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Long-term warming increased carbon sequestration capacity in a humid subtropical forest

Running head: Warming improves subtropical forest C stock

Xujun Liu [1, 2, #], Zhiyang Lie [1, 2, #], Peter B. Reich [3], Guoyi Zhou [1], Junhua Yan [1, 2], Wenjuan Huang [4], Yingping Wang [5], Josep Peñuelas [6, 7], David T. Tissue [8], Mengdi Zhao [1, 2], Ting Wu [1, 2], Donghai Wu[1, 2], Wenfang Xu[1, 2], Yuelin Li [1, 2], Xuli Tang [1, 2], Shuyidan Zhou [1, 2], Ze Meng [1, 2], Shizhong Liu [1, 2], Guowei Chu [1, 2], Deqiang Zhang [1, 2], Qianmei Zhang [1, 2], Xinhua He [1, 9, 10], Juxiu Liu [1, 2, *]

[1] Key Laboratory of Vegetation Restoration and Management of Degraded Ecosystems, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China

[2] Guangdong Provincial Key Laboratory of Applied Botany, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China

[3] Institute for Global Change Biology and School for Environment and Sustainability, University of Michigan, Ann Arbor, MI, USA

[4] Department of Ecology, Evolution, and Organismal Biology, Iowa State University, Ames, IA 50011, USA

[5] CSIRO Oceans and Atmosphere, PMB 1, Aspendale, Victoria 3195, Australia

[6] CSIC, Global Ecology Unit CREAF-CSIC-UAB, Bellaterra, 08193 Catalonia, Spain

[7] CREAF, Cerdanyola del Valles, 08193 Catalonia, Spain

[8] Hawkesbury Institute for the Environment, Western Sydney University, Penrith, NSW 2751, Australia

[9] School of Biological Sciences, University of Western Australia, Perth, WA 6009, Australia

[10] Department of Land, Air and Water Resources, University of California at Davis, CA 95616, USA

[#] Xujun Liu and Zhiyang Lie contributed equally to this study.

[*] Corresponding author

Author for correspondence:

Juxiu Liu, Tel.: +86 20 3720 2219; Fax: 86 20 3725 2615; e-mail: ljxiu@scbg.ac.cn

Abstract: Tropical and subtropical forests play a crucial role in global carbon (C) pools, and their responses to warming can have significant impact on C-climate feedback and predictions of future global warming. Despite earth system models projecting reductions in land C storage with warming, the magnitude of this response varies greatly between models, particularly in tropical and subtropical regions. Here, we conducted a field ecosystem-level warming experiment in a subtropical forest in southern China, by translocating mesocosms (ecosystem composed of soils and plants) across 600 m elevation gradients with temperature gradients of 2.1℃ (moderate warming), to explore the response of ecosystem C dynamics of the subtropical forest to continuous 6-year warming. Compared with the control, the ecosystem C stock decreased by 3.8% under the first year of 2.1°C warming; but increased by 13.4% by the sixth year of 2.1°C warming. The increased ecosystem C stock by the sixth year of warming was mainly attributed to a combination of sustained increased plant C stock due to the maintenance of a high plant growth rate and unchanged soil C stock. The unchanged soil C stock was driven by compensating and offsetting thermal adaptation of soil microorganisms (unresponsive soil respiration and enzyme activity, and more stable microbial community), increased plant C input, and inhibitory C loss (decreased C leaching and inhibited temperature sensitivity of soil respiration) from soil drying. These results suggest that the humid subtropical forest C pool would not necessarily diminish consistently under future long-term warming. We highlight that differential and asynchronous responses of plant and soil C processes over relatively long-term periods should be considered when predicting the effects of climate warming on ecosystem C dynamics of subtropical forests.

Keywords: Warming, Carbon stock, Carbon fluxes, Carbon sequestration capacity, Thermal acclimation, Subtropical forest

1 Introduction

The global mean temperature is predicted to increase by 1.5–2°C by the end of the 21st century in the context of low carbon (C) emissions, with significant impacts on key ecosystem processes, such as C cycle (Chuckran et al., 2020; Dolezal et al., 2020; Nottingham et al., 2020) and land C-climate feedbacks. Tropical and subtropical forests play an essential role in global C cycle, because they store roughly two-thirds of global terrestrial plant biomass C (Gatti et al., 2021; Pan et al., 2011), and one-third of global soil C (Jackson et al., 2017). Even small changes in C stocks and C sequestration capacity of tropical forests under projected climate warming may elicit significant effects on atmospheric C dioxide (CO_2) concentrations and thus climate change processes (Mitchard, 2018; Sullivan et al., 2020; Soong et al., 2021). However, whether tropical and subtropical forests will increase or decrease C stock under future warming scenarios is still debated (Mitchard, 2018; Sullivan et al., 2020; Pennisi, 2020). Similarly, the fate of subtropical and subtropical forest C stock and/or C sequestration capacity remains highly uncertainty among different earth system models (Cox et al., 2013).

The magnitude and direction of ecosystem C stock and/or C sequestration capacity depends on the effects of warming on photosynthetic C uptake relative to ecosystem respiratory C losses (Wood et al., 2012). However, field experiments in tropical and subtropical forests involve partial warming methods that only increase the temperature of plants or soil, rather than ecosystem-level warming (Song et al., 2019), challenging the identification of C sequestration capacity of ecosystem responses to warming in forest ecosystems. Temperature gradients within the tropics may provide some clues to assess the potential response of tropical and subtropical forests to increased

temperature by substituting space for time (Raich et al., 2006; Girardin et al., 2014). For example, a study in Hawaii tropical forests along an elevation gradient found that the higher C loss of soil in the warmer forests could be offset by the higher plant C inputs (Girardin et al., 2014). Meta-analyses of the effects of temperature on C storage and fluxes across a broad range of tropical forest sites also found that total net primary production (NPP), litter production, tree growth, and below-ground C allocation all increase with increasing mean annual temperature (ranging from 10°C to 28°C; Silver, 1998; Raich et al., 2006), which could be offset by increased soil C loss with warming (Raich et al., 2006). Consequently, although recent evidence suggests that the temperature sensitivity of soil C in tropical forests is comparable to that in temperate forests (Nottingham et al., 2020), ignoring potential positive plant C inputs may overestimate soil C loss and lead to an underestimation of tropical and subtropical forest C sequestration capacity under future warming. Therefore, field experimental warming of both plants and soils is needed to enable a comprehensive response of whole forest ecosystems. However, there are no field warming experiments that incorporate both plants and soils conducted in tropical or subtropical forests, which hinders a comprehensive understanding of future changes in the C sequestration capacity of tropical and subtropical forest (Nottingham et al., 2020; Walker et al., 2020).

Another challenge in characterizing the fate of C stock and/or C sequestration capacity of tropical and subtropical forest to future climate warming is the absence of evidence whether the tropical and subtropical forest could adapt to long-term warming (Raich et al., 2006; Wood et al., 2012). The duration of experiment warming may alter the response trajectory of tropical forest C stock and/or C sequestration. It has been shown that temperate forest plants exhibit thermal acclimation to long-term warming in both their photosynthetic and respiratory metabolism (Sendall

et al., 2015; Reich et al. 2016; Bermudez et al., 2021), as well as the soil microorganisms can also produce thermal acclimation to inhibit the consumption of the soil C (Melillo et al., 2017; Guo et al., 2020). However, to date, the duration of warming experiments conducted in tropical and subtropical forests has been relatively short (Nottingham et al., 2020), which might not be sufficient to capture whether the C dynamics of tropical and subtropical forest can adapt to long-term warming. Moreover, the acclimation of C processes in plants and soil may be asynchronous due to their different sensitivities to temperature (Wang et al., 2019). Although many studies demonstrate that soil microorganisms and subsequent soil C stock could rapidly adapt to warming (e.g., several months; Romero-Olivares et al., 2015; Melillo et al., 2017; Guo et al., 2020), it remains unclear whether soil microorganisms and plants exhibit similar degrees of acclimation and at similar rates. In humid tropical and subtropical forests, soil respiration is more responsive to environmental impacts than photosynthesis in terms of impact on annual C sequestration (Yan et al., 2013). Thus, quantifying the potential asynchronous thermal adaptation of plants and soil microorganisms responses to warming over longer periods in tropical and subtropical forests is required to predict the effects of climate warming on C sequestration capacity.

The lack of warming experiments and insufficient duration of warming in tropical and subtropical forests make it difficult to optimize the prediction of earth system models regarding the tropical and subtropical forest C stock and/or C sequestration capacity under future climate warming scenarios (Cavaleri et al., 2015). Here, we conducted a continuous 6-year field warming experiment in a subtropical forest by translocating mesocosms across altitudes, to quantify C sequestration capacity in both the short-term (the first 1–2-year experiment period, used hereafter) and long-term (the 5–6-year experiment period, used hereafter). The simultaneous warming manipulation of both

plants and soils in the subtropical forest mesocosms provides the opportunity to assess the response of ecosystem C sequestration capacity to warming, which is essential for developing more accurate predictions of the overall impacts of warming on forest C sequestration capacity. Building on our previous research that found that warming enhanced plant growth (Wu et al., 2020a; Lie et al., 2021), but reduced surface soil C in short-term warming (Fang et al., 2020), our main objective was to determine the long-term effects of subtropical forest ecosystem warming on plant and soil C dynamics and their contribution to C sequestration in this system.

2 Materials and Methods

2.1 Study site

This study was conducted at the Dinghushan Biosphere Reserve (23°09'21" to 23°11'30"N, 112°30'39" to 112°33'41"E) in southern China, which exhibits a subtropical monsoon climate. The mean annual temperature at the altitude of 600 m over the past 10 years is approximately 21.4°C, ranging from mean average temperatures of 13.2°C in Winter (January) to 27.3°C in Summer (July). The mean annual precipitation is approximately 1800 mm, with ~80% falling during the wet season (April to September). The dominant forest type at 600 m above sea level in the area is mountain evergreen broadleaf forest (MEBF), and the dominant plant species of MEBF include *Ardisia lindleyana*, *Itea chinensis*, *Machilus breviflora*, *Myrsine seguinii*, *Schima superba*, and *Syzygium rehderianum*. The soils are classified as Ultisols, developed from sandstone and shale bedrocks at approximately 70-80 cm deep (Fang et al., 2020; Lie et al., 2021; Zhou et al., 2023).

2.2 Experimental design

In accordance with temperature variations along elevations, the warming treatments were initiated by translocating forest mesocosms (containing *in-situ* soil and dominant plant species) from a high-altitude site to nearby high-altitude sites and two low-altitude sites (Fig. 1). In May 2012, we established study plots at three altitudes at Dinghu Mountain to represent contrasting climate warming scenarios, where plots at 600 m represented current conditions (control) and those at 30 and 300 m represented two future temperatures. These three study plots have similar aspects, orientation, and annual rainfall (Lie et al., 2021). Three open (ambient) mesocosms (3 m length × 3 m width × 0.8 m depth) were installed at each altitude, where the belowground section was a concrete brick wall covered with ceramic tiles that contained an aperture at the top of the wall and another at the base for the collection of surface water and underground runoff, respectively, in buckets. Soils collected from the MEBF (600 m) from each of three depths (0–20, 20–40, and 40–70 cm) were homogenized and then added by layer into each chamber located at 600, 300 and 30 m, respectively. Then, six 1-year-old seedlings (~4 mm base diameter and ~30 cm height) of each of six dominant tree species (*Ardisia lindleyana*, *Itea chinensis*, *Machilus breviflora*, *Myrsine seguinii*, *Schima superba*, and *Syzygium rehderianum*) in the MEBF were randomly transplanted into each mesocosm, along with four 0.2 × 0.2 m litter traps. The plots were not planted with other vegetation, and other new species were removed when they emerged. In summary, despite variations in habitat conditions other than temperature across different elevation gradients, this experiment ensured the mesocosms at three altitudes have similar habitat conditions except for temperature through rigorous site selection and the method of relocating plants and soil.

From May 2013 to December 2018, soil temperature and moisture at 5-cm depth were measured hourly using Campbell 109 constantan-copper thermocouples (Campbell Scientific,

Logan, UT, USA) and time-domain reflectometer probes (CS616, Campbell Scientific), respectively, and recorded using Campbell Scientific CR1000 data loggers. Throughout the whole experimental period (December 2013–December 2018), monitoring data indicates that the experiment successfully achieved warming (Fig. S1). The mean monthly soil temperature at altitudes of 300 m and 30 m were 1.0 ± 0.1°C and 2.1 ± 0.1°C warmer, respectively, compared to the control site (altitude of 600 m; $P < 0.05$).

2.3 Sampling and preprocessing

In December (2013 to 2018), we measured the base diameter and tree height of each species and randomly collected some fully developed leaves. In June 2014, 2015, and 2018, we harvested one plant of each species as root, stem, and leaf tissues. Fallen litter was collected monthly from litter traps from 2013 to 2018. The leaf, stem, root, and litter samples were oven-dried at 60°C till constant dry weight. The measurements of C-related parameters, including C, nitrogen (N), and phosphorus (P) in plant and soil, soil bulk density, soil pH, soil enzymes, microbial biomass C (MBC) and N (MBN), and microbial community structure are listed in Supporting Information Text S1.

Soil samples were collected by four soil depths (0–10, 10–20, 20–40, and 40–70 cm) using a 3.5-cm diameter auger in December (2013 to 2018), each soil sample was derived from a mixture of five subsamples of the same soil depth in each plot. Soil samples were sieved through a 2-mm mesh and divided into two parts: one was stored at 4°C prior to analysis of microbial biomass C and phospholipid fatty acid (PLFA) and the other was air-dried prior to soil physicochemical analysis. Soil bulk density was measured using the cutting-ring method in December (2013 and 2018).

Using a Li-Cor 6400XT infrared gas analyzer (Li-COR, Inc., Lincoln, Nebraska, USA)

connected to a Li-COR 6400–09 soil respiration chamber (9.55 cm diameter), soil respiration was measured fortnightly (2013–2014 and 2017–2018); each measurement date was considered to be the midpoint of that sampling period. Furthermore, the soil respiration measurements were taken within a three-hour period (9:00 am to noon), because this period has been proven to be close to the daily average values (Tang et al., 2006). In order to distinguish between heterotrophic respiration and autotrophic respiration, the samples of gas, soil, and root were collected when soil respiration was measured during each period in 2018, and their $\delta^{13}C$ values were measured using an Isotope Ratio Mass Spectrometer (IsoPrime 100, UK). Details about autotrophic and heterotrophic respiration measurements have been provided in the Supporting Information Text S2

The volume of leached water collected in the buckets was recorded following each rainfall event. Water samples were monthly collected (2013–2018) from the buckets after a thorough stir and stored at 4°C prior to the analysis of dissolved organic C (DOC) using a total organic C analyzer (TOC-VCSH, Shimadzu, Japan) within a week of collection.

2.4 Estimation of plant, soil and ecosystem C stocks

Plant C stock (t ha^{-1}) was estimated by summing the total C stocks of different species in the mesocosm (Eqs 1 and 2):

$$\text{C stock of each species} = \sum_{i=1} \text{tissue C concentration}_i \times \text{tissue biomass}_i \quad (1)$$

$$\text{Ecosystem plant C stock} = \frac{\sum_{j=1} \text{C stock of each species}_j}{10^6 \times S} \quad (2)$$

where i refers to different plant individuals of the same tree species; j is for species; and S is mesocosm area (ha). The tissue biomass of different plant species was calculated based on the

allometric equation involving the basal diameter and height (Table 1), and detailed information regarding the establishment of these equations has been previously introduced by Wu et al. (2020a).

Soil C stock (t ha^{-1}) across the four soil depths (0–10, 10–20, 20–40, and 40–70 cm) was calculated as (Eq. 3):

$$\text{Soil C stock} = \sum((1-\delta_i) \times \rho_i \times C_i \times T_i/10) \quad (3)$$

where i is soil depth; δ_i is proportional volume (%) of soil particles with diameter >2 mm; ρ_i is bulk density (g cm^{-3}); C_i is soil C concentration (g kg^{-1}); and, T_i is soil thickness (cm). A complete assessment of ecosystem C stock should take into account C stocks from plants, soil, litter, soil microorganisms and animals. We could not measure the complete C stock of the ecosystem; however, plant and soil C stocks represent most of ecosystem C, so the ecosystem C stock in this study is approximate and estimated as the sum of plant C and soil C stock. We determined plant C accumulation and soil C content, the methods were shown in the text S1.

2.5 Estimation of plant growth rate, annual litterfall, soil respiration, and dissolved organic C leaching

Plant growth rate (t C ha^{-1} yr^{-1}) was calculated as the difference in December biomass of a mesocosm between two consecutive years. Annual litterfall (t C ha^{-1} yr^{-1}) was the sum of monthly (January–December) litterfall. Annual soil respiration (t C ha^{-1} yr^{-1}) was the sum of monthly soil respiration, and ecosystem C loss from leaching (t C ha^{-1} yr^{-1}) was calculated as the 12-monthly products of runoff and its organic C concentration for both surface water and drainage water.

2.6 Estimation of warming effect size and temperature sensitivity

The warming effect size of these C-related variables was calculated as the response ratio (Eq.

4):

$$\text{Effect size} = \frac{X_W - \bar{X}_C}{\bar{X}_C} \quad (4)$$

where X_w and X_C represent the mean value in the warming treatment and control, respectively.

Then, we calculated temperature sensitivities of plant growth, annual litterfall, soil respiration and DOC leaching as the response magnitude per 1.0°C increase in temperature (Eq. 5):

$$\text{Temperature sensitivity} = \frac{Ln\,(X_W) - Ln\,(X_C)}{T_w - \bar{T}_c} \quad (5)$$

where X_w and X_C represent the mean value in the warming treatment and control, respectively; T_w and T_C represent the annual mean temperature in the warming treatment and the control, respectively.

2.7 Statistical analyses

One-way ANOVA was used to test the differences in C stock (plant, soil, and ecosystem) and C fluxes (plant growth rate, litterfall, soil respiration and leached water C losses) among different warming treatments (control, 1.0°C warming and 2.1°C warming) in the same year, as well as the temporal (short term *vs*. long term) differences in the warming effect size. General linear models were used to fit the bivariate plots of soil temperature with the C stocks and C fluxes. Linear mixed-effects models were used to estimate the effects of warming and warming duration on the plant, soil, and ecosystem C stocks, and annual C fluxes for plant growth rate, litterfall, soil respiration and leached water C losses; warming treatment and duration were treated as fixed factors, with plot as a random factor. Year was a continuous variable and treatment was a nominal variable. All analyses were performed in R (version 3.6.1), and the results were plotted using Origin Pro. 2021 (https://www.originlab.com/).

3 Results

3.1 Plant, soil and ecosystem C stocks

The plant C stock was positively correlated with annual air temperature among all years, and its slope increased with warming duration (Fig. S2). Compared with the control, plant C stock increased on average by 49.8% under the sixth year of 1.0°C warming and 75.0% under the sixth year of 2.1°C warming ($P < 0.001$; Fig. 2a).

Soil C stock was negatively correlated with annual soil temperature in Years 1–2, while its relationship turned to positive in Years 3–6 (Fig. S2). The topsoil (0–10 cm) C stock was negatively correlated with annual soil temperature, but its slope decreased with warming duration. The relationships of deeper soil C stock (40–70 cm) and soil annual temperature were inconsistent between short-term and long-term (Years 5–6) warming (Fig. S3). Compared with the control, the 2.1℃ warming reduced soil C stock only in Years 1–2 ($P < 0.05$) and had no effect on the other years. However, warming effects on soil C stocks varied with soil depth (Table S2). The 2.1℃ warming reduced topsoil C stock throughout the experiment ($P < 0.05$), with less negative effects on other soil layers, especially deeper soils (40–70 cm) that barely responded (Table S1).

Ecosystem C stock was negatively correlated with annual soil temperature in Years 1–2, while its relationship turned to positive in the other years (Fig. S2). Compared with the control, ecosystem C stocks decreased by 5.2% for 1.0°C warming and 3.8% for 2.1°C warming in the first year, but increased by 9.1% and 13.4% for 1.0°C and 2.1°C warming, respectively, in the sixth year ($P < 0.05$; Fig. 2c).

3.2 Ecosystem C fluxes and temperature sensitivity

The plant growth rate was positively correlated with annual air temperature, and its slope has almost no variation across all years (Fig. S4). As compared with the control, the plant growth rate increased by 1.77 t C ha^{-1} yr^{-1} and 1.83 t C ha^{-1} yr^{-1} under the first and sixth year of 2.1°C warming, respectively ($P < 0.001$; Fig. 3a). There was no significant changes in temperature sensitivity of plant growth rate under warming for both short-term and long-term (Fig. 3b).

A positive relationship between annual litterfall with annual air temperature was found for both short-term and long-term warming (Fig. S4). Compared with the control, annual litterfall increased significantly by 33% and 12% under short-term and long-term 2.1°C warming, respectively ($P < 0.001$), and changes in annual litterfall were not significant under 1.0°C warming (Fig. 3c). Temperature sensitivity of annual litterfall increased under short-term 2.1°C warming and approached to zero during long-term 2.1°C warming ($P < 0.05$; Fig. 3d). There were no significant changes in temperature sensitivity of annual litterfall under both short-term and long-term 1.0°C warming.

Soil respiration was positively correlated with annual soil temperature for both short-term and long-term warming, but its slope decreased with warming duration (Fig. S5). Soil respiration increased under 2.1°C warming, with greater increases under short-term warming (75%) than under long-term warming (12%; Fig. 3e). Temperature sensitivity of soil respiration increased greatly under short-term warming but was nearly zero under long-term warming ($P < 0.01$; Fig. 3f). Furthermore, the increase in soil respiration under long-term warming was mainly driven by autotrophic respiration rather than heterotrophic respiration (Fig. S6). Specifically, autotrophic respiration increased by 1.5 times under 2.1°C warming ($P < 0.001$), while heterotrophic respiration

did not change (Fig. S6).

Leached water DOC remains almost unchanged with the increase of soil temperature in the Years 1–2 (Its slope is almost zero), but its relationship turns to weak negative in the years 5–6 (Fig. S4). Leached water DOC decreased by 46% and 34% under long-term 1.0°C and 2.1°C warming, respectively, but there was no change under short-term warming (Fig. 3g) relative to the control. The temperature sensitivity of leached water DOC was not affected by warming (Fig. 3h).

3.3 C-related parameters under warming

C-related variables in plants and soils that significantly responded to warming were more commonly observed during short-term warming compared with long-term warming, suggesting longer-term acclimation of these processes (Fig. 4). For instance, among plant parameters (litter quality, root biomass, and root to shoot ratio), litter C/N ratio and root biomass significantly responded to 2.1°C warming, with a more positive response under short-term warming than under long-term warming. Among soil parameters under 2.1°C warming, the soil moisture content was unchanged under short-term warming, but it decreased significantly under long-term warming (Fig. 4 and S1). The total phospholipid fatty acids (PLFA), bacterial biomass, Gram-positive bacterial biomass (G^+), Gram-negative bacterial biomass (G^-), available P and cellobiohydrolase were significantly decreased under short-term warming but not under long-term warming. The activities of polyphenol oxidase and peroxidase were significantly increased under short-term warming, while their responses disappeared under long-term warming. In contrast, the bacterial/fungal ratio, arbuscular mycorrhizal fungi/ectomycorrhizal fungi ratio (AM/ECM ratio) and MBC/MBN ratio showed significantly negative responses to long-term warming and no response to short-term

warming.

4 Discussion

4.1 Ecosystem C sequestration increased under 2.1℃ long-term warming

Compared with the control, increased ecosystem C stock of the subtropical forest under 2.1°C long-term warming could be achieved by a combination of sustained increased plant C stocks due to the maintenance of a high plant growth rate exceeding the decreased soil C stocks caused by soil respiration (Fig. 2, 3 and 5). Although the plants in the experiment were young seedlings, similar behavior of increased plant growth rate in warmer conditions have been observed in mature trees in some tropical and subtropical forests with comparable temperatures (Girardin et al., 2014), demonstrating that the C sequestration capacity of the subtropical forest might increase under 2.1℃ long-term warming. In contrast to the long-term warming effects, short-term warming reduced the C sequestration capacity because enhanced soil respiration reduced soil C sequestration more than increased plant growth rate promoted plant C sequestration. The results suggested that C sequestration of plants and soils responded asynchronously to sustained warming, where the responses of soils but not plants varied between short-term and long-term warming. The changes in C sequestration capacity in various warming periods contrasted with studies in grasslands that often found the positive effects of short-term warming on both plant C stocks and soil C losses disappeared in long-term warming (Wang et al., 2019). The results are also in contrast to most Earth system models that predict reductions in C sequestration of tropical and subtropical forests under climate change due to the continuous large soil C loss (Cox et al., 2013; Mitchard, 2018; Nottingham et al., 2020). Considering that few studies have addressed warming of forest plants and soils over

short-term and long-term periods in the field, our findings provide greater insight into the complexities of ecosystem C sequestration capacity responses that vary with short-term and long-term warming.

4.2 The subtropical forest plants maintained a higher C sequestration capacity through warming duration

We found that the humid subtropical forest plants in the warming treatment continuously maintained a higher C sequestration capacity than the control throughout the warming period: constant higher plant growth rate and sustained increase in plant C stocks (Fig. 2 and 5). The following three factors explain this sustained increase in plant C sequestration capacity of the humid subtropical forest throughout the warming period. First, the monthly maximum air temperature due to warming (< 30.0°C, Fig. S1) did not exceed the thermal threshold of the plants in the region (Asia), where the tropical and subtropical plants have been reported to continue sequestering large amounts of C even as temperatures slightly rise (Sullivan et al., 2020). Second, the enhanced leaf photosynthetic rates and stomatal conductance were sustained under both short-term (Li et al, 2016) and long-term warming (Wu et al., 2020b), leading to the increased plant growth rate. Another possible explanation could be related to the alleviation of nutrient limitation, particularly P that is considered as the limit nutrient for tropical and tropical forest productivity (Sardans et al., 2012). Our previous studies found that warming could sustain the plant P demand for high growth through enhancing the soil P mineralization and dissolution (Lie et al., 2022). Thus, plants under moderate warming might maintain higher C stocks through physiological regulation and accelerated nutrient cycling, compared with the control.

4.3 The warming-induced increase in soil C loss disappeared under long-term warming

Our finding of decreased soil C stock in response to warming, especially in Years 1–2, was consistent with other short-term (~2 years) warming experiments in temperate coniferous (Melillo et al., 2002; Pries et al., 2017) and tropical forests (Nottingham et al., 2020). The decreased soil C sequestration capacity was mainly associated with enhanced microbial activity (e.g., enhanced soil respiration) and subsequent microbial C decomposition (e.g., enhanced C-acquiring enzyme activity (polyphenol oxidase and peroxidase)), despite the absence of independent heterotrophic respiration data during short-term warming. Reduced microbial C assimilation efficiency (greater respiration with lower microbial biomass, Fig. 3e and 4), mainly during short-term warming, also contributed to the decreased soil C sequestration capacity. Moreover, a reduction in substrate quality may have contributed to decreased soil C sequestration capacity. For instance, we found that warming had negative effects on, available P, nitrate N, and MBC; however, warming had a positive effect on litter C/N ratio, which might lead to greater microbial consumption of energy for nutrient acquisition (e.g., through enhanced enzyme activity; Frey et al., 2008; Melillo et al., 2017). Meanwhile, warming-induced drought might decrease the diffusion of DOC, thereby decreasing the substrate availability in forest soils (Allison et al., 2008).

We found that the negative effect of warming on the soil C stock capacity disappeared under long-term warming (Fig. 2 and 5), which could be explained by the thermal acclimation of soil microorganisms, the offset of cumulative C inputs from heightened plant growth, and the reduction in leached water C and temperature sensitivity of soil respiration caused by soil drying (Fig. 3 and 4). In this study, there was no response of soil heterotrophic respiration to long-term warming (Fig.

S6), and the temperature sensitivity of soil respiration decreased with warming duration (Fig. 3 and S2), which is similar to the results observed in temperate forests and cultivation experiments (Bradford et al. 2010; Romero-Olivares et al. 2015; Melillo et al., 2017). In addition, we found that the C-related enzyme activity did not respond to long-term warming (Fig. 4), and the soil microbial community tends towards fungal dominance (Fig. 4) that may have higher C assimilation efficiency (Soares & Rousk, 2019) and become more stable at our site (Zhou et al., 2023) relative to the responses under short-term warming. These results suggest that soil microorganisms have thermal acclimation to long-term warming, thus reducing the soil C loss caused by soil respiration.

In addition, the alleviation of nutrient deficiency (e.g., available P, nitrate N, and microbial biomass N) with warming duration, may also prevent microorganisms from decomposing soil C for nutrient acquisition. Moreover, cumulative prior inputs of C from heightened plant growth under long-term warming may also offset prior decreases in soil C stocks. Although the plant C input was not quantified in this study, some clues may evidence the increase in plant C input under warming: higher annual litterfall and root biomass (Fig. 3 and 4), faster litter decomposition rate (Liu et al., 2017;), and the recovery of soil C stock to the same level to the control under long-term warming (Fig. 2). Furthermore, we found that the soil in the warming treatment became drier compared to the control under long-term warming (Fig. S1). Soil drying can decrease the leaching of soil C through surface and groundwater (Fig. 3 and 4) and inhibit soil respiration and the sensitivity of soil respiration to temperature (Ni et al., 2017), thereby decreasing soil C loss.

4.4 Implications

In this study, we provide insights into how warming affects C cycling in subtropical systems.

Contrary to the large ecosystem C loss observed in soil-only warming experiments (Carey et al., 2016; Baccini et al., 2017; Nottingham et al., 2020), our findings suggest that the increased plant C stocks can partially offset the increased soil C losses under moderate warming (Fig. 5). This implies that manipulating only soil temperatures may introduce a bias in assessing the impact of warming on the C sequestration capacity of ecosystems. Furthermore, our study indicates that microbial composition and time-varied soil respiration may be crucial for tracking the trajectory of the C sequestration capacity of subtropical forests under warming (Fig. 5). Current model predictions of C sequestration capacity in subtropical forests might be underestimated if the model was mainly based on short-term experimental observations (Cox et al., 2013) and lacked consideration of microbial thermal acclimation. We suggest that future studies should include more diverse climatic conditions, soil types, and community composition to improve predictions of long-term warming effects on C dynamics in subtropical forests (Raich et al., 2006).

It should be noted that although we found that the humid subtropical forests still have high C sequestration potential under warming, this potential may not be fully realized in future responses. Firstly, despite the mesocosms in our experiment containing dominant plants and soil from natural forests, there are still some differences in terms of community composition and structure, soil depth, and microclimate between the mesocosms and the natural forest environment. These differences may not impact the assessments of the warming effects on C sequestration capacity of subtropical forests, while they might limit the robust estimates of ecosystem C dynamics. Thus, cautions should be taken when extrapolating these experimental results to other tropical and subtropical forests. Secondly, the extent of future warming may surpass the warming tested in this study, potentially exceeding the biological threshold temperatures and resulting in the degradation of tropical and

410 subtropical forests. Finally, other global change factors such as precipitation changes, N deposition
411 and fires can increase the vulnerability of forest C sequestration to warming (Sullivan et al., 2020).
412 Therefore, we call for the continued efforts in subtropical forest protection and low C emission
413 maintenance, and the urgency of more ecosystem-level field warming experiments in various
414 subtropical forests, as well as the long-term interactive experiments of warming and other climate
415 change factors.

416 5 Conclusion

417 Our 6-year field experiment in southern China sheds light on the effects of warming on the C
418 dynamics of a humid subtropical forest. Compared to the control, we found that the subtropical
419 forest had a higher ecosystem C sequestration capacity under long-term 2.1°C warming. The
420 increase in plant C stock was greater than the soil C loss under long-term 2.1°C warming, potentially
421 due to thermal acclimation of soil microorganisms (unresponsive soil respiration and enzyme
422 activity, and more stable microbial community), increased plant C input, and decreased C leaching
423 from soil drying. This suggests that subtropical forests, particularly those that are relatively young,
424 may be able to continue sequestering C under future moderate warming. Moreover, considering
425 plant and soil responses to both short-term and long-term warming can help improve model
426 predictions of ecosystem C under warming and provide a deeper understanding of the underlying
427 mechanisms at play.

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Conflict of interest

The authors declare no competing interests

Data availability statement

The data that support the findings of this study are openly available in figshare since the manuscript has been received.

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Table 1 Estimates of biomass of the six tree species. B: biomass (kg); D: basal diameter (cm); H: tree height (cm).

Species	Leaf	Stem	Root
Ardisia lindleyana	$B = 4E\text{-}05\times(D^2H)^{2.8695}$	$B = 0.0002\times(D^2H)^{2.5533}$	$B = 31.649\times(D^2H)^{-0.099}$
Itea chinensis	$B = 6.6199\times(D^2H)^{0.5068}$	$B = 0.9724\times(D^2H)^{0.7159}$	$B = 8.3192\times(D^2H)^{0.5361}$
Machilus breviflora	$B = 3.4487\times(D^2H)^{0.5098}$	$B = 1.2104\times(D^2H)^{0.6108}$	$B = 2.8922\times(D^2H)^{0.5408}$
Myrsine seguinii	$B = 2.4273\times(D^2H)^{0.4548}$	$B = 0.1738\times(D^2H)^{0.8661}$	$B = 0.6093\times(D^2H)^{0.6243}$
Schima superba	$B = 7.5092\times(D^2H)^{0.4173}$	$B = 6.2352\times(D^2H)^{0.4866}$	$B = 7.9756\times(D^2H)^{0.5109}$
Syzygium rehderianum	$B = 9.985\times(D^2H)^{0.3064}$	$B = 21.52\times(D^2H)^{0.2721}$	$B = 16.654\times(D^2H)^{0.2496}$

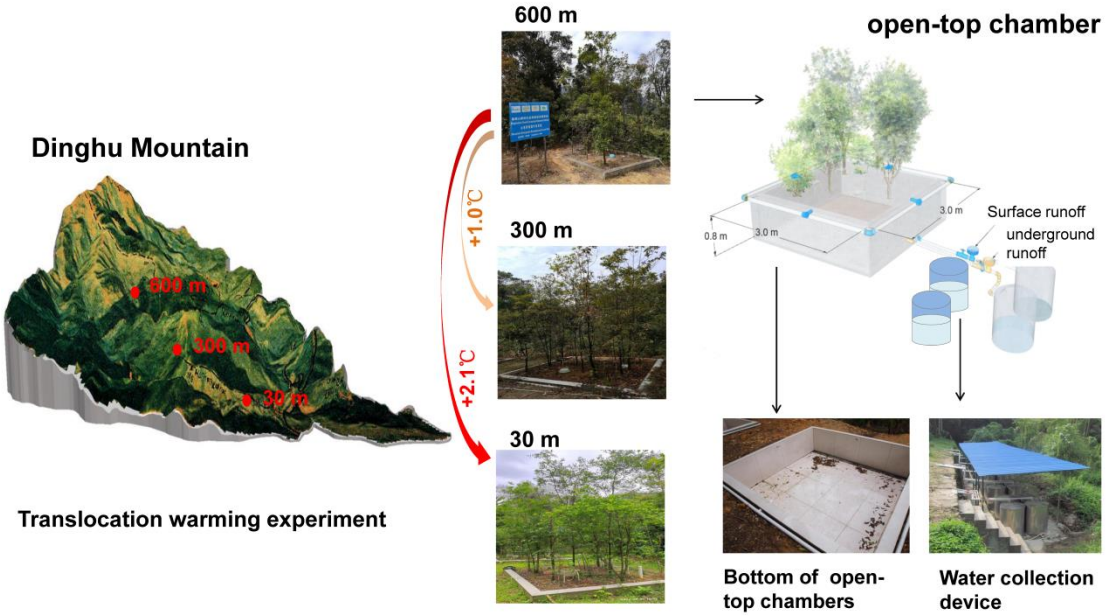
Fig. 1 The scheme of warming experimental design. To conduct a warming experiment, we used natural temperature differences at three elevations by translocating ecosystem-scale macrocosms (soils and tree seedlings) from the high-elevation site (600 m) to the two lower-elevation sites (300 m and 30 m). In each site, three open-top chambers were contected.

Fig. 2 Effects of warming on C stocks of forest plant (a), soil (0–10 cm; b), and ecosystem (c), from 2013 to 2018. W: warming; D: warming duration. $^{***}P < 0.001$; *ns*: not significant. Data are means ± SE ($n = 3$).

Fig. 3 Effects of warming on C fluxes in plant growth rate (a), annual litterfall (c), soil respiration (e), DOC leaching (g), and sensitivity (b, d, f, and h) from 2013 to 2018. W: warming; D: warming duration. $^{***}P < 0.001$; $^{*}P < 0.05$; $^{\dagger}P < 0.10$; *ns*: not significant. Data are means ±SE (n = 3).

Fig. 4 Responses of parameters to short-term (Years 1–2) and long-term warming (Years 5–6) ($n = 6$). Points are mean standardized effect size of warming; error bars are 95% confidence intervals; vertical dashed line represents weighted effect size = 0. R/S ratio: root/shoot ratio; MBC: microbial biomass carbon; MBN: microbial biomass nitrogen; ECM: ectomycorrhizal fungi; AMF: arbuscular mycorrhizal fungi; G^{+}: gram-positive bacteria; G^{-}: gram-negative bacteria; BG: β-glucosidase; CBH: cellobiohydrolase; NAG: N-acetylglucosaminidase; PPO: polyphenol oxidase; POD: peroxidase.

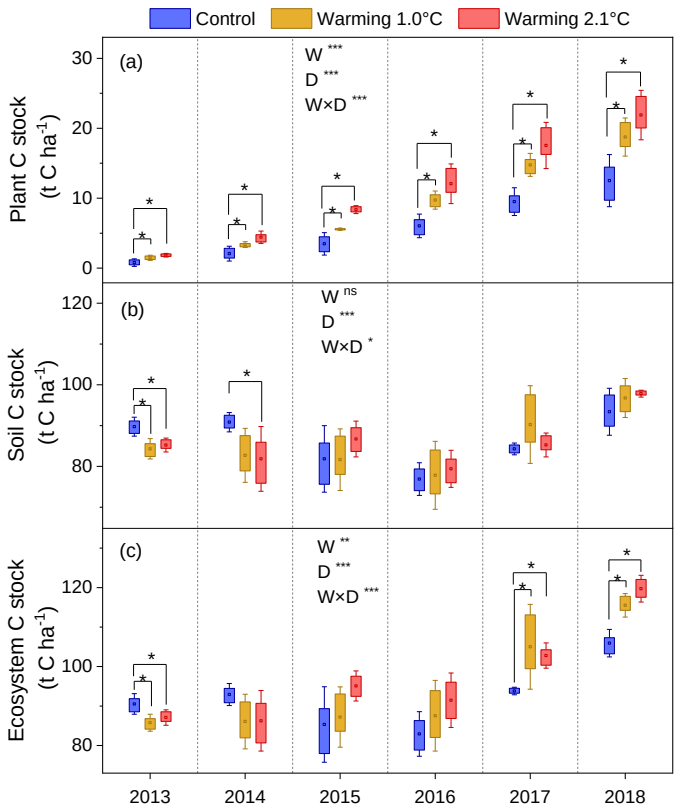
Fig. 5 Schematic of the C cycle in tropical forests under short (blue font) and long-term (red font) warming. Values in boxes are percent changes. *ns*: no significant. difference between ambient (control) and warming.



638

639

640 **Fig. 1**



641

642 **Fig. 2**

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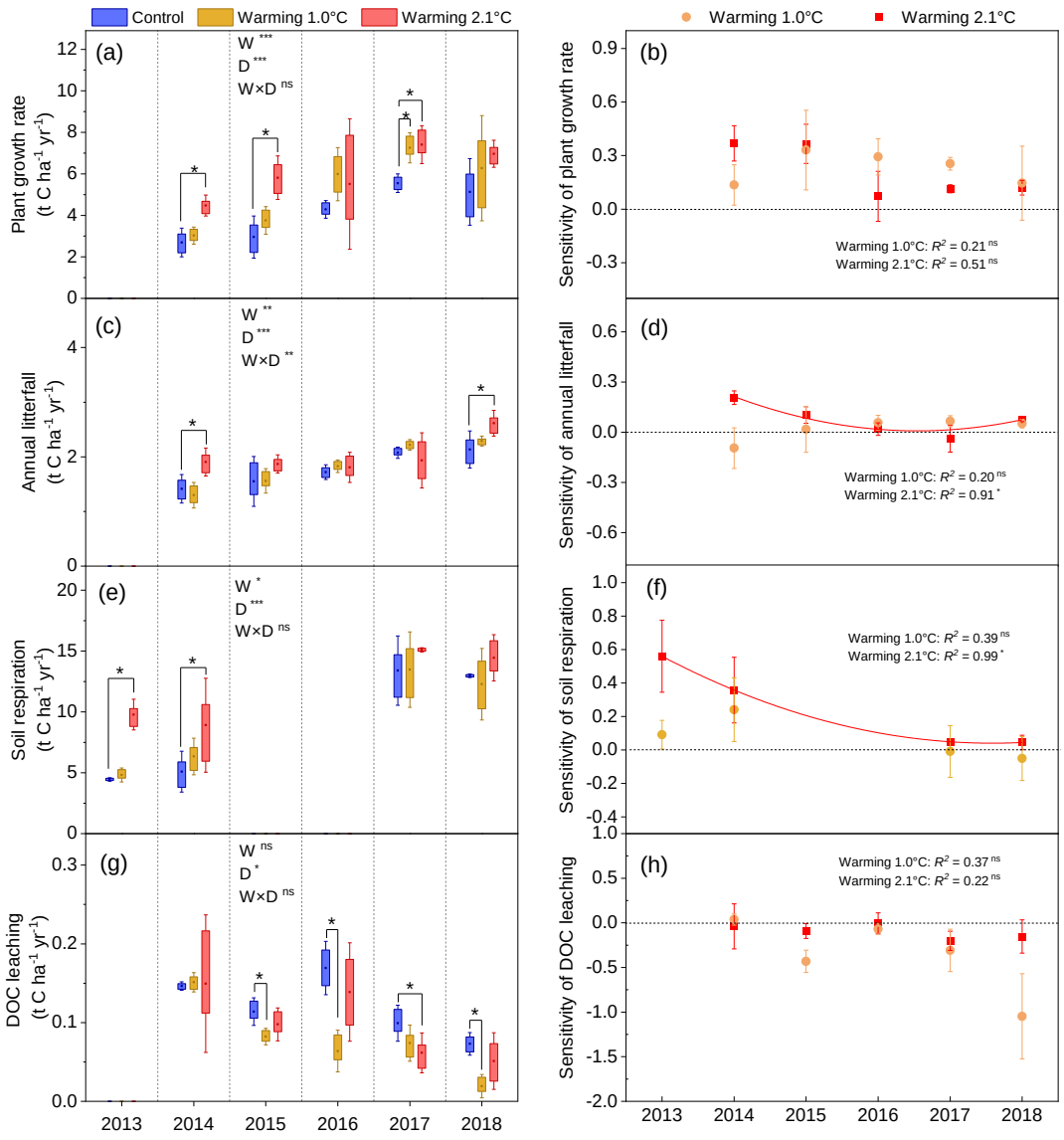
Control
Warming 1.0°C
Warming 2.1°C
(a)
W ***
D ***
W×D ns
Plant growth rate (t C ha-1 yr-1)
(b)
Sensitivity of plant growth rate
Warming 1.0°C: R2 = 0.21 ns
Warming 2.1°C: R2 = 0.51 ns
(c)
W **
D ***
W×D **
Annual litterfall (t C ha-1 yr-1)
(d)
Sensitivity of annual litterfall
Warming 1.0°C: R2 = 0.20 ns
Warming 2.1°C: R2 = 0.91 *
(e)
W *
D ***
W×D ns
Soil respiration (t C ha-1 yr-1)
(f)
Sensitivity of soil respiration
Warming 1.0°C: R2 = 0.39 ns
Warming 2.1°C: R2 = 0.99 *
(g)
W ns
D *
W×D ns
DOC leaching (t C ha-1 yr-1)
(h)
Sensitivity of DOC leaching
Warming 1.0°C: R2 = 0.37 ns
Warming 2.1°C: R2 = 0.22 ns
2013
2014
2015
2016
2017
2018

Fig. 3

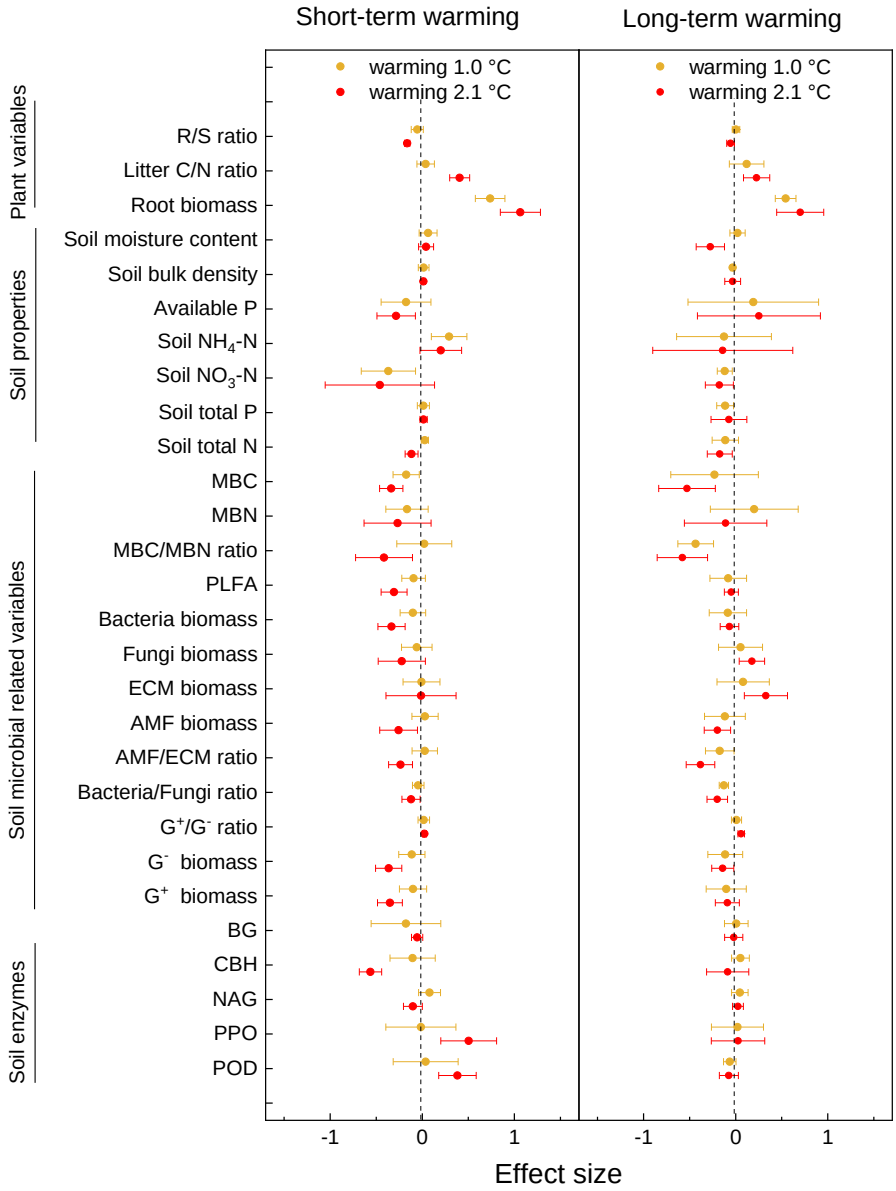
Short-term warming
Long-term warming
warming 1.0 °C
warming 2.1 °C
Plant variables
Soil properties
Soil microbial related variables
Soil enzymes
R/S ratio
Litter C/N ratio
Root biomass
Soil moisture content
Soil bulk density
Available P
Soil NH4-N
Soil NO3-N
Soil total P
Soil total N
MBC
MBN
MBC/MBN ratio
PLFA
Bacteria biomass
Fungi biomass
ECM biomass
AMF biomass
AMF/ECM ratio
Bacteria/Fungi ratio
G+/G- ratio
G- biomass
G+ biomass
BG
CBH
NAG
PPO
POD
-1
0
1
Effect size

Fig. 4

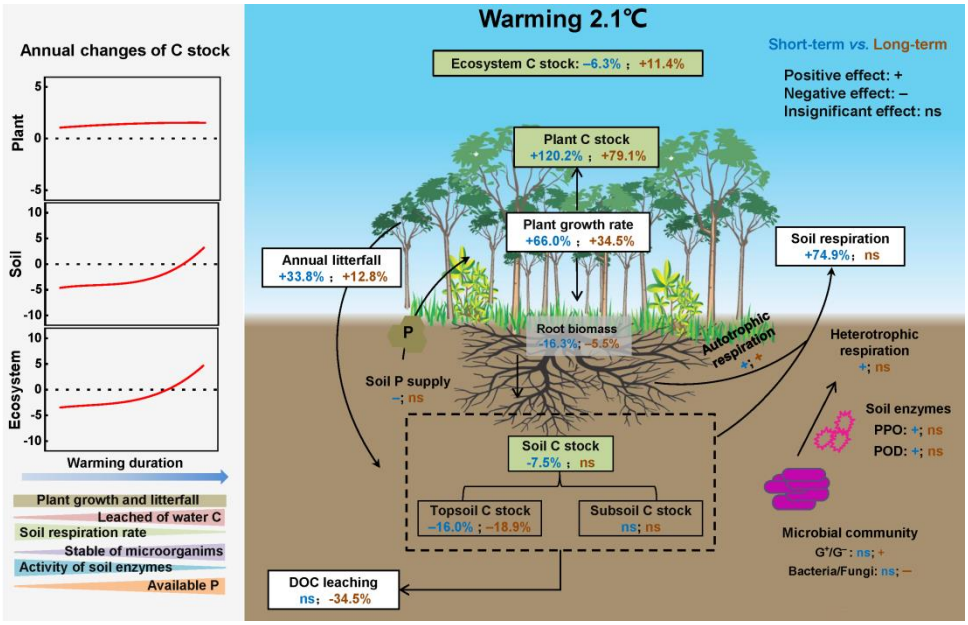
Annual changes of C stock
Plant
Soil
Ecosystem
Warming duration
Plant growth and litterfall
Leached of water C
Soil respiration rate
Stable of microorganims
Activity of soil enzymes
Available P
Warming 2.1℃
Short-term vs. Long-term
Positive effect: +
Negative effect: –
Insignificant effect: ns
Ecosystem C stock: –6.3% ; +11.4%
Plant C stock
+120.2% ; +79.1%
Plant growth rate
+66.0% ; +34.5%
Annual litterfall
+33.8% ; +12.8%
Soil respiration
+74.9%; ns
P
Root biomass
-16.3%; –5.5%
Autotrophic respiration
Heterotrophic respiration
+; ns
Soil P supply
–; ns
Soil enzymes
PPO: +; ns
POD: +; ns
Soil C stock
-7.5% ; ns
Topsoil C stock
–16.0% ; –18.9%
Subsoil C stock
ns; ns
Microbial community
G+/G–: ns; +
Bacteria/Fungi: ns; –
DOC leaching
ns; -34.5%

Fig. 5