have shown that in forest (Allison and Treseder, 2008; Xu *et al.*, 2010) and grassland (Murata *et al.*, 1999; Bai *et al.*, 2013) ecosystems, soil respiration is significantly enhanced by elevated soil temperature through altered soil microbial turnover and activity. In addition, the amount of CO_2 released from soil organic carbon (SOC) decomposition may be altered by easily utilizable C addition, termed the priming effect (PE) (Kuzyakov *et al.*, 2000). However, the interactive effect of PE and global warming on soil respiration remains unclear, and understanding their interactions and the underlying mechanisms is vital for predicting future soil C dynamics under climate change (Perveen *et al.,* 2014).

Many incubation studies using ^{13}C or ^{14}C labelling techniques have shown that the addition of external organic C (e.g. plant litter and glucose) significantly accelerated or inhibited the decomposition rate of native SOC and induced a strong positive or negative PE (Kuzyakov *et al.*, 2000). Typically, positive PE is primarily induced by the activation of soil microbes caused by the addition of available substrates (Bol *et al.*, 2003; Kuzyakov, 2010; Li *et al.*, 2017b), whilst possible negative PE-inducing mechanisms include the toxicity of the added substrates to microorganisms and the associated inhibition of enzyme activity (Blagodatskaya and Kuzyakov, 2008). The direction and extent of PE may be affected by incubation duration, addition quality, and soil properties. For instance, soils with a higher SOC content (Kuzyakov *et al.*, 2000; Luo *et al.*, 2015; Finley *et al.*, 2018) and fine texture (Luo *et al.*, 2016) exhibited a stronger PE. Moreover, the extent of PE is generally higher at the early stages of incubation than at the later stages (Zhang and Wang, 2012; Zhang *et al.*, 2013; Chao *et al.*, 2019).

Under climate-warming conditions, the effects of soil temperature on the extent of PE must be explored. Over a decade ago, Kuzyakov (2010) predicted that the extent of PE may be lower at higher incubation temperatures. This assumption appears to be reasonable because at higher temperatures, the level of native SOC decomposition may be difficult to increase further through labile substrate addition (Guenet *et al.*, 2010; Kuzyakov, 2010; Zhang *et al.*, 2013). Indeed, some laboratory studies conducted at different incubation temperatures have reported that the extent of positive PE was much lower at higher temperatures than at lower temperatures (Frøseth and Bleken, 2015; Wang *et al.*, 2016a, 2016b). In addition, PE is generally lower in soils with higher nitrogen availability (Hartley *et al.*, 2010; Zhang and Wang, 2012; Chen *et al.*, 2014), and the level of soil N availability can be enhanced by elevated temperature (Butler *et al.*, 2012; Averill and Waring, 2018; Chen *et al.*, 2018a, 2018b). In this context, PE is expected to be weaker at higher temperatures according to the 'N mining theory' (Fontaine *et al.*, 2003). However, other studies have shown that elevated incubation temperatures significantly enhanced the extent of PE (Zhu and Cheng, 2011; Thiessen *et al.*, 2013; Streit *et al.*, 2014; Li *et al.*, 2017a, 2017b), and a similar extent of PE at different incubation temperatures has also been reported (Ghee *et al.*, 2013; Hopkins *et al.*, 2014; Guttières *et al.*, 2021). This inconsistency in results indicates that more studies are warranted to explore the response of PE to warming and the underlying mechanisms, particularly under field conditions.

Our current understanding of the PE of SOC and its driving factors has mainly been derived from laboratory incubation experiments. Typically, in such experiments, soil sampled from the field is incubated at a constant temperature after the addition of fresh organic C. In this approach, mimicking the seasonal dynamics of soil physicochemical conditions and interactions between plants and microbes is difficult due to depletion of fast-cycling C and microbial adaptation to certain temperatures during incubation. Thus, the direct PE and its extent under field conditions cannot be assessed reliably, and whether the importance of PE is overestimated in incubation experiments remains unknown. However, limited field studies have been conducted on PE due to the difficulties in ^{13}C isotope measurements of soil respiration. In such studies, ^{13}C isotope measurements of soil respiration is essential to separate CO_2 flux-derived native SOC and added fresh substrates. In laboratory-based incubation experiments, this is achieved by flushing the incubation jar

with CO_2-free air to avoid interference from the existing CO_2, which is difficult under field conditions. Recent studies have shown that a technique based on Keeling plots allows for easy ^{13}C isotope measurements of soil respiration under field conditions (Keeling, 1958). Consequently, this technique has been widely used to measure ^{13}C isotopes for tree stem respiration (Salomón *et al.*, 2019) and to determine C isotope composition of ecosystem respiration (Fontaine *et al.*, 2003; Kodama *et al.*, 2011; Ravn *et al.*, 2017). Thus, combined with ^{13}C-labeled substrate addition, this approach can facilitate the assessment of PE under field conditions.

Temperate forests play a pivotal role in national C budgets and climate systems (Wang and Yang, 2007; Sui *et al.*, 2013; Wang *et al.*, 2016a). Generally, soil respiration in such ecosystems is limited by soil N availability and is highly responsive to elevated temperatures (Melillo *et al.*, 2011; Butler *et al.*, 2012). In the present study, we added ^{13}C-labelled glucose, a labile C substrate commonly present in plant exudates, to the soil to mimic root exudates. In a temperate forest, we conducted an experiment to assess the direction and extent of PE of soil respiration induced by glucose addition and tested the effect of warming on the extent of PE on an experimental platform. We performed an *in situ* experiment by setting warming control at 2 °C and 4 °C above the ambient value using a Qingyuan warming platform to alleviate the possible C limitation and induce N limitation. We hypothesised that (1) the soil respiration rate would be higher in the warming plots than in the control plots; (2) soil respiration would be significantly enhanced by glucose addition due to altered soil microbial community composition and activity; and (3) the extent of PE would be weaker in the warming plots due to improved soil N availability.

MATERIALS AND METHODS

Site description

The warming experiment was conducted in the Qingyuan Forest of the Chinese Ecosystem Research Network (CERN), Chinese Academy of Sciences, which is located in Qingyuan County, Liaoning Province, Northeast China (41°51′, 124°54′E). The region experiences a continental monsoon climate. The mean annual temperature for this site is 4.5 °C, and average annual precipitation is 811 mm (Zhu *et al.*, 2007). The dominant soil type found is Udalfs with clay loamy texture (sand = 25.6%, silt = 51.2%, clay = 23.2%). The study site is a typical temperate mixed coniferous broad-leaf forest. The dominant tree species, including *Juglans mandshurica*, *Quercus mongolica*, and *Larix kaempferi*, coexist in the canopy layer (Duan *et al.,* 2022).

Experimental design

In 2018, nine experimental plots (2 × 3 m^2) were established and randomly subjected to three warming treatments with three replicates to simulate different warming scenarios: control (CK), low-intensity warming (LW, +2 °C), and high-intensity warming (HL, +4 °C). A 1 m buffer was designed between the adjacent plots. Within each warming plot, rod-shaped infrared heaters (8 mm diameter × 151 cm length, 2000 W; 240 V Model, HS-2420 from Kalglo Electronics Co. Inc., USA) with equilateral triangle housing were installed to mimic the natural climate warming process (Kimball, 2005, Kimball *et al.,* 2012). The IR heaters were suspended 2 m above the ground level. One rod-shaped infrared heater was installed for LW and two for HW. A feedback control system was used to achieve the target warming between the warmed and control plots (Duan *et al.*, 2022). Soil temperatures at two points in each plot (at 5 cm mineral depth) were recorded using probes (PT100, Comity Inc, Beijing, China). Volumetric moisture contents at two points (0--10 cm mineral soil layer) in each plot were measured using probes (AQ, Computer Network Information Center, Chinese Academy of Sciences, Beijing, China).

Glucose addition and soil respiration measurement

Warming treatment on the experimental plot was initiated on 1 May 2019. Three months later, on August 18, two plastic chambers (25 cm diameter and 20 cm height) were installed in each plot. The chambers were inserted 5 cm into the soil to ensure an airtight seal. Glucose solution (glucose concentration = 12.17 g L^{-1}; average $\delta^{13}C$ in labelled glucose = 581‰; evenly spread on the soil surface in the form of liquid) was added into one of the chambers, and the other was used as control (CK). Soil respiration was measured at 1, 3, 6, 10, 15, 21, 28, 35, 45, and 58 days after the addition of ^{13}C-labelled glucose. During each measurement, the chambers were sealed by closing the manual valve, and three gas samples were collected at 0, 40, and 80 min. CO_2 concentration and $\delta^{13}C$ were analysed using a high-precision isotopic CO_2 Cavity Ring-Down Spectrometer (Picarro G2131-i Analyser; Picarro, Inc., Santa Clara, CA, USA). The experiment was completed on 14 October 2019 owing to the snow cover.

Calculation and quantification of the PE

Linear regression modelling was applied to analyse the changes in CO_2 concentration in each chamber over a 40 min period to obtain the slopes (i.e. CO_2 production rate) of the association between CO_2 concentration and time. The slope is rational when the regression coefficients are >0.9 (Metcalfe *et al.*, 2007):

$$F = \Delta C / \Delta t \times 273 / (273 + T) / 22.4 \times V / A$$

where F is the CO_2 flux (μmol m^{-2} s^{-1}), ΔC is the change in CO_2 concentration over a period of time (Δt), T is the air temperature inside the chambers, 22.4 (L) is the molar volume of an ideal gas at a standard temperature and pressure, V is the chamber volume (m^3), and A is the chamber area (m^2).

The mass balance equation was used to separate glucose-derived CO_2 from the soil-derived CO_2:

$$C_G = C_t \frac{(\delta_t - \delta_s)}{(\delta_G - \delta_s)} \quad (1)$$

$$C_s = C_t - C_G \quad (2)$$

where C_t is the total CO_2 from soil respiration ($C_t = C_G + C_S$) during the given time period, C_G is the amount of CO_2 derived from glucose mineralisation, C_S is the amount of CO_2 derived from native soil respiration, δ_t is the ^{13}C value of CO_2 emitted from the chamber containing the soil and glucose mixture, and δ_G and δ_S are the $\delta^{13}C$ values of glucose and soil, respectively.

The PE induced by glucose addition on native soil organic matter (SOM) mineralisation was defined as the % change compared with the CO_2 released from the control. The magnitude of PE was calculated using the following equation:

$$PE(\%) = (Rs_{\text{soil with glucose}} - Rs_{\text{control soil}}) / Rs_{\text{control soil}} \times 100$$

where $Rs_{\text{control soil}}$ is the CO_2 emitted by the control soil, and $Rs_{\text{soil with glucose}}$ is the CO_2 released from the soil treated with glucose.

Soil sampling and analysis of physiochemical properties

At the end of the experiment, soil samples from 0 to 10 cm depth were collected from each chamber and passed through a 2 mm sieve. Subsamples of each soil sample were separated to determine gravimetric water content and soil chemical properties. Soil mineral N (NH_4^+-N and NO_3^--N) and total and available

phosphorus concentrations were measured according to the methods described by Lu (2000) using a continuous flow analyser (AA3, Seal Analytical, Germany). SOC and total N contents were analysed using an elemental analyser (Vario Macro Elementar, GmbH, Germany). The total P concentration was determined using the colorimetric method described by Lu (2000). The potential activities of five soil extracellular enzymes involved in the acquisition of C (e.g. β-glucosidase, peroxidase, and polyphenol oxidase), N (leucine aminopeptidase), and P (acid phosphatase) were assayed using a microplate spectrophotometer. Detailed methods for phospholipid-derived fatty acids (PLFAs) and soil enzyme activity analyses are provided in the Supplementary Material.

Statistical analysis

Repeated measures ANOVA was performed to analyse the effects of warming, glucose addition and the interaction between warming and glucose on SOM mineralisation, glucose mineralisation, and PE. All statistical analyses were performed in SPSS 21.0 (version 21.0, SPSS Inc., Chicago, IL, USA) at the significance level of $P < 0.05$.

RESULTS

Effect of warming on soil temperature and moisture

During the experimental period, the daily mean temperature of soil in CK plots showed a large temporal variance, ranging from 6.51 °C to 17.94 °C. Soil temperature differences in the LW, HW, and control plots remained relatively constant during the experiment (Fig. 1a). At the end of October, the measurement of soil respiration and the associated ^{13}C isotope abundance was stopped because of snow cover. The effect of warming on soil moisture was not significant (Fig. 1b).

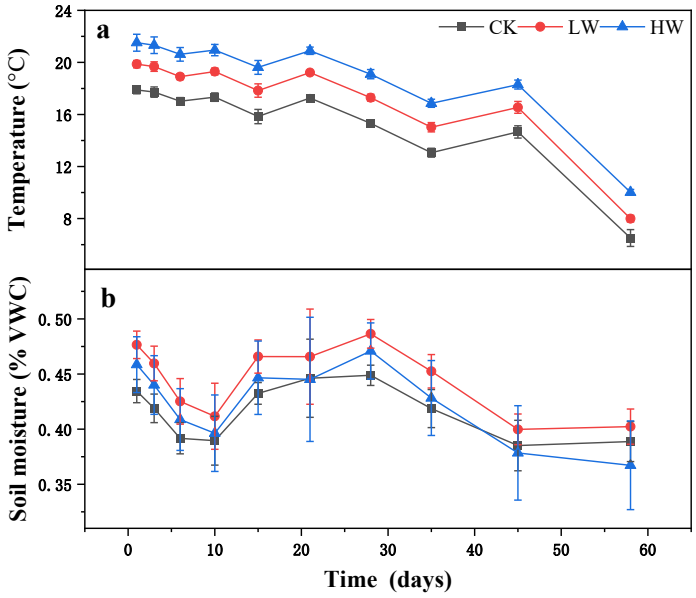

Fig. 1 Daily variations of temperature at the 5 cm depth of the mineral soil temperature (a) and soil volume moisture content in the 0--10 cm soil (b) during the experimental period (18 August to 14 October 2019). CK = control with no warming; LW = low-level warming treatment; HW = high-level warming treatment. Error bars represent the standard deviation of the means (n = 3).

Effect of soil warming on glucose mineralisation

The ^{13}C-labelled glucose mineralisation was clearly reflected by the ^{13}C abundance of respired CO_2, indicating that glucose mineralisation contributed to soil CO_2 efflux. The rate of glucose mineralisation decreased sharply in the first 15 days and then remained low and stable. However, the effect of soil warming on glucose mineralisation was not significant (Fig. 2).

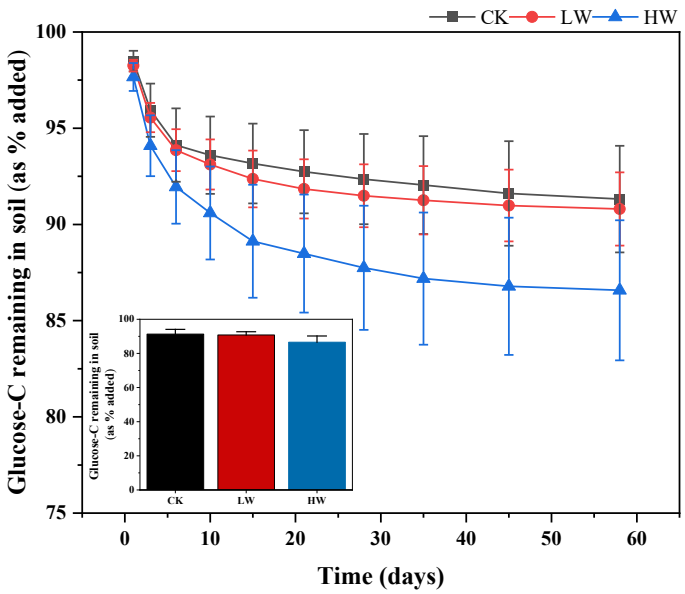

Fig. 2 Effects of temperature treatments on the mineralization rate of ^{13}C-labelled glucose during the experimental period (18 August to 14 October 2019). CK = control with no warming; LW = low-level warming treatment; HW = high-level warming treatment. Insert diagram shows the final mineralization rate of ^{13}C-labelled glucose. Error bars represent the standard deviation of the means (n = 3).

Effect of glucose addition and warming on soil respiration

The response of soil respiration to warming was related to the warming level. While soil respiration did not differ significantly between the LW and control plots, it was significantly higher (61.92%) in HW compared to the control plots (Fig. 3). ^{13}C-labeled glucose was used to distinguish the source of CO_2 and determine the SOM decomposition rate and PE intensity. No obvious trend of SOM decomposition over time was recorded during the experiment. The SOM decomposition rate did not decrease rapidly with rapid glucose mineralisation, and in CK, LW, and HW, the trends of SOM decomposition rate were basically the same. The addition of glucose significantly increased the rate of SOM decomposition rate from 88.97% to 165.4%. Moreover, a statistically significant interaction between glucose addition and temperature was observed for SOM decomposition (Fig. 3).

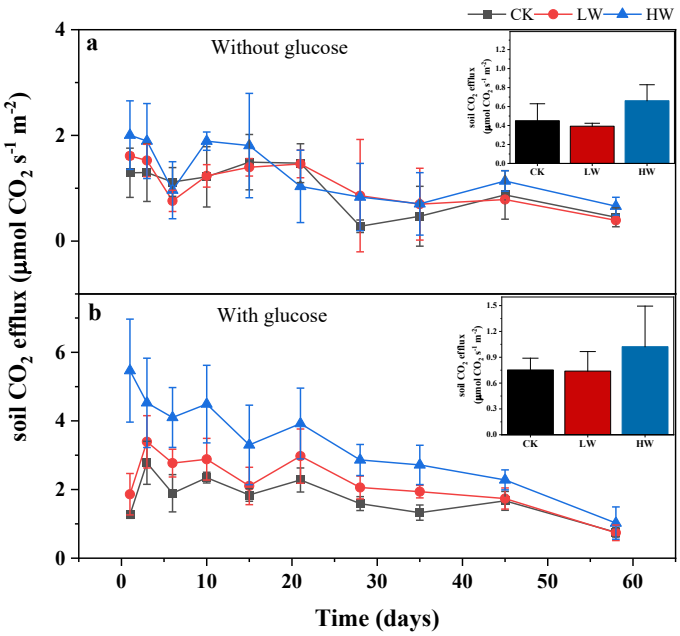

Fig. 3 CO_2 production from soil with water (a) or with ^{13}C-labelled glucose addition (b). CK = control with no warming; LW = low-level warming treatment; HW = high-level warming treatment. Insert diagram shows the final mineralization rate of soil. Error bars represent the standard deviation of the means (n = 3).

The effect of temperature increase on PE was non-significant. On the other hand, PE intensity increased by 12.57% in LW and 121.17% in HW compared to that in CK. Therefore, PE did not significantly impact the temperature treatment owing to large variations in the field. Nonetheless, a strong PE was observed, indicating that PE is real and very important. Overall, a positive PE was noted under both temperature treatments during the experiment (Fig. 4). As time progressed, although glucose was decomposed, PE did not weaken, indicating that glucose lasts for a prolonged period *in situ* (Figs. 2 and 4).

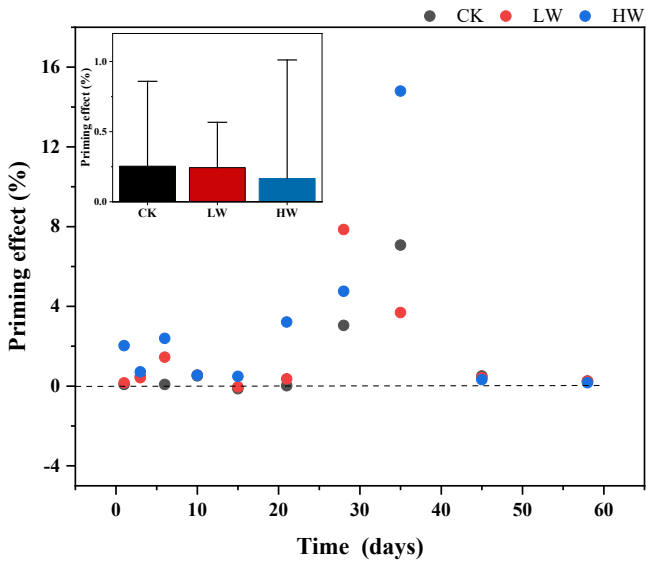

Fig. 4 Dynamics of the priming effect in different treatments. CK = control with no warming; LW = low-level

warming treatment; HW = high-level warming treatment. Insert diagram shows the final priming effect. Error bars represent the standard deviation of the means (n = 3).

Effects of glucose addition n and warming on soil physicochemical properties

Warming and glucose addition were not observed to have any significant effects on soil physicochemical properties (except inorganic N), soil enzyme activities, and soil microbial community structures (Figs. 5, 6, and 7). In contrast, a statistically significant decrease in soil N availability (–31.65% relative to the control plots) was observed in the glucose addition treatments. Additionally, warming significantly increased soil N availability in the LW and HW treatments by 6.46% and 32.22%, respectively. However, there was no significant interaction between warming and glucose addition on soil N availability (Fig. 5).

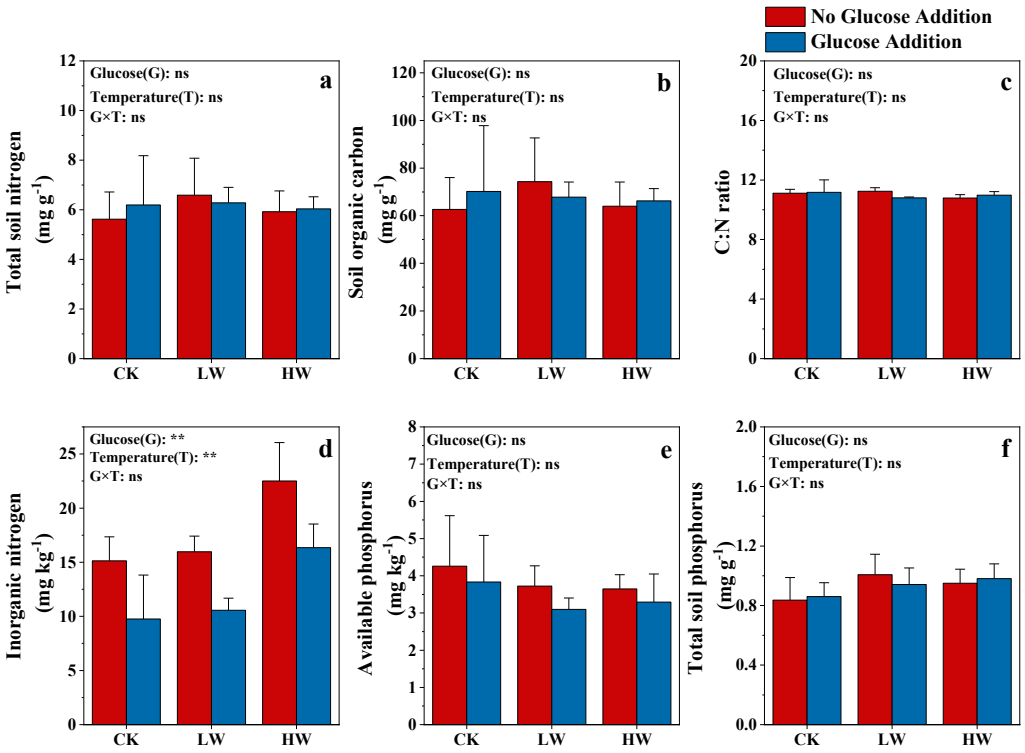

Fig. 5 Effect of warming and glucose addition on soil properties (0--10 cm) after a 55-day experiment. CK = control with no warming; LW = low-level warming treatment; HW = high-level warming treatment. Error bars represent the standard deviation of the means (n = 3). ns, non-significant; *$P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

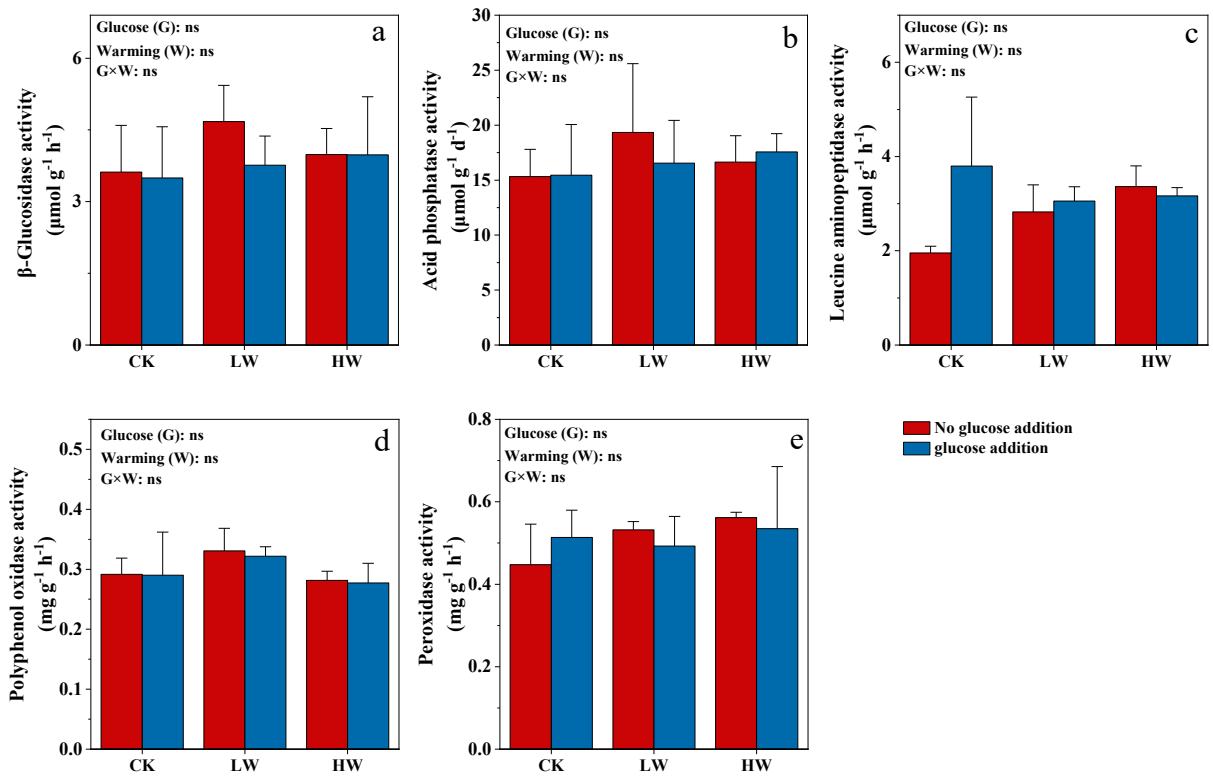

Fig. 6 Effect of warming and glucose addition on potential activity of specific soil (0--10 cm) enzymes involved in C, N and P acquisition after a 55-day experiment. CK = control with no warming; LW = low-level warming treatment; HW = high-level warming treatment. Error bars represent the standard deviation of the means (n = 3). ns, non-significant; *P < 0.05; ** P < 0.01; *** P < 0.001.

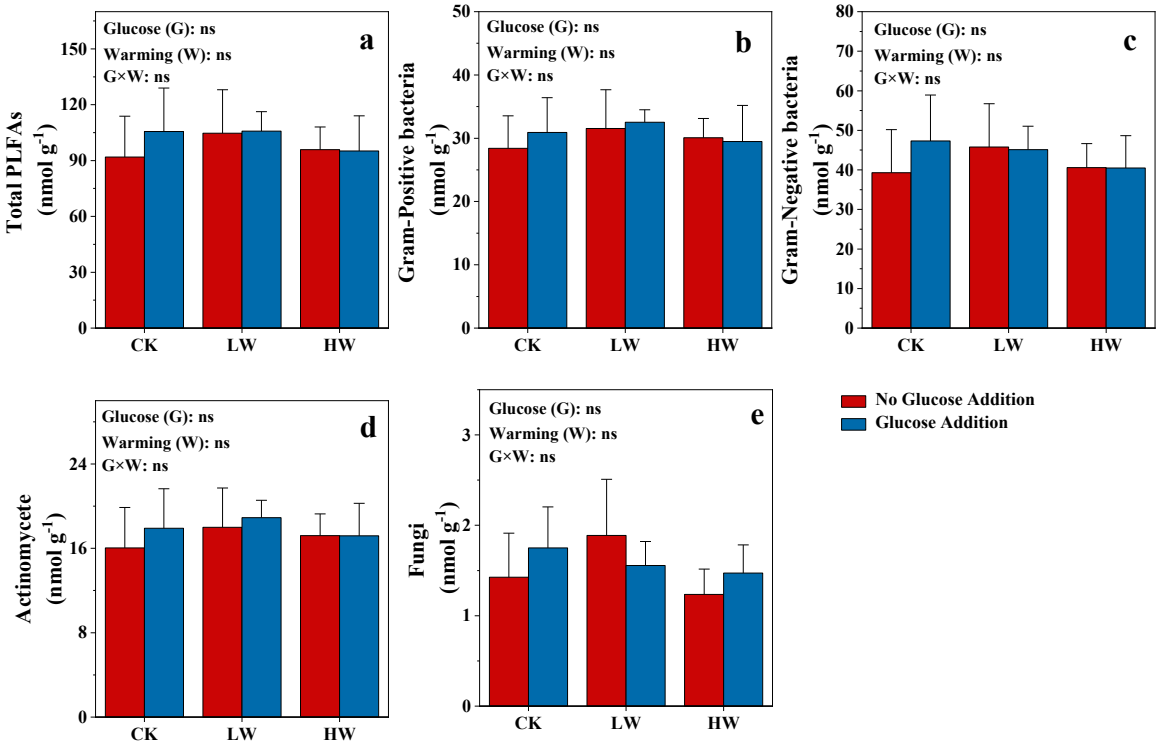

Fig. 7 Effect of warming and glucose addition on the total PLFAs content (a), specific PLFAs groups content (b-e) of soil (0--10 cm) after a 55-day experiment. CK = control with no warming; LW = low-level warming treatment; HW = high-level warming treatment. Error bars represent the standard deviation of the means (n = 3). ns, non-significant; *P < 0.05; ** P < 0.01; *** P < 0.001.

DISCUSSION

In the present study, we assessed the extent and direction of PE in a temperate forest and found that soil respiration was significantly increased by glucose addition, confirming the importance of PE in regulating soil C turnover and cycling these ecosystems. In addition, *in situ* warming tended to increase the extent of PE, although this regulation was not significant due to large spatial variations in soil nutrient status under field conditions.

Effect of warming on glucose mineralisation

Using the ^{13}C stable isotope technique, we quantified the response of glucose mineralisation to *in situ* warming in a temperate forest. Across all warming treatments, glucose mineralisation rapidly in the early stage of the experiment (<15 d) and remained stable in later stages (Fig. 2). This trend consistent with the results obtained in laboratory-based incubation studies (Zhang and Wang, 2012; Gunina and Kuzyakov, 2015; Chao *et al.*, 2019). When highly available glucose enters the soil, soil microbes rapidly consume this labile substrate (Werth and Kuzyakov, 2010). Subsequently, however, the decomposition rate of glucose decreases gradually due to depletion of the substrate. Of note, only 8.68% (CK), 9.2% (LW), and 13.42% (HW) of the added glucose was released as CO_2 at the end of experiment, although the decomposition rate had dropped to a very low level (<0.1% day^{-1}). Most of the glucose remaining in the soil was protected through mineral association and physical protection of structural compounds in aggregates (Cotrufo *et al.*, 2013; Haddix *et al.*, 2016).

Furthermore, glucose mineralisation was not significantly affected by warming during the experimental period. This result is contrary to previous reports. For instance, Guttières *et al.* (2021) reported that elevated temperatures significantly increased the amount of CO_2 derived from added fresh wheat straw by 12%. The different responses of the decomposition process may be related to the C quality of substrates used in different studies. Based on the kinetic theory, the temperature sensitivity of microbial decomposition is inversely related to substrate C quality (Loranger *et al.*, 2002; Fierer *et al.*, 2005; Davidson *et al.*, 2006; Wang *et al.*, 2016a). The low temperature sensitivity of glucose owing to its low activation energy may contribute to its similar decomposition rate at different temperatures, as observed in the present study. Indeed, similar results have been reported in previous studies involving different incubation temperatures. For instance, Ghee *et al.* (2013) reported that the rate of glucose mineralisation was similar at different incubation temperatures. In addition, the relatively lower temperature gradient in the present study (only 4 °C increase in the HW plots) and the high spatial variance in soil nutrient status under field conditions may have masked the effect of warming on glucose mineralisation.

Effect of in situ warming on soil respiration in a temperate forest

In the present study, an increase in temperature significantly increased soil respiration *in situ*; this effect was particularly strong in the HW plots. This result is not surprising because temperature is known to be one of the major drivers of soil microbial decomposition (Curiel Yuste *et al.*, 2007; Gregorich *et al.*, 2017), and

soil respiration is generally more responsive to increased temperatures at higher than at lower latitudes (Song *et al.*, 2014; Muñoz *et al.*, 2016). Many field warming experiments have shown that soil respiration significantly increases due to warming, at least in the short term (Raich and Schlesinger, 1992; Lloyd and Taylor, 1994; Davidson *et al.*, 1998; Rodeghiero and Cescatti, 2005; Romero-Olivares *et al.*, 2017). For instance, Phillips *et al.* (2016) reported that in a grassland ecosystem, the soil respiration rate was 27% higher in heated plots (3.5 °C above the ambient value) than in control plots. This increase was close to our result of 61.92% increase in soil respiration in the HW plots (Fig. 3). The observed increase in the soil respiration rate in the warming plots may be attributed to the following reasons. First, the increased soil nutrient availability under the warming treatment can improve soil microbial activity (Song *et al.*, 2011) and enhance soil respiration derived from SOC decomposition. Indeed, a significant increase in the inorganic N concentration was observed in the warming plots (Fig. 5). Second, increased soil respiration may result from increased autotrophic respiration derived from plant roots (Li *et al.*, 2016). Although root biomass under different treatments was not measured in the present study, previous studies have shown that the warming effect on soil respiration is primarily mediated by increased root biomass (Zhou *et al.*, 2011; Luan *et al.*, 2013; Wang *et al.*, 2021). Other studies have shown that the warming effect on soil respiration is mediated by changes in soil microbial community composition and activity (Chen *et al.*, 2015, 2021; Xu and Yuan, 2017). However, this may not be the case in the present study, since no significant changes in soil microbial biomass and enzyme activities were observed in the warming plots (Figs. 6 and 7). Increased temperature had no significant effect on microbial community structure and soil enzyme activity, which may be due to the delayed response of microbial communities to climate warming (Rinnan *et al*., 2007, 2009; Zhang *et al.*, 2016; Johnston *et al.*, 2019) and high spatial heterogeneity in the field. This finding is consistent with the results of previous studies (Allison and Treseder, 2008; Steinweg *et al.,* 2013; Jing *et al.,* 2014; Weedon *et al.,* 2014). Similar to our results, Peng *et al.* (2020) found a significant increase in soil respiration in warming plots of an alpine meadow ecosystem, albeit without any change in bacterial or fungal biomass.

Soil respiration in the LW plots was not significantly different from that in the CK plots. This phenomenon has been commonly explained by warming-induced soil drying in some studies (Fu *et al.*, 2013; Quan *et al.*, 2019). For instance, previous studies have reported that experimental warming could significantly reduce the level of soil moisture and dampen the positive effects of warming on soil respiration (Pendall *et al.*, 2013). However, this may not be the case in the present study because no soil moisture reduction was observed in the warming plots (Fig. 1), and microbial activity may not have been water-limited during the experimental period in the studied ecosystem. This may primarily be due to the small temperature increase in the LW treatment and the high spatial variance in soil nutrient status under field conditions.

Effect of glucose addition on soil respiration in temperate forest

Using the Keeling plot technique, we accurately measured the ^{13}C isotope of soil respiration *in situ* and assessed the direction and extent of PE under field conditions. Glucose addition significantly altered soil respiration (Fig. 3) and induced a strong positive PE in the studied temperate forest (Fig. 4). Across all warming levels, the average soil respiration increased by 88.97 to 165.4% due to glucose addition during the experimental period. This increase is within the range reported in laboratory-based incubation studies. In a meta-analysis, Zhang *et al.* (2013) reported that external organic C addition significantly altered native SOC decomposition rate, ranging from 95.1% inhibition to 1207% stimulation. Thus, our results confirmed the occurrence and importance of PE under field conditions at varying environmental temperatures, despite the large spatial variability in soil nutrient availability and soil microbial community composition, in a forest ecosystem. Similar to that in laboratory incubation experiments, the PE observed in the present study may be induced by the 'N mining' mechanism. Generally, in forest ecosystems, soil microbial growth and activity

are energy-limited (Traoré *et al.*, 2016; Chen *et al.*, 2018a) and increase after glucose addition. The concentration of inorganic N decreased owing to microbial growth and rapid consumption (Fig. 5). Soil microbes produce more extracellular enzymes to mine N from soil organic matter (SOM) decomposition, thus producing a positive PE. Surprisingly, no significant responses of soil microbial biomass and enzyme activity to glucose addition were observed in the present study. This may largely be because the PLFA analysis used in the present study could only indicate living microbial groups. However, the C use efficiency and growth of soil microbes may be more important for regulating PE (Manzoni *et al.*, 2012). Therefore, different active microorganisms that utilise the added glucose or native SOM should be distinguished in future studies.

In previous laboratory-based incubation experiments, PE tended to be relatively high at the early stage and gradually decreased to a gentle trend over time (Zhang *et al.*, 2013; Chao *et al.*, 2019). This may be primarily attributed to the gradual consumption of added substrate over time, which leads to the weakening of PE. Meanwhile, in the present study, we found that warming caused positive PE to occur more easily, although there was no clear trend of change. This result is consistent with that of a previous study (Ghee *et al.,* 2013), which found that the rates of SOM mineralisation increased with increasing temperature, and the magnitude of PE did not change significantly. When ^{13}C glucose is consumed, activated microorganisms utilise recalcitrant substrates (Kuzyakov and Bol, 2006) and produce extracellular enzymes via co-metabolism to increase PE (Blagodatskaya and Kuzyakov, 2008). In this context, real PE occurs and reaches a peak value at approximately days 30--40. Our results showed that there was still more glucose in the soil at the end of the experiment, which may have led to persistent PE (Fig. 3). However, uncontrolled temperature variations in the field, which are shaped by seasonal climate and diurnal variations, coupled with the continuous input of litter and root exudates, renders the mechanism of field PE elusive, warranting further research in the future.

Before starting the experiment, we hypothesised that the extent of PE induced by glucose addition would be lower in the warming plots (Fontaine *et al.*, 2004, 2011; Zhang *et al.*, 2013). However, we observed that the effect of warming on PE was not significant, and there was even a weak increasing trend of the extent of PE. Although a significantly higher soil N availability was observed in the warming treatments (Fig. 5), this was not translated into a lower PE through N mining. In contrast, previous studies have demonstrated that a faster release of labile C from glucose mineralisation under higher temperatures may promote microbial activity, leading to a stronger PE through microbial co-metabolic decomposition of SOC (Lenka *et al.*, 2019), which may offset the 'N mining' effect. Similar findings were reported by Guttières *et al.* (2021), who found that basal SOM mineralisation at 20 °C was 38% higher than that at 15 °C, although the extent of PE induced by glucose was not affected by incubation temperature. Our results indicate that the extent of PE may not be responsive to elevated temperatures under future climate change. However, additional studies are warranted to confirm this speculation.

Advantages and disadvantages of the field assessment of PE

Caution is recommended when extrapolating the results of the present study to other ecosystems. First, soil respiration derived from heterotrophic and autotrophic respiration was not separated in the present study. In addition, due to the limitation of field experimental conditions, we did not conduct a root-cutting treatment; hence, the impact of spatial heterogeneity of roots on experimental results cannot be excluded. This may also be responsible for the low response of microorganisms to glucose addition. The responses of tropical forests play a critical role in regulating the feedback between climate and atmospheric CO_2 and the extent to which this process should be explored. Owing to the limitation of *in situ* warming technology, we could only monitor and ensure that the warming range in the 0--5 cm soil layer was constant and reached the experimental target value, but we could not guarantee temperature increase across the whole soil profile.

Additional experiments are warranted in the future to test the response of the whole soil profile to the predicted warming in forests and other ecosystems.

ACKNOWLEDGEMENT

This research was supported by the National Natural Science Foundation of China [No. U22A20612 and No. 41877092]; the National Key Research and Development Program of China [No. 2022YFF1303003 and No. 2016YFA0600802]; K.C.Wong Education Foundation [No. GJTD-2018-07], Research and Development Project of Scientific Instruments and Equipment of Chinese Academy of Sciences [No. YJKYYQ20190054], Liaoning Vitalization Talents Program [No. XLYC1902016]. We greatly thank Xiaoming Fang, Jin Li for their help on the field and soil sampling, Pan Yin, Kaiyan Zhai for data analysis and paper writing. We are grateful to the QingYuan Forest Station of Chinese Ecosystem Research Network for providing the experimental sites and relevant supports.

SUPPLEMENTARY MATERIAL

Supplementary material for this article can be found in the online version.

CONTRIBUTION OF AUTHORS

Silong WANG and Weidong ZHANG contribute equally to this work. Silong WANG and Weidong ZHANG share co-last authorship.

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SUPPLEMENTARY MATERIAL

SUPPLEMENTARY METHODS

Phospholipid-derived fatty acid (PLFA) analyses

Phospholipid-derived fatty acids (PLFAs), used to evaluate soil microbial communities, were extracted following the method described by (Buyer and Sasser, 2012). Briefly, 3.0 g of freeze-dried room temperature soil was used with a mixture of chloroform-methanol-citrate buffer (1:2:0.8 by vol.) at pH 4.0 and included an internal standard of 19:0 phosphatidylcholine (Avanti Polar Lipids, Alabaster, AL). After evaporation under a stream of nitrogen the lipids were separated on a solid-phase extraction column and the phospholipids eluted with 10 ml of methanol. After evaporation under nitrogen the phospholipids were transesterified to fatty acid methyl esters, extracted into 2 ml of hexane, evaporated, and analyzed by gas chromatography (GC). Identification and quantification of fatty acid methyl ester biomarkers were done on an Agilent 6890N GC with an Agilent Ultra 2 column (25 m long, 0.2 mm internal diameter, and 0.33 μm film thickness) utilizing a flame ionization detector. The system was controlled with MIS Sherlock (MIDI, Inc., Newark, DE, USA) and Agilent ChemStation software. Following standard convention and to allow comparisons with prior research, quantities of PLFA biomarkers were reported as nmol PLFA g^{-1} dry soil. Microbial biomass was calculated as the sum of all PLFAs detected in the sample. The PLFAs detected are summarised in Table SI.

TABLE SI

Summary of PLFA biomarkers summed to represent microbial groups.

Microbial group category	Peaks			
Fungi	18:2 w6c			
Gram Negative	10:0 2OH	10:0 3OH	12:0 2OH	12:1 w4c
	12:1 w8c	13:1 w3c	13:1 w4c	13:1 w5c
	14:0 2OH	14:1 w5c	14:1 w7c	14:1 w8c
	14:1 w9c	15:1 w5c	15:1 w6c	15:1 w7c
	15:1 w8c	15:1 w9c	16:0 2OH	16:1 w3c
	16:1 w4c	16:1 w5c DMA	16:1 w6c	16:1 w7c
	16:1 w7c DMA	16:1 w9c	16:1 w9c DMA	17:0 cyclo w7c
	17:1 w3c	17:1 w4c	17:1 w5c	17:1 w6c
	17:1 w7c	17:1 w8c	17:1 w9c	18:1 w3c
	18:1 w5c	18:1 w6c	18:1 w7c	18:1 w8c
	18:1 w9c	19:0 cyclo 9,10 DMA	19:0 cyclo w6c	19:0 cyclo w7c
	19:0 cyclo w9c	19:1 w6c	19:1 w7c	19:1 w8c
	19:1 w9c	20:0 cyclo w6c	20:1 w4c	20:1 w6c
	20:1 w8c	20:1 w9c	21:1 w3c	21:1 w4c
	21:1 w5c	21:1 w6c	21:1 w8c	21:1 w9c
	22:0 cyclo w6c	22:1 w3c	22:1 w5c	22:1 w6c
	22:1 w8c	22:1 w9c	24:1 w7c	24:1 w9c
	11:0 anteiso	11:0 iso	12:0 anteiso	12:0 iso

Gram Positive	13:0 anteiso	13:0 iso	14:0 anteiso	14:0 iso
	14:1 iso w7c	15:0 anteiso	15:0 iso	15:1 anteiso w9c
	15:1 iso w6c	15:1 iso w9c	16:0 anteiso	16:0 iso
	17:0 anteiso	17:0 iso	17:1 iso w9c	18:0 iso
	19:0 anteiso	19:0 iso	20:0 iso	22:0 iso
Actinomycetes	16:0 10-methyl	17:0 10-methyl	17:1 w7c 10-methyl	18:0 10-methyl
	19:1 w7c 10-methyl	20:0 10-methyl	18:1 w7c 10-methyl	

Enzyme assays

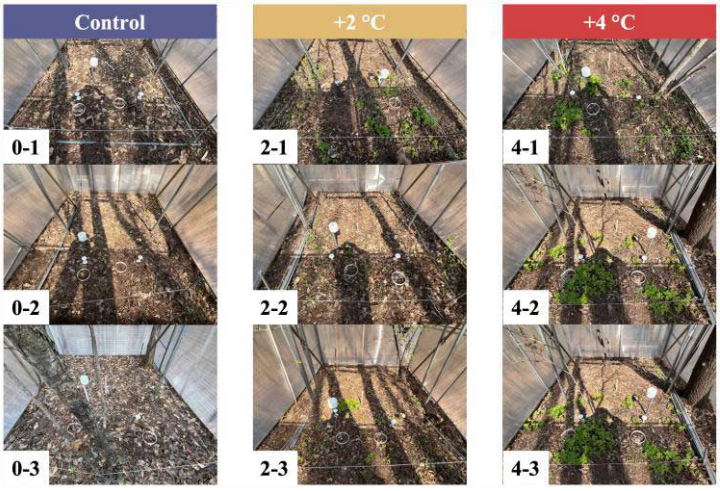
Soil β-glucosidase activity was measured by the S-β-GC Kit (Solarbio Science & Technology Co., Ltd., Beijing, China; BC0165) method using ρ-nitrophenyl β-D-glucopyranoside as substrate of air-dry soil under incubation (1 h, 37 ℃). The ρ-nitrophenol form was determined at 400 nm with the enzyme labelled instrument (Bio Tek Instruments Inc, Epoch). The unit of soil β-glucosidase activity (μmol g^{-1} h^{-1}) was defined as μmol ρ-nitrophenol g^{-1} of soil h^{-1}.

Soil peroxidase activity and polyphenol oxidase activity was measured by the S-POD Kit (Solarbio Science & Technology Co., Ltd., Beijing, China; BC0895) and the S-PPO Kit (Solarbio Science & Technology Co., Ltd., Beijing, China; BC0115) method using pyrogallol as substrate of air-dry soil under incubation (1 h, 30 ℃). The purpurogallin form was determined at 430 nm using an enzyme-labelled instrument (BioTek Instruments Inc., Epoch). The units of soil peroxidase activity (mg g^{-1} h^{-1}) and polyphenol oxidase activity (mg g^{-1} h^{-1}) were defined as mg purpurogallin g^{-1} of soil h^{-1}.

Soil leucine aminopeptidase activity was measured by the S-LAP Kit (Solarbio Science & Technology Co., Ltd., Beijing, China; BC4025) method using L-leucine-ρ-nitroaniline as substrate of air-dry soil. After incubating in the water bath at 37 ℃ for 1 h, the sample was immediately incubated in boiling water for 5 min. The ρ-nitroaniline form was determined at 405 nm with the enzyme labelled instrument (Bio Tek Instruments Inc, Epoch). The unit of soil leucine aminopeptidase (μmol g^{-1} h^{-1}) was defined as μmol ρ-nitroaniline g^{-1} of soil h^{-1}.

Soil acid phosphatase activity was measured by the S-ACP Kit (Solarbio Science & Technology Co., Ltd., Beijing, China; BC0145) method using disodium phenyl phosphate as substrate of air-dry soil under incubation (24 h, 37 °C). The phenol form was determined at 660 nm using an enzyme-labelled instrument (BioTek Instruments Inc., Epoch). The unit of acid phosphatase activity (μmol g^{-1} d^{-1}) was defined as μmol phenol g^{-1} of soil day^{-1} at 37 ℃.

SUPPLEMENTARY FIGURES



Experimental platform for infrared radiation warming in the QingYuan Forest Station of Chinese Ecosystem Research Network

SUPPLEMENTARY REFERENCES

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