**Differential responses of soil respiration to soil warming and experimental
throughfall reduction in a transitional oak forest in central China**

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Abstract

Examining responses of soil respiration to climate change is crucial for understanding future terrestrial carbon (C) cycling. However, the interaction between climate warming and precipitation reduction on soil respiration has not been well documented. This study aimed to determine the impacts of soil warming and throughfall reduction on soil respiration and its components (heterotrophic respiration and autotrophic respiration).

A field manipulation experiment with soil warming and throughfall reduction was conducted in an oak natural forest (*Quercus aliena*) at a transitional climatic zone in central China during the growing seasons (May–November) in 2011 and 2012. Soil temperature was elevated by 1.23–1.66 °C relative to the ambient environment by using infrared heaters, and throughfall was reduced by 50 % through roof interception.

There were significant interactive effects of soil warming and throughfall reduction on soil respiration and autotrophic respiration in both 2011 and 2012. Soil warming substantially elevated soil respiration by 32.0–46.3 % and autotrophic respiration by 57.8–63.2 % without throughfall reduction, respectively, but suppressed both of them with throughfall reduction. Throughfall reduction increased soil respiration by 16.2–37.2% and autotrophic respiration by 62.9–97.7% under ambient temperature, whereas decreased them by 13.7–29.2% and 22.6–51.9% under soil warming.

Heterotrophic respiration was significantly increased by soil warming while showed little effect by throughfall reduction or its interactions with soil warming. The offset of the positive warming effect on soil respiration under throughfall reduction may be attributed mainly to the changes in soil microbial biomass and fine root biomass induced by throughfall reduction.

Our observations suggest that either climate warming or precipitation reduction may increase soil CO₂ emission, but this stimulation in oak forests will be largely counteracted if climate warming accompanies with simultaneous precipitation reduction at the climatic transitional zone.

Keywords: Autotrophic respiration, Heterotrophic respiration, Microbial biomass, Carbon cycling, Climate warming, Drought

1. Introduction

Increasing global temperature and changing precipitation regime associated with global climate change is expected to exacerbate and engender regional drought events, especially in mid-latitude and subtropical dry regions (IPCC, 2013), which will most likely alter the structure and function of forest ecosystems, and consequently affect terrestrial carbon (C) cycling (Rustad et al., 2001; Lin et al., 2011; Wu et al., 2011; Xia et al., 2014). Most field evidence suggests that climate warming generally stimulates plant growth and ecosystems C flux (e.g. NPP, ecosystem photosynthesis,

and ecosystem respiration), whereas decreased precipitation shows the opposite effects (Wu et al., 2011; Zhou et al., 2013).

Soil respiration is the second-largest terrestrial C flux in the global C cycle (Bond–Lamberty and Thomson, 2010), and therefore, any potential change in the rate of soil respiration induced by biotic (Högberg et al., 2001, 2008; Caquet et al., 2012) or abiotic factors (Flanagan and Johnson, 2005; Flanagan et al., 2013) will have strong impact on the global C cycling and subsequently climate change (Davidson and Janssens, 2006; Phillips et al., 2012). Therefore, a deep insight into soil respiration would enhance our ability to predict terrestrial C fluxes and C sink potential under future climate change.

Soil respiration is strongly influenced by soil temperature and water availability (Verhoef et al., 1996), and these two environmental factors are closely related to climate warming and changing precipitation patterns (Rustad et al., 2001; Zhou et al., 2006; Schindlbacher et al., 2012). Numerous studies have examined the responses of soil respiration to soil warming and precipitation reduction in forest ecosystems (Sotta et al., 2007; Xu et al., 2010). A meta-analysis showed that experimental warming significantly increased soil respiration in forest ecosystems (Rustad et al., 2001; Wu et al., 2011), as a result of increased decomposition of soil organic C (SOC). However, soil respiration was suppressed by experimental warming in a semi-arid Pannonian sand forest-steppe ecosystem (Lellei-Kovács et al., 2008). The differential responses of soil respiration to rising temperature could be attributed to interactions with other

environmental factors, such as soil water availability (Wang et al., 2014). Throughfall reduction was reported to reduce soil respiration in a temperate mixed beech–spruce forest (Nikolova et al., 2009) and in a tropical *terra firme* forest (Sotta et al., 2007), whereas elevate soil respiration in the wet tropical rain forest (Cleveland et al., 2010). Wu et al. (2011) found that decreased precipitation, regardless of vegetation types, could reduce soil respiration by 12 %. These contradictory results have been attributed to the difference in soil texture, plant root, and soil microbial activities (Scott et al., 2007). Even though individual effects of increasing temperature and reduced precipitation on soil respiration have been widely studied, it remains unclear how soil respiration responds to the concurrent changes in temperature and precipitation, and whether they interacted with each other to affect soil respiration.

One of the key challenges to evaluate the role of soil respiration in global C cycling is that soil respiration is a combined flux comprised of autotrophic respiration of roots and associated rhizosphere community, and heterotrophic respiration from the decomposition process of SOC (Kuzyakov, 2002; Jassal and Black, 2006; Hinko–Najera et al., 2015; Song et al., 2015). Autotrophic respiration is strongly affected by the allocation of recently assimilated photosynthates to belowground and root activity (Högberg et al., 2001; Shi et al., 2011), whereas heterotrophic respiration is dependent on the microbe activity and substrate availability, such as C input from litterfall, root turnover, and soil C content (Hanson et al., 2000; Hinko–Najera et al., 2015). The two components represent different ecological and biological processes

and respond differently to changes in environment drivers, including temperature and water (Boone et al., 1998; Scott–Denton et al., 2006; Schindlbacher et al., 2009; Wang et al., 2014), and thus, have profound implications on the soil C storage (Boone et al., 1998; Luan et al., 2011a). For example, respiration from bulk soil was higher than that from planted soils under soil warming (Hartley et al., 2007). However, the two respiration components displayed similar levels of elevation under warming treatment in a mature Norway spruce forest (Schindlbacher et al., 2009). In a temperate broadleaved evergreen eucalypt forest, throughfall reduction strongly suppressed autotrophic respiration, but had no effect on heterotrophic respiration (Hinko-Najera et al., 2015). Therefore, without partitioning and quantifying soil respiration components, soil total respiration measurements alone provide little insight into terrestrial C cycling and are insufficient to evaluate its response to future climate change (Kuzyakov, 2002; Wang et al., 2014).

Forests dominated by oak species (*Quercus* spp.) are the largest forest biome in area and in vegetation C storage among all forest types in China, which covers a total area of 15.5×10^6 ha (15.2% of all forests; Fang et al., 1996) and storing 835.94 Tg vegetation C (22.4% of all forests; Wang et al., 2001). Therefore, oak forests play an important role in controlling ecosystem C cycling and regional climate changes in China, and have been reported to be strong C sinks in terrestrial ecosystems (Fang et al., 2001). Previous studies in oak forest ecosystems have found that the rising temperature can stimulate soil respiration (Luan et al., 2014), whereas experimental

drought can reduce aboveground biomass (Ogaya et al., 2003) and soil respiration (Asensio et al., 2007). In addition, oaks are dominantly associated with ectomycorrhizal, which can produce extracellular enzymes to degrade soil organic matter (Phillips et al., 2013), and thus exert influence on the response of soil respiration to climate change. Whether these C sinks will persist or become sources under climate change is uncertain and may depend largely on the responses of soil respiration to climate change (Deng et al., 2012).

We conducted a field manipulation experiment by soil warming and throughfall reduction in a temperate oak natural forest from 2010 to 2012, in order to examine the responses of soil respiration to climate warming and changing precipitation pattern. The study site was located at a transitional climatic zone from northern subtropical to warm-temperate climate in central China, because the transitional climatic zone was illustrated to be more susceptible to changing climate than the other regions (Mahlstein et al., 2013). Previous study in this area indicates that the seasonal variability of soil respiration is dependent on soil temperature but independent of soil moisture as a result of the relative sufficient rainfall (Luan et al., 2011a). Soil warming can reduce soil moisture through stimulating evapotranspiration, leading to elevated root production (Majdi and Öhrvik, 2008) and soil microbial activity (Schindlbacher et al., 2012). Thus, we first hypothesized that soil warming should increase soil respiration by elevating both heterotrophic and autotrophic respiration. Given that throughfall reduction can partially decrease throughfall and improve soil

aeration condition, and probably increase plant root and soil microbial activity (Liu et al., 2014), our second hypothesis is that throughfall reduction also elevate soil respiration through enhancing both heterotrophic and autotrophic respiration.

However, a combination of throughfall reduction and soil warming may aggravate soil water deficits and suppress plant and soil microbial metabolism. Therefore, our third hypothesis is that soil respiration under the combined treatment should be lower than that under any single factor.

2. Materials and Methods

2.1. Site description

The study site is located at the Baotianman Nature Reserve (111°47'–112°04' E, 33°20'–33°36'N), Henan Province, central China. The mean annual temperature is 15.1 °C, with monthly air temperatures ranging from 1.5 °C in January to 27.8 °C in July (at 1400 m a.s.l.). The mean annual precipitation is approximately 894 mm with 60 % of rainfall falling in summer season (from June to August). Upland soils are dominated by Haplic luvisol soils (Luan et al., 2011a). The typical forest in this area is the warm–temperate forest composed of temperate deciduous broadleaf trees as well as some subtropical tree species due to its transitional location in the northern transitional subtropical to warm–temperate climate.

The experiment was carried out in an oak natural forest (58 years old), dominated by *Quercus aliena* var. *acuteserrata*, which accounts for 66 % of the overstory. Other non–dominant tree species included *Carpinus cordata* BL., *Lindera obtusiloba* BL.,

Acer spp., and *Dendrobenthamia hupehensis* Fang. Mean diameter at breast height (DBH) of the stand was 20.1 ± 4.5 cm. Stand density and basal area were 1276.7 tree·ha⁻¹ and 33.5 m²·ha⁻¹, respectively. The average bulk density and soil organic C content at 10 cm surface layer were 0.70 ± 0.09 g cm⁻³ and 41.20 ± 1.71 g kg⁻¹, respectively. Soil capillary porosity ranged from 0.50 to 0.62 (Luan et al., 2011b).

2.2. Experimental design

A factorial design of temperature and precipitation was established in March 2011, laid out four treatments (control (C), throughfall reduction (T), soil warming (W), and throughfall reduction plus soil warming (TW)). Twelve (three replicates) 3 m × 4 m plots separated by 3 m wide buffer strips were established and were randomly assigned to the treatments. We dug trenches and cut off all roots to estimate heterotrophic and autotrophic components of soil respiration (Hanson et al., 2000; Jassal and Black, 2006; Hinko–Najera et al., 2015).

2.3. Soil warming

In each warming plot, one 165 cm×15 cm MSR–2420 infrared heater (Kalglo Electronics Inc., Bethlehem, PA, USA) with a radiation output of 100 Watts m⁻² was suspended in the middle of each plot at the height of 2.25 m above the ground (and under the throughfall reduction roof in the combined treatment plots) (Xia et al., 2009; Aguilos et al., 2011). The heaters had been operating continuously, 24 hours per day and 365 days per year since May 1, 2011 (yet data used in this study is only from May to November). In agreement with Wan et al. (2002), the effects of infrared radiators

on soil temperature were spatially uniform within the warming plots ($CV=3\%$, Fig. A.1). In the unheated control plot, one ‘dummy’ heater made of metal flashing of the same shape and size as the infrared radiator was suspended 2.25 m above the surface to simulate shading effects of heaters.

2.4. Throughfall reduction

Throughfall was reduced by $\sim 50\%$ by covering 50 % of the plot area with interception roof. We installed a $4\text{ m} \times 5\text{ m}$ interception roof covered with eight $0.25\text{ m} \times 5\text{ m}$ plastic sheets mounted to iron wire frames in each throughfall reduction plot with 0.5 m distance to the plot edge in the forest understory (Yahdjian et al., 2002; Borken et al., 2006). Gaps of 0.2 m between adjacent plastic sheets allowed air to circulate through the roof, minimizing temperature and humidity effects. The plastic sheets were transparent and able to transmit 60–70% of photosynthetically active radiation. The roof was kept approximately $10\text{--}15^\circ$ slope by setting the two sides at different heights (3.5 m for one side and 2.5 m for the other) to ensure quick drainage of ambient rainfall by gravity.

Excluded precipitation from each roof was drained into eight 30 L PVC tanks, and the water was dumped out to the nearest valley 70 m away following each rainfall event. The plastic sheets were swept every 4–5 days to transfer accumulated litter onto the forest floor beneath.

Throughfall was partially excluded from the treatment plots during the growing seasons (May–November). The plastic sheets were removed during the snow seasons

to avoid damage of the roof frame from snowpack. They were put back in place before the next rain season started. Due to less than 9 % of rainfall falling during winter time, we consider that the removal of the frame during winter will not have much impact on our experiment treatment. Rainfall was measured in forest understory using throughfall collectors. To eliminate surface water flow and lateral movement of soil water into the plots, a 0.5 m deep trench was excavated and lined with double-layer plastic sheets on the uphill side of the experimental site (Heisler–White et al., 2008). The plastic sheets were kept 0.2 m above ground surface, and then the trench was refilled. Spatial heterogeneity of soil moisture was low under the throughfall reduction treatment ($CV = 13\%$, Fig. A.1) so that potential effects of surface water flow and lateral movement of soil water can be ignored.

2.5. Trenching

We established two subplots (3 m×2 m) within each 3 m×4 m experimental plot. One subplot was randomly selected for the trenching treatment to determine soil heterotrophic respiration. Trenches of 0.2 m wide and 0.7 m deep (below which few root existed, Luan et al., 2011a) were excavated around each subplot in March 2011. After lining the trench with 2 mm thick plastic sheet (compound crystal film), soil was refilled into the trench. All aboveground plants were carefully removed with minimal soil disturbance, and the trenched subplots were then kept free of seedlings and herbaceous vegetation by periodical manual removal throughout the experiment.

2.6. Soil CO₂ efflux measurement

At each subplot, two polyvinyl chloride (PVC) collars (19.6 cm inner diameter, 8 cm height) were inserted to a depth of 5 cm along diagonal direction by a 0.3 m buffer to edge in March 2011. A total of 48 PVC collars were installed for all plots. Soil surface CO₂ flux was measured using a Li-8100 soil CO₂ flux system (LI-COR Inc., Lincoln, NE, USA). The measurements were made once a week from May to November in 2011 and 2012.

2.7. Soil temperature and moisture measurements

Soil temperature at 5 cm depth was measured adjacent to each respiration collar with a portable temperature probe connected with the Li-8100. Soil moisture (volumetric water content, % v/v) at 0–5 cm was measured with a portable time domain reflectometer MPKit-B soil moisture gauge (NTZT Inc., Nantong, China) at three points close to each collar. Soil temperature and moisture were also investigated at the time of soil respiration measurement.

2.8. Fine root biomass and soil microbial biomass measurements

Fine root (< 2 mm diameter) biomass (FRB) was determined using soil cores (Pregitzer et al., 2008), and was sampled once per year in July 2011 and July 2012, respectively. For each sampling, three soil cores of 70 mm in diameter were taken along the diagonal direction in each plot to a 30 cm depth to cover the most active fine tree roots. Each fine root core was first sieved by 2 mm mesh, and fresh soil samples were collected for further analysis in laboratory. The remaining root samples from the soil core were washed and hand sorted to separate living fine roots from dead roots,

stem materials and litter fractions based on the morphology and color (Peichl and Arain, 2006). Live FRB was measured after oven drying at 70 °C for 72 hours.

Fresh soil sample sieved by 2 mm mesh was used for soil microbial biomass C (MBC) and nitrogen (MBN) analysis. MBC and MBN were estimated by chloroform fumigation–extraction method (CFE; Vance et al., 1987). Twenty grams of dry–weight–equivalent soil samples were fumigated with CHCl_3 for 48 hours at 25 °C, and then the fumigated and un–fumigated samples were extracted with 60 ml of 0.5 M K_2SO_4 after shaking for 30 minutes with a reciprocal shaker. K_2SO_4 extracts were filtered through 0.45 μm filters and frozen at –20 °C before analyzing for extractable C and N by a total organic C analyzer (TOC–VCSH, Shimadzu Corp. Japan). MBC and MBN were calculated from the differences between extractable C and N contents in the fumigated and un–fumigated soil samples using conversion factors of 0.45 (k_{EC}) (Wu et al., 1990) and 0.54 (k_{EN}) (Brookes et al., 1985; Vance et al., 1987), respectively. Air dried soil samples were passed through a 0.25 mm mesh sieve for SOC and total N (TN) analysis. SOC was analyzed by the dichromate oxidation method (Nelson and Sommers, 1996), and TN was analyzed using the Kjeldahl method (Bremner, 1996).

2.9. Data analysis

Autotrophic respiration was estimated by subtracting heterotrophic respiration (trenched subplots) from soil respiration (un – trenched subplots) in each plot. The apparent temperature sensitivities of soil respiration, heterotrophic respiration, and

autotrophic respiration were described by means of a Q_{10} function (Janssens and Pilegaard, 2003):

$$R = R_{10} \times Q_{10}^{((T-10)/10)} \quad (1)$$

Where R represents soil respiration, heterotrophic respiration, and autotrophic respiration, respectively; T is the soil temperature at 5 cm depth; R_{10} and Q_{10} were fitted parameters.

The impacts of soil warming, throughfall reduction, and annual variation (year) on soil respiration and its components, temperature sensitivity, and soil microclimate were tested using three-way ANOVA. Since significant interactions between soil warming and throughfall reduction on soil respiration and autotrophic respiration (all $P < 0.001$; Table 1), one-way ANOVA was then carried out separately to test effects of soil warming and throughfall reduction on different respiration components. We presented the effects (relative change, %) of soil warming with throughfall reduction as $(TW-T)/T \times 100$, and without throughfall reduction as $(W-C)/C \times 100$, respectively. The impact of throughfall reduction with and without soil warming was calculated as $(TW-W)/W \times 100$ and $(T-C)/C \times 100$, respectively (Klein et al., 2004). To determine which factors drive the changes in soil respiration in response to soil warming and throughfall reduction, linear regression analysis was used between soil CO_2 efflux and soil temperature, soil moisture, FRB, SOC, TN, MBC, and MBN. Stepwise multiple regressions were further conducted to ascertain the contribution of the above factors in influencing soil CO_2 efflux. Statistical analyses were carried out using SPSS 10.0

(SPSS Science, Birmingham, UK).

3. Results

3.1. Soil temperature and moisture

Soil warming treatment significantly increased soil temperature by 1.03–1.65 °C in 2011 and by 0.97–1.66 °C in 2012 for un-trenched subplots (all $P < 0.01$; Fig. 1a, b), and by 1.26–1.38 °C in 2011 and by 1.23–1.55 °C in 2012 for trenched subplots (all $P < 0.01$; Table 1, Fig. 1 c, d). Throughfall reduction significantly elevated soil temperature in the untrenched subplots by 0.08 °C in 2011 and 0.14 °C in 2012 for plots without soil warming (T–C; both $P < 0.05$), and by 0.36 °C in 2011 and 0.07 °C in 2012 with soil warming (TW–W, both $P < 0.05$; Fig. 1a, b).

In the un-trenched subplots, soil warming reduced soil moisture by 10.71% in 2011 and 12.12% in 2012 (W – C, both $P < 0.01$), and throughfall reduction had a comparable effect with warming, with a reduction of 11.25 % in 2011, and 9.30 % in 2012 (T – C, both $P < 0.01$; absolute difference). In the trenched subplots, soil warming reduced soil moisture by 5.07 % in 2011 and 6.27 % in 2012 without throughfall reduction (W–C), and by 3.92 % in 2011 and 4.73 % in 2012 with throughfall reduction (TW–T, all $P < 0.05$; Fig. 1g, h). Throughfall reduction lowered soil moisture by 4.8 % in 2011 and 8.0 % in 2012 under ambient temperature (T – C), and by 3.6 % in 2011 and 6.4 % in 2012 when combined with soil warming (TW – W, all $P < 0.05$; Fig. 1g, h) in the trenched subplots.

3.2. Soil CO₂ efflux

There were strong inter-annual variations in soil respiration and heterotrophic respiration during the two growing seasons (both $P < 0.05$; Table 1), with higher values in 2011 than 2012 (Fig. 2). Soil warming significantly interacted with throughfall reduction to affect the mean annual soil respiration ($T \times W$: $P < 0.01$; Table 1). Soil warming substantially stimulated soil respiration by 32.0 % in 2011 and 46.3 % in 2012 under ambient precipitation ($W-C$, both $P < 0.05$; Fig. 3a, b), but suppressed it in 2011 and 2012 when throughfall was partially excluded ($TW-T$, Fig. 3a, b). Throughfall reduction increased soil respiration by 16.2 % in 2011 and 37.2 % ($P < 0.05$) in 2012 under ambient temperature ($T-C$, Fig. 3a, b), whereas decreased it by 29.2 % ($P < 0.05$) in 2011 and 13.7 % in 2012 under warming condition ($TW-W$, Fig. 3a, b).

Soil warming significantly increased heterotrophic respiration (W : $P < 0.05$), whereas throughfall reduction and its interaction with soil warming showed no effects on heterotrophic respiration (all $P > 0.05$, Table 1). Soil warming significantly increased heterotrophic respiration by 37.7 % without throughfall reduction ($P < 0.05$, $W-C$), and by 29.0 % with throughfall reduction in 2012 ($P < 0.05$, $TW-T$, Fig. 3c, d).

There were significant interactive effects of soil warming and throughfall reduction on autotrophic respiration ($P < 0.05$; Table 1), even though the main effects of soil warming or throughfall reduction were not significant (both $P > 0.05$; Table 1). Soil warming significantly increased autotrophic respiration by 57.8 % in 2011 and 67.7 % in 2012 under ambient precipitation ($W-C$, both $P < 0.05$; Fig. 3c, d), but suppressed

it by 53.4% in 2011 ($P < 0.01$) and 36.1% in 2012 ($P > 0.05$) when throughfall was partly excluded (TW–T, Fig. 3c, d). Throughfall reduction substantially stimulated autotrophic respiration without soil warming by 62.9 % and 97.7 % in 2011 and 2012 (T – C, both $P < 0.05$; Fig. 3a, b), but suppressed it with soil warming by 51.9 % in 2011 ($P < 0.01$) and 22.6 % in 2012 (TW – W; Fig. 3a, b).

Soil warming significantly interacted with throughfall reduction to affect relative contributions of heterotrophic respiration to soil respiration (HR/SR) in 2011 and 2012 (both $P < 0.05$, Fig. 4). Throughfall reduction decreased HR/SR by 12.6 – 13.1% under ambient temperature but increased it by 6.6 – 11.1% under soil warming (Fig. 4).

3.3. Changes in Q_{10} induced by treatments

The main effects of soil warming and throughfall reduction on the Q_{10} of soil respiration and its components were not significant ($P > 0.05$ for both T and W; Table 2). Significant interactive effects of throughfall reduction and year on the Q_{10} values of soil respiration and heterotrophic respiration ($P < 0.05$ for Y×T; Table 2) were observed. Throughfall reduction significantly enhanced Q_{10} of soil respiration by 1.2 in 2012 ($P < 0.01$), but substantially decreased Q_{10} values of heterotrophic respiration by 0.4 in 2011 ($P < 0.05$, Fig. 5). In addition, Q_{10} values of autotrophic respiration were significantly higher than those of soil respiration and heterotrophic respiration in both years ($P < 0.05$, Fig. 5).

3.4. Dependence of soil CO₂ efflux on biotic and abiotic factors

In this study, soil respiration and heterotrophic respiration increased exponentially with soil temperature (both $P < 0.001$), but showed no relationship with soil moisture (Fig. 6a, b, c, d). Autotrophic respiration was positively correlated with soil temperature ($P < 0.001$), but negatively correlated with soil moisture ($P < 0.05$, $R^2 = 0.22$; Fig. 6e, f). Regardless of different treatments and years, soil respiration was linearly and positively correlated with MBC ($R^2 = 0.17$, $P < 0.05$) and MBN ($R^2 = 0.27$, $P < 0.01$; Fig. 7a, b). Regression analyses showed that soil respiration for all treatments was positively correlated with FRB over the two years ($R^2 = 0.25$, $P < 0.05$; Fig. 7c). Across all treatments, heterotrophic respiration showed positive and linear dependence upon MBN ($R^2 = 0.37$, $P < 0.01$; Fig. 7e). In addition, autotrophic respiration was marginally correlated to FRB ($R^2 = 0.13$, $P = 0.08$; Fig. 7f).

Stepwise multiple regression analyses revealed that autotrophic respiration alone explained 59 % ($P < 0.001$) of the variation in soil respiration, and additional 40 % variation could be attributed to heterotrophic respiration across the 12 plots (Table 3). Among the biotic and abiotic environmental factors, MBN (Partial $R^2 = 0.30$) and soil temperature (Partial $R^2 = 0.04$) together explained 34 % variation ($P = 0.005$) in soil respiration. Soil temperature alone explained 21 % of the variation in heterotrophic respiration, and the combination of SOC and FRB accounted for additional 14 % of the variation (Table 3). For autotrophic respiration, the variability could be ascribed to soil temperature (25 %) alone across the two seasons.

4. Discussion

4.1. Effect of soil warming on soil CO₂ efflux

The increase in soil respiration (32.0–46.3%) under soil warming in this study was greater than the 20 % reported by Rustad et al. (2001), but comparable to other warming studies in forests (27–47%; Melillo et al., 2002; Niinistö, 2004; Schindlbacher et al., 2009). However, we did not observe a declining response of soil respiration to warming (acclimation) during the first two years experiment, as reported by Melillo et al. (2002) and Niinistö et al. (2004). Similar to Hartley et al. (2007) and Schindlbacher et al. (2009), we found an increase in heterotrophic respiration under soil warming, but the increment (13.5–37.7%) was lower than reported in cool temperate mixed forest (74.0%, Aguilos et al., 2011). In addition, soil warming caused a 57.8–67.7 % increase in autotrophic respiration, which was in line with Schindlbacher et al.'s (2009) result (56–75%). Thus, our results support the first hypothesis that soil warming should stimulate soil respiration by elevating both heterotrophic respiration and autotrophic respiration.

The positive response of soil respiration to soil warming may be attributed mainly to the elevated FRB (increased by 26.3–30.5%) and microbial biomass (increased by 57–70%) in oak forests (Table A.1). Our observation is consistent with the earlier study by Bai et al. (2010), in which a 17 % increase in root standing crop with soil warming resulted in either direct stimulation of root growth or indirect enhancement of root and mycorrhizal respiration. Similarly, Allen and Vu (2009) reported that both fine roots and total root biomass were increased with increased soil temperature.

Previous studies with short-term experimental warming (< 3 years) showed that warming had no obvious effect on microbial biomass (Biasi et al., 2008; Xu et al., 2010), whereas long-term (> 3 years) manipulated soil warming induced significant increase or decrease in microbial biomass (Frey et al., 2008; Belay-Tedla et al., 2009). In this study, however, *in situ* soil warming stimulated soil microbial biomass (MBC and MBN) under ambient precipitation in both trenched and un-trenched plots (Table A.1). The possible mechanism underlying this phenomenon may be that reduced soil water availability due to soil warming should not be a limiting factor influencing microbial activity in transitional climatic zone under the annual average precipitation of approximately 900 mm. Also, soil substrate may not limit biological activity due to abundant litter fall input (about 11.23 t ha⁻¹yr⁻¹; Liu et al., 2003).

4.2. Effects of throughfall reduction on soil CO₂ efflux

The finding that throughfall reduction increased soil respiration by elevating autotrophic respiration, but showed little effect on heterotrophic respiration, partially supports our second hypothesis. Our results agree with Cleveland et al. (2010) who found that throughfall reduction caused an increased soil respiration in a tropical rain forest in Costa Rica. Similar increases in soil respiration were also observed under throughfall reduction in Tapajós National rainforest in Brazil (Davidson et al., 2008). In contrast, some earlier studies in tropical forests reported that soil respiration showed either reduction with decreasing soil water content (Sotta et al., 2007; Nikolova et al., 2009; Suseela and Dukes, 2013) or no change with throughfall

reduction (Davidson et al., 2008). As Sotta et al. (2007) suggested, different responses of soil CO₂ efflux to soil water content may be explained by variations in the soil texture, root distribution, and soil microbes among different ecosystems. In this study, soil moisture (all > 20 % v/v) was relatively higher than that reported by Sotta et al. (2007) and Nikolova et al. (2009) even though soil water availability was significantly reduced by throughfall reduction. In addition, the loamy soil with good water holding capacity at this site (Shi et al., 2005) could weaken the effect of water stress resulting from throughfall reduction on microbial and root activities. Therefore, increased soil respiration under throughfall reduction in this study should be attributed mainly to the improved aerobic processes as a result of the reduced water-filled pore spaces (Liu et al., 2014). A negative correlation between soil moisture and soil respiration was reported in this area (Luan et al., 2011a,b), also suggesting that higher soil water content may suppress the release of soil CO₂ flux. Thus, the positive effects of throughfall reduction on soil respiration may be the result of improved root and microbe activities under a relatively optimal range of soil moisture.

The similar changes in soil respiration and autotrophic respiration under throughfall reduction suggest that throughfall reduction-induced increases in soil respiration should result mainly from root/rhizosphere respiration, rather than from microbial respiration (Fig. 4). This is in disagreement with earlier studies in which reduced throughfall decreased autotrophic respiration but not heterotrophic respiration in a dry temperate evergreen forest (Hinko-Najera et al., 2015). Consistent with our findings,

however, experimental drought had also been found to decrease heterotrophic respiration, but tended to increase autotrophic respiration in a mixed deciduous forest (Borken et al., 2006; Risk et al., 2012). The negative responses of autotrophic respiration to soil water availability in this study (Fig. 6f) may be caused by the positive response in root biomass (Fig. 7). Reduced water availability in surface soil probably increased root biomass in order to search for more water deeper in the soil profile (Joslin et al., 2000). The stimulation of FRB by decreased precipitation (Table A.1) has also been observed in central European mixed stands, where reduced soil water apparently elevated fine root growth of *Fagus* during a dry mid–summer (Leuschner et al., 2001). Similarly, Steinberg et al. (1990) and Kramer (1995) reported that improved aerobic processes resulting from decreased soil water content increased root/shoot ratios of several tree species through increased C allocation to the roots.

Unlike some previous studies (Davidson and Janssens, 2006; Cleveland et al., 2010), we found that throughfall reduction had no effect on heterotrophic respiration. This result is in agreement with Hinko–Najera et al. (2015) and may be explained by the insignificant changes in soil microbial activity and substrate availability under throughfall reduction (Table A.1). Lower soil moisture induced by throughfall reduction can decrease the diffusivity of dissolved organic C in the soil as the soil dries (Davidson et al., 2008; Cleveland et al., 2010). In addition, soil temperature and soil C availability (SOC and root biomass) could be the main controllers on heterotrophic respiration, which was proved by our multi – regression analysis (Table

3). The results of this study imply that moderate water reduction (decreased by 5–10% v/v compared to control plots) will not affect soil microbial activity under throughfall reduction in the trenched plots.

4.3. Interactive effect of soil warming and throughfall reduction on soil CO₂ efflux

The interactive effect between warming and throughfall reduction (Table 1) suggests that the warming-induced soil CO₂ emission by soil warming could be offset, to a certain degree, by the decreased soil water availability due to throughfall reduction (Figure 3). Our findings are in line with the study in an old Norway spruce (*Picea abies*) forest in Austria (Schindlbacher et al., 2012), in which warming induced C losses from soils were likely to have been offset by reduced soil respiration during and after summer drought.

Differential responses of soil respiration to single– and two–factor treatments can be explained by the changes in biotic and abiotic factors induced by different treatments (Table 3 and Figure 7). For example, under throughfall reduction conditions, soil warming further intensified soil water stress, which may exceed the threshold that is suitable for the metabolism of plant roots, resulting in decrease in C allocation to belowground (Meier and Leuschner, 2008). This can be supported by the suppression of FRB under soil warming with throughfall reduction (TW – T; Table A.1) and decreased contribution of autotrophic respiration to soil respiration (Figure 4). On the other hand, the greatly reduced soil moisture may constrain microbial activity and slow down the decomposition of plant litter and soil organic matter

(Schimel et al., 1999), which can also be proved by the reduced MBC and MBN under combined treatment (TW – T; Table A.1). In contrast to the decreased contribution of heterotrophic respiration to soil respiration under single factor treatments, the contribution of heterotrophic respiration was enhanced under combined treatment.

4.4. Trenching effect

As one of the root reduction approaches (Hanson et al., 2000; Kuzyakov, 2002), the trenching method has been widely used in forest ecosystems to separate auto- and heterotrophic parts of soil respiration (Yi et al., 2007; Luan et al., 2011a; Savage et al., 2013). One of the main drawbacks is the priming effect resulting from the decomposition of dead roots following the trench treatment (Hanson et al., 2000; Ngao et al., 2007). We believe this process is unlikely to affect our estimation of heterotrophic respiration due to its minor contribution (5%) to soil respiration after 3 months of trenching in this area (Luan et al., 2011a). In addition, we found a higher level of soil moisture (3.7–9.4%, absolute difference) in the trenched plots compared with the un-trenched plots, which may lead to an overestimation of heterotrophic respiration (Yan et al., 2010). Following Saiz et al. (2007) and Yan et al. (2010) simulating the relationship between heterotrophic respiration and soil temperature combined with moisture, an exponential–quadratic function was used to determine this over-estimation error. Our results showed that the heterotrophic respiration contribution decreased by 6.5% after this correction. Although some over- or

under-estimation may be inevitable, we feel that our trenching method is reasonable in this study and able to help partition respiration components in oak forests.

4.5. Seasonal variation of soil CO₂ efflux and Q₁₀

Similar to previous studies (Schindlbacher et al., 2009; Luan et al., 2011a), we observed seasonal variations in soil respiration, heterotrophic respiration, and corresponding temperature sensitivities. Seasonal fluctuation of soil CO₂ efflux may be attributed to the differences in various factors such as soil microclimate and labile C supply from plants and soil microbial activity (Högberg et al., 2001; Schindlbacher et al., 2009). In this study, the positive linear correlations between soil respiration and MBC and FRB may have contributed, to some extent, to the seasonal variation because higher MBC and FRB were found in 2012. The reduced Q₁₀ of soil respiration and heterotrophic respiration for all treatments in 2012 probably resulting from the relatively low soil moisture content due to the less-than-average (890 mm) annual precipitation (680 mm for 2012). Similar influences of soil water content on Q₁₀ have been observed in Douglas-fir forest (Jassal et al., 2008) and hardwood forest (Davidson et al., 2008). Differential influences of throughfall reduction on Q₁₀ between 2011 and 2012 may be explained by the variability in root biomass and MBC. The higher increases in FRB and MBC induced by throughfall reduction in 2012 (Table A.1) may exert a positive effect on Q₁₀ of soil respiration and heterotrophic respiration (Janssens et al., 2003).

4.6 Implications for soil CO₂ emission from oak forests

As the largest forest biome and forest C storage, oak forests play an important role in regulating regional C cycling in China. we estimate that the annual soil CO₂ emission from oak forests in China may increase by 0.033-0.046 Gt C under the warming scenario of *ca* 1.2–1.7°C (temperature increase) and by 0.017-0.029 Gt C under the drought scenario of *ca* 50% precipitation reduction, respectively. However, precipitation reduction may decrease soil CO₂ emission by 0.009-0.032 Gt C under soil warming based on the simulation scenario in this study. The rough estimation indicates that soil pool of oak forest may transform from C source to sink if climate warming is simultaneously coupled with precipitation reduction. In fact, carbon assimilation and emission processes from plants were not studied in this study; their responses to warming and decreased precipitation could largely shape the response of soil CO₂ emission to climate change. Therefore, aboveground C processes should be taken into consideration when assessing the response of soil C cycling to future climate change.

5. Conclusions

In a warm temperate oak forest at the transitional climatic zone in central China, we observed that throughfall reduction substantially interacted with soil warming to affect soil respiration and autotrophic respiration. Both throughfall reduction and soil warming elevated soil respiration and autotrophic respiration, whereas negative effects occurred when both throughfall reduction and soil warming were applied simultaneously. Positive responses of heterotrophic respiration were observed under

soil warming, but not under throughfall reduction. Our findings suggest that soil CO₂ emission in transitional oak forests may be stimulated under either precipitation reduction or climate warming, but this stimulation may be weakened if climate warming is simultaneously coupled with precipitation reduction.

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References

- Aguilos, M., Takagi, K., Liang, N., Watanabe, Y., Goto, S., Takahashi, Y., Mukai, H., Sasa, K., 2011. Soil warming in a cool–temperate mixed forest with peat soil enhanced heterotrophic and basal respiration rates but Q₁₀ remained unchanged. *Biogeosci. Discuss.* 8, 6415–6445.
- Allen, L.H., Vu, J.C.V., 2009. Carbon dioxide and high temperature effects on growth of young orange trees in a humid, subtropical environment. *Agric. For.*

Meteorol. 149(5), 820–830.

Andrews, J.A., Harrison, K.G., Matamala, R., Schlesinger, W.H., 1999. Separation of root respiration from total soil respiration using carbon-13 labeling during free-air carbon dioxide enrichment (FACE). *Soil Sci. Soc. Am. J.* 63(5), 1429–1435.

Asensio, D., Penuelas, J., Ogaya, R., Llusà, J., 2007. Seasonal soil and leaf CO₂ exchange rates in a Mediterranean holm oak forest and their responses to drought conditions. *Atmosph. Envir.* 41(11), 2447–2455.

Bai, W., Wan, S., Niu, S., Liu, W., Chen, Q., Wang, Q., Zhang, W., Han, X., Li, L., 2010. Increased temperature and precipitation interact to affect root production, mortality, and turnover in a temperate steppe: implications for ecosystem C cycling. *Global Change Biol.* 16(4), 1306–1316.

Belay-Tedla, A., Zhou, X., Su, B., Wan, S., Luo, Y., 2009. Labile, recalcitrant, and microbial carbon and nitrogen pools of a tallgrass prairie soil in the US Great Plains subjected to experimental warming and clipping. *Soil Biol. Biochem.* 41(1), 110–116.

Biasi, C., Meyer, H., Rusailimova, O., Hämmerle, R., Kaiser, C., Baranyi, C., Daims, C., Lashchinsky, N., Barsukov, P., Richter, A., 2008. Initial effects of experimental warming on carbon exchange rates, plant growth and microbial dynamics of a lichen-rich dwarf shrub tundra in Siberia. *Plant Soil* 307(1), 191–205.

Bond-Lamberty, B., Thomson, A., 2010. Temperature-associated increases in the global soil respiration record. *Nature* 464(7288), 579–82.

Boone, R.D., Nadelhoffer, K.J., Canary, J.D., Kaye, J.P., 1998. Roots exert a strong

influence on the temperature sensitivity of soil respiration. *Nature* 396(6711), 570–572.

Borken, W., Savage, K., Davidson, E.A., Trumbore, S.E., 2006. Effects of experimental drought on soil respiration and radiocarbon efflux from a temperate forest soil. *Global Change Biol.* 12(2), 177 – 193.

Bremner J.M., 1996. Nitrogen – total. In: Sparks D.L. (ed) *Methods of soil analysis*. SSSA Book Ser, Madison, pp:1085 – 1122.

Brookes, P., Landman, A., Pruden, G., Jenkinson, D., 1985. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. *Soil Biol. Biochem.* 17(6), 837–842.

Caquet, B., De Grandcourt, A., Thongo, M., Epron, D., Kinana, A., Saint, A., Nouvellon, Y., 2012. Soil carbon balance in a tropical grassland: Estimation of soil respiration and its partitioning using a semi–empirical model. *Agric. For. Meteorol.* 158, 71–79.

Cleveland, C.C., Wieder, W.R., Reed, S.C., Townsend, A.R., 2010. Experimental drought in a tropical rain forest increases soil carbon dioxide losses to the atmosphere. *Ecology* 91(8), 2313–2323.

Davidson, E.A., Janssens, I.A., 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature* 440(7081), 165–73.

Davidson, E.A., Nepstad, D.C., Ishida, F.Y., Brando, P.M., 2008. Effects of an experimental drought and recovery on soil emissions of carbon dioxide, methane,

nitrous oxide, and nitric oxide in a moist tropical forest. *Global Change Biol.* 14(11), 2582–2590.

Deng, Q., Hui, D.F., Zhang, D.Q., Zhou, G.Y., Liu J.X., Liu, S.Z., Chu, G.W., Li, J., 2012. Effects of precipitation increase on soil respiration: a three-year field experiment in subtropical forests in China. *PloS one* 7(7), e41493.

Fang, J.Y., Liu, G., Xu, S.L., X., 1996. Biomass and net production of forest vegetation in China. *Acta Ecologica Sinica* 16(5), 497–508.

Fang, J.Y., Chen, A.P., Peng, C.H., Zhao, S.Q., Cil, J., 2001. Changes in forest biomass carbon storage in China between 1949 and 1998. *Science* 292, 2320–2322.

Flanagan, L.B., Johnson, B.G., 2005. Interacting effects of temperature, soil moisture and plant biomass production on ecosystem respiration in a northern temperate grassland. *Agric. For. Meteorol.* 130(3), 237–253.

Flanagan, L.B., Sharp, E.J., Letts, M.G., 2013. Response of plant biomass and soil respiration to experimental warming and precipitation manipulation in a Northern Great Plains grassland. *Agric. For. Meteorol.* 173, 40–52.

Frey, S., Drijber, R., Smith, H., Melillo, J., 2008. Microbial biomass, functional capacity, and community structure after 12 years of soil warming. *Soil Biol. Biochem.* 40(11), 2904–2907.

Högberg, P., Högberg, M.N., Göttlicher, S.G., Beston, N.R., Keel, S.G., Metcalfe, D.B., Campbell, C., Schindlbacher, A., Hurry, V., Lundmark, T., 2008. High temporal resolution tracing of photosynthate carbon from the tree canopy to forest soil

microorganisms. *New Phytol.* 177(1), 220–228.

Högberg, P., Anders, B., Nina, T., Andrew F.S., Ekblad, A., Högberg, M.N., Nyberg, G.O., Mikael, R., David, J., 2001. Large-scale forest girdling shows that current photosynthesis drives soil respiration. *Nature* 411(6839), 789–792.

Hanson, P., Edwards, N., Garten, C., Andrews, J., 2000. Separating root and soil microbial contributions to soil respiration: a review of methods and observations. *Biogeochemistry* 48(1), 115–146.

Hartley, I.P., Heinemeyer, A., Evans, S.P., Ineson, P., 2007. The effect of soil warming on bulk soil vs. rhizosphere respiration. *Global Change Biol.* 13(12), 2654–2667.

Heisler-White, J.L., Knapp, A.K., Kelly, E.F., 2008. Increasing precipitation event size increases aboveground net primary productivity in a semi-arid grassland. *Oecologia* 158(1), 129–140.

Hinko-Najera, N., Fest, B., Livesley, S. J., Arndt, S., 2015. Reduced throughfall decreases autotrophic respiration, but not heterotrophic respiration in a dry temperate broadleaved evergreen forest. *Agric. For. Meteorol.* 200, 66–77.

IPCC, 2013. Working Group I Contribution to the IPCC Fifth Assessment Report. *Climate Change 2013: The Physical Science Basis Summary for Policymakers*, Cambridge, UK/ New York, NY, USA.

Janssens, I.A., Pilegaard, K., 2003. Large seasonal changes in Q_{10} of soil respiration in a beech forest. *Global Change Biol.* 9(6), 911–918.

-
- Jassal, R.S., Black, T.A., 2006. Estimating heterotrophic and autotrophic soil respiration using small–area trenched plot technique: theory and practice. *Agric. For. Meteorol.* 140(1), 193–202.
- Jassal, R.S., Black, T.A., Novak, M.D., Gaumont- Guay, D., Nesic, Z., 2008. Effect of soil water stress on soil respiration and its temperature sensitivity in an 18- year- old temperate Douglas- fir stand. *Global Change Biol.* 14(6), 1305–1318.
- Joslin, J., Wolfe, M., Hanson, P., 2000. Effects of altered water regimes on forest root systems. *New Phytol.* 147(1), 117–129.
- Klein, J.A., Harte, J., Zhao, X.Q., 2004. Experimental warming causes large and rapid species loss, dampened by simulated grazing, on the Tibetan Plateau. *Ecol. Lett.* 7(12), 1170–1179.
- Kramer, K., 1995. Phenotypic plasticity of the phenology of seven European tree species in relation to climatic warming. *Plant Cell Environ.* 18(2), 93–104.
- Kuzyakov, Y., 2002. Separating microbial respiration of exudates from root respiration in non–sterile soils: a comparison of four methods. *Soil Biol. Biochem.* 34(11), 1621–1631.
- Lellei–Kovács, E., Kovács–Láng, E., Kalapos, T., Botta–Dukát, Z., Barabás, S., Beier, C., 2008. Experimental warming does not enhance soil respiration in a semiarid temperate forest–steppe ecosystem. *Commun. Ecol.* 9(1), 29–37.
- Leuschner, C., Backes, K., Schipka, F., Schmitt, U., Terborg, O., Runge, M., 2001. Drought responses at leaf, stem and fine root levels of competitive *Fagus sylvatica* L.

and *Quercus petraea* (Matt.) Liebl. trees in dry and wet years. For. Ecol. Manag.

149(1–3), 33–46.

Lin, X., Zhang, Z., Wang, S., Hu, Y., Xu, G., Luo, C., Chang, X., Duan, J., Lin, Q., Xu, B., 2011. Response of ecosystem respiration to warming and grazing during the growing seasons in the alpine meadow on the Tibetan plateau. Agric. For. Meteorol. 151(7), 792–802.

Liu, Y., Liu, S.R., Wang, J.X., Zhu, X.L., Zhang, Y.D., Liu, X.J., 2014. Variation in soil respiration under the tree canopy in a temperate mixed forest, central China, under different soil water conditions. Ecol. Res. 29(2), 133–142.

Liu, Y., Wu, M., Guo, Z., Jiang, Y., Liu, S., 2003. Study of element cycle of *Quercus acutidentata* forest ecosystem in Baotianman Natural Reserve. Acta Ecol. Sin. 23(8), 1488–1497.

Luan, J., Liu, S., Wang, J., Zhu, X., Shi, Z., 2011a. Rhizospheric and heterotrophic respiration of a warm–temperate oak chronosequence in China. Soil Biol. Biochem. 43(3), 503–512.

Luan, J., Liu, S., Zhu, X., Wang, J., 2011b. Soil carbon stocks and fluxes in a warm–temperate oak chronosequence in China. Plant soil 347(1–2), 243–253.

Luan, J., Liu, S., Chang, S.X., Wang, J., Zhu, X., Liu, K., Wu, J., 2014. Different effects of warming and cooling on the decomposition of soil organic matter in warm–temperate oak forests: a reciprocal translocation experiment. Biogeochemistry, 121(3), 551–564.

Mahlstein, I., Daniel, J.S., Solomon, S., 2013. Pace of shifts in climate regions increases with global temperature. *Nat. Clim. Change* 3, 739–743.

Majdi H., Öhrvik, J., 2008. Interactive effects of soil warming and fertilization on root production, mortality, and longevity in a Norway spruce stand in Northern Sweden. *Global Change Biol.* 10(2), 182–188.

Meier, I.C., Leuschner, C., 2008. Belowground drought response of European beech: fine root biomass and carbon partitioning in 14 mature stands across a precipitation gradient. *Global Change Biol.* 14(9), 2081–2095.

Melillo, J.M., Steudler, P.A., Aber, D.J., Newkirk, K., Lux, H., Bowles, F.P., Catricala, C., Magill, A., Ahrens, T., Morrissea, S., 2002. Soil warming and carbon–cycle feedbacks to the climate system. *Science* 298, 2173–2176.

Nelson D.W., Sommers L.E., 1996. Total carbon, organic carbon, and organic matter. In: Sparks D.L. (ed) *Methods of soil analysis*. Soil Sci. Soc. Am. Inc., Madison, pp: 961 – 1010.

Ngao, J., Longdoz, B., Granier, A., Epron, D., 2007. Estimation of autotrophic and heterotrophic components of soil respiration by trenching is sensitive to corrections for root decomposition and changes in soil water content. *Plant Soil* 301(1–2), 99–110.

Niinistö, S.M., Silvola, J., Kellomäki, S., 2004. Soil CO₂ efflux in a boreal pine forest under atmospheric CO₂ enrichment and air warming. *Global Change Biol.* 10, 1363–1376.

Nikolova, P.S., Raspe, S., Andersen, C.P., Mainiero, R., Blaschke, H., Matyssek, R., Häberle, K.H., 2009. Effects of the extreme drought in 2003 on soil respiration in a mixed forest. *Europ. J. For. Res.* 128(2), 87–98.

Ogaya, R., Peñuelas, J., Martínez-Vilalta, J., Mangirón, M., 2003. Effect of drought on diameter increment of *Quercus ilex*, *Phillyrea latifolia*, and *Arbutus unedo* in a holm oak forest of NE Spain. *For. Ecol. Manag.* 180 (1), 175–184.

Peichl, M., Arain, M.A., 2006. Above–and belowground ecosystem biomass and carbon pools in an age–sequence of temperate pine plantation forests. *Agric. For. Meteorol.* 140(1), 51–63.

Phillips, R.P., Brzostek, E., Midgley, M.G., 2013. The mycorrhizal–associated nutrient economy: a new framework for predicting carbon–nutrient couplings in temperate forests. *New Phytol.* 199(1), 41–51.

Phillips, R.P., Meier, I.C., Bernhardt, E.S., Grandy, A.S., Wickings, K., Finzi, A.C., 2012. Roots and fungi accelerate carbon and nitrogen cycling in forests exposed to elevated CO₂. *Ecol. Lett.* 15(9), 1042–1049.

Pregitzer, K.S., Burton, A.J., King, J.S., Zak, D.R., 2008 Soil respiration, root biomass, and root turnover following long–term exposure of northern forests to elevated atmospheric CO₂ and tropospheric O₃. *New Phytol.* 180, 153–161.

Risk, D., Nickerson, N., Phillips, C., Kellman, L., Moroni, M., 2012. Drought alters respired $\delta^{13}\text{C}$ CO₂ from autotrophic, but not heterotrophic soil respiration. *Soil Biol. Biochem.* 50, 26–32.

Rustad, L.E., Campbell, J.L., Marion, G.M., Norby, R.J., Mitchell, M.J., Hartley, A.E., Cornelissen, J., Gurevitch, J., GCTE–NEWS, 2001. A meta–analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. *Oecologia* 126, 543–562.

Saiz, G., Black, K., Reidy, B., Lopez, S., Farrell, E.P., 2007. Assessment of soil CO₂ efflux and its components using a process–based model in a young temperate forest site. *Geoderma* 139(1), 79–89.

Savage, K., Davidson, E.A., Tang, J., 2013. Diel patterns of autotrophic and heterotrophic respiration among phenological stages. *Global Change Biol.* 19(4), 1151–1159.

Schimel, J.P., Gulledge, J.M., Clein–Curley, J.S., Lindstrom, J.E., Braddock, J.F., 1999. Moisture effects on microbial activity and community structure in decomposing birch litter in the Alaskan taiga. *Soil Biol. Biochem.* 31(6), 831–838.

Schindlbacher, A., Wunderlich, S., Borken, W., Kitzler, B., Zechmeister–Boltenstern, S., Jandl, R., 2012. Soil respiration under climate change: prolonged summer drought offsets soil warming effects. *Global Change Biol.* 18(7), 2270–2279.

Schindlbacher, A., Zechmeister–Boltenstern, S., Jandl, R., 2009. Carbon losses due to soil warming: Do autotrophic and heterotrophic soil respiration respond equally? *Global Change Biol.* 15, 901–913.

Shi, W.Y., Tateno, R., Zhang, J.G., Wang, Y.L., Yamanaka, N., Du, S., 2011.

Response of soil respiration to precipitation during the dry season in two typical forest stands in the forest–grassland transition zone of the Loess Plateau. *Agric. For. Meteorol.*, 151(7), 854–863.

Shi, Z., Cheng, R., Liu, S., Jiang, Y., 2005. Community structure and species diversity of *Platycarya Strobilacea* forest in Baotianman, Haenan Province. *J. Mount. Sci.* 23(3), 374–380.

Scott - Denton, L.E., Rosenstiel, T. N., Monson, R.K., 2006. Differential controls by climate and substrate over the heterotrophic and rhizospheric components of soil respiration. *Global Change Biol.* 12(2), 205–216.

Song, W., Chen, S., Zhou, Y., Wu, B., Zhu, Y., Lu, Q., Lin, G., 2015. Contrasting diel hysteresis between soil autotrophic and heterotrophic respiration in a desert ecosystem under different rainfall scenarios. *Sci. Rep.* 5, 16779. [http:// doi.org /10.1038 /srep16779](http://doi.org/10.1038/srep16779)

Sotta, E.D., Veldkamp, E., Schwendenmann, L., Guimaraes, B.R., Paixao, R.K., Ruivo, M., da Costa, L., Carlos, A., Meir, P., 2007. Effects of an induced drought on soil carbon dioxide (CO₂) efflux and soil CO₂ production in an Eastern Amazonian rainforest, Brazil. *Global Change Biol.* 13(10), 2218–2229.

Steinberg, S., Miller, J., McFarland, M., 1990. Dry matter partitioning and vegetative growth of young peach trees under water stress. *Funct. Plant Biol.* 17(1), 23–36.

Suseela, V., Dukes, J. S., 2013. The responses of soil and rhizosphere respiration to

simulated climatic changes vary by season. *Ecology* 94 (2), 403–413

Vance, E., Brookes, P., Jenkinson, D., 1987. An extraction method for measuring soil microbial biomass C. *Soil Biol. Biochem.* 19(6), 703–707.

Verhoef, A., Allen, S.J., De Bruin, H.A., Jacobs, C.M., Heusinkveld, B.G., 1996. Fluxes of carbon dioxide and water vapour from a Sahelian savanna. *Agric. For. Meteorol.* 80(2), 231–248.

Wan, S., Luo, Y., Wallace, L., 2002. Changes in microclimate induced by experimental warming and clipping in tallgrass prairie. *Global Change Biol.* 8(8), 754–768.

Wan, S., Norby, R.J., Ledford, J., Weltzin, J.F., 2007. Responses of soil respiration to elevated CO₂, air warming, and changing soil water availability in a model old- field grassland. *Global Change Biol.* 13(11), 2411–2424

Wang, X., Feng, Z., Ouyang, Z., 2001. Vegetation carbon storage and density of forest ecosystems in China. *China J. Appl. Ecol.* 12(1), 13–16.

Wang, X., Liu, L.L., Piao, S.L., Janssens, I.A., Tang, J.W., Liu, W.X., Chi, Y., Wang, J., Xu, S., 2014. Soil respiration under climate warming: differential response of heterotrophic and autotrophic respiration. *Global Change Biol.* 20(10), 3229–3237.

Wu, J., Joergensen, R., Pommerening, B., Chaussod, R., Brookes, P., 1990. Measurement of soil microbial biomass C by fumigation–extraction—an automated procedure. *Soil Biol. Biochem.* 22(8), 1167–1169.

Wu, Z., Dijkstra, P., Koch, G.W., Penuelas, J., Hingate, B.A., 2011. Responses of

terrestrial ecosystems to temperature and precipitation change: a meta - analysis of experimental manipulation. *Global Change Biol.* 17(2), 927–942.

Xia, J., Chen, J., Piao, S., Ciais, P., Luo, Y., Wan, S., 2014. Terrestrial carbon cycle affected by non-uniform climate warming. *Nat. Geosci.* 7, 173–180.

Xia, J., Niu, S., Wan, S., 2009. Response of ecosystem carbon exchange to warming and nitrogen addition during two hydrologically contrasting growing seasons in a temperate steppe. *Global Change Biol.* 15(6), 1544–1556.

Xu Y., Gao, X.J., Giorgi, F., 2009. Regional variability of climate change Hot-Spots in East Asia. *Adv. Atmosph. Sci.* 26(4), 783–792.

Yahdjian, L., Sala, O.E., 2002 A rainout shelter design for intercepting different amounts of rainfall. *Oecologia* 133, 95–101.

Yan, L., Chen, S., Huang, J., Lin, G., 2010. Differential responses of auto - and heterotrophic soil respiration to water and nitrogen addition in a semiarid temperate steppe. *Global Change Biol.* 16(8), 2345–2357.

Yi, Z., Fu, S.L., Yi, W.M., Zhou, G.Y., Mo, J.M., Zhang, D.Q., Ding M.M., Wang, X.M., Zhou, L.X., 2007. Partitioning soil respiration of subtropical forests with different successional stages in south China. *For. Ecol. Manag.* 243(2), 178–186.

Zhou, X., Sherry, R.A., An, Y., Wallace, L.L., Luo, Y., 2006. Main and interactive effects of warming, clipping, and doubled precipitation on soil CO₂ efflux in a grassland ecosystem. *Global Biogeochem. Cycles* 20(1), 12.

Zhou, G., Peng, C., Li, Y., Liu, S., Zhang, Q., Tang, X., Liu, J., Yan, J., Zhang, D.,

Chu, G., 2013. A climate change-induced threat to the ecological resilience of a subtropical monsoon evergreen broad-leaved forest in Southern China. *Global Change Biol.* 19(4), 1197-1210.

Tables and Figures

Table 1. Results (*P*-values) of ANOVA on the effects of year (Y), throughfall reduction (T), soil warming (W), and their interactions on soil respiration (SR) and its components (heterotrophic respiration, HR, and autotrophic respiration, AR), soil temperature, and soil moisture in the trenched (ST_t and SM_t) and un-trenched plots (ST_u and SM_u).

Source of variation	SR	HR	AR	ST _u	SM _u	ST _t	SM _t
Y	0.008	0.027	0.169	0.012	0.003	0.827	<0.001
T	0.615	0.335	0.788	0.012	0.001	0.735	0.001
W	0.210	0.036	0.621	<0.001	<0.001	<0.001	0.002
Y×T	0.246	0.612	0.373	0.795	0.294	0.939	0.303
Y×W	0.561	0.761	0.636	0.958	0.967	0.980	0.719
T×W	0.002	0.643	0.001	0.301	0.110	0.219	0.628
Y×T×W	0.559	0.959	0.419	0.853	0.663	0.513	0.936

Table 2. Results (*P*-values) of three-way ANOVA on the effects of year (Y), throughfall reduction (T), soil warming (W), and their interactions on the temperature sensitivity (Q₁₀) of soil respiration (SR), heterotrophic respiration (HR), and autotrophic respiration (AR).

Source of variations	Q ₁₀ of SR	Q ₁₀ of HR	Q ₁₀ of AR
Y	<0.001	0.001	0.242
T	0.141	0.663	0.072
W	0.240	0.705	0.331
Y×T	0.013	0.013	0.415
Y×W	0.730	0.150	0.892
T×W	0.060	0.633	0.445
Y×T×W	0.833	0.850	0.430

Table 3 Summary of stepwise multiple regression showing dependence of soil respiration (SR) on heterotrophic respiration (HR) and autotrophic respiration (AR), and environmental factors (soil microbial biomass C, MBC; soil organic C, SOC; soil temperature, ST; fine root biomass, FRB) that influence SR and its components. Data of soil CO₂ flux, ST, and SM are mean values of July, when soil samples were collected and analyzed in 2011 and 2012.

Dependent variable	Independent variables	Partial R ²	Adjust R ²	P value
SR	AR	0.59	0.59	<0.001
	HR	0.40	0.99	<0.001
SR	MBN	0.30	0.30	0.003
	ST	0.04	0.34	0.005
HR	ST	0.21	0.21	0.014
	SOC	0.09	0.30	0.010
	FRB	0.05	0.35	0.009
AR	ST	0.25	0.25	0.008

Figure legends

Fig. 1. Seasonal dynamics of soil temperature (a, b, c, d; ST) and moisture (e, f, g, h; SM) for the un-trenched and trenched plots in 2011 and 2012. C, control; T, throughfall reduction; W, soil warming; TW, throughfall reduction plus soil warming.

Fig. 2. Seasonal dynamics of soil respiration (SR), heterotrophic respiration (HR), and autotrophic respiration (AR) in 2011 (a, c, e) and 2012 (b, d, f). C, control; T,

throughfall reduction; W, soil warming; TW, throughfall reduction plus soil warming.

Fig. 3. Relative changes (%) in soil respiration (SR), heterotrophic respiration (HR), and autotrophic respiration (AR) induced by throughfall reduction without soil warming (T – C) and with soil warming (TW – W), and by soil warming without throughfall reduction (W – C) and with throughfall reduction (TW – T) in 2011 and 2012. *, $P < 0.05$; **, $P < 0.01$.

Fig. 4. Relative contribution of heterotrophic respiration to soil respiration (HR/SR) under different treatments. C, control; T, throughfall reduction; W, soil warming; TW, throughfall reduction plus soil warming. Different letters represent significant difference at $P < 0.05$.

Fig. 5. Temperature sensitivity (Q_{10}) of soil respiration (a), heterotrophic respiration (b), and autotrophic respiration (c) in 2011 and 2012 under the treatments of control (C), throughfall reduction (T), soil warming (W), and throughfall reduction plus soil warming (TW). Different letters represent significant difference at $P < 0.05$.

Fig. 6. Dependences of soil respiration (SR, a and b), heterotrophic respiration (HR, c and d), and autotrophic respiration (AR, e and f) on soil temperature (a, c, and e) and moisture (b, d, and f). Each point represents monthly average value of each treatment

across the two growing seasons.

Fig. 7. Dependences of soil respiration (SR; a, b, and c), heterotrophic respiration (HR; d and e), and autotrophic respiration (AR; f) on microbial variables (MBC and MBN) and fine root biomass (FRB) in different treatments across the two seasons. Data of soil CO₂ flux were mean values of July, when soil samples were collected and analyzed in 2011 and 2012.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5

Fig. 6.

Fig. 7.

Appendices

Table A.1. Relative changes (%) in fine root biomass (FRB) and microbial variables (MBC and MBN) induced by throughfall reduction (T) and soil warming (W) in 2011 and 2012. T–C, effect of throughfall reduction under ambient temperature; TW–W, effect of throughfall reduction under warming; W–C, effect of soil warming under ambient precipitation; TW–T, effect of soil warming under throughfall reduction.

Treatment	2011					2012				
	FRB	Un-trenched		Trenched		FRB	Un-trenched		Trenched	
		MBC	MBN	MBC	MBN		MBC	MBN	MBC	MBN
T–C	15.9**	82.2**	64.8***	7.2	23.4	37.7**	106.8***	134.5***	2.8	14.4
TW–W	–13.6**	–5.9	–14.2	–23.9*	–29.9*	–19.1*	–3.5	–8.6	–42.3*	–40.9*
W–C	26.3***	57.7*	60.6**	49.3**	53.6*	30.5**	63.0*	70.3**	60.7*	75.3**
TW–T	–5.8	–18.5	–16.5*	5.9	–12.8	–23.3**	–23.9*	–33.6**	–9.7	–9.5

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Fig. A.1. Spatial patterns of soil surface (5 cm) temperature (a, b) and moisture (c, d) in the throughfall reduction plus soil warming plot (TW2) based on the mean value of data investigated in 15 May and 20 Jul 2011. Subplots a, c are trenched plots, b, d are un-trenched plots. Size of circle represents magnitude of measured soil temperature and moisture.