Effects of experimental throughfall reduction and soil warming on fine root biomass
and its decomposition in a warm temperate oak forest

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Abstract

Fine root dynamics play a critical role in regulating carbon (C) cycling in terrestrial ecosystems. Examining the responses of fine root biomass and its decomposition to altered precipitation pattern and climate warming is crucial to understand terrestrial C dynamics and its feedback to climate change. Fine root biomass and its decomposition rate were investigated in a field manipulation experiment with throughfall reduction and soil warming conducted in a warm temperate oak forest. Throughfall reduction significantly interacted with soil warming in affecting fine root biomass and its decomposition. Throughfall reduction substantially increased fine root biomass and its decomposition in unheated plots, but showed negative effects in warmed plots. Soil warming significantly enhanced fine root biomass and its decomposition under ambient precipitation, but exhibited a negative effect under throughfall reduction. Differences in fine root biomass among different treatments could be largely attributed to soil total nitrogen (N), while fine root decomposition rate was more depended on microbial biomass C and N. Our observations indicate that decreased precipitation may offset the positive effect of soil warming on fine root biomass and decomposition.

Key words: Climate change, Microbial biomass, Precipitation, Drought, Soil moisture, Soil temperature

1. Introduction

One aspect of global climate change is changing patterns of precipitation (Easterling et al., 2000; Davidson et al., 2004; Gimbel et al., 2015), drought events will be more common and exert a greater impact on most of terrestrial ecosystems functioning (Hasibeder et al., 2015). Global warming induced by increased levels of atmospheric greenhouse gases has elevated the global mean temperature by 0.76 °C since 1850 and is expected to continue to increase by another 1.0-3.7 °C by the end of this century (IPCC, 2014). Potential changes in fine root biomass and its decomposition caused by climate change not only have an influence on soil C sequestration, but will likely regulate atmosphere CO₂ concentration (Pritchard et al., 2001; Bytnerowicz et al., 2007; Beier et al., 2008). Therefore, knowledge about the responses of fine root biomass and decomposition patterns to climate change is of significance to make convincing projections on terrestrial C cycling (Rustad, 2008).

As an important process of C cycling in terrestrial ecosystems (Mao et al., 2011), fine root biomass contributes the primary input of organic C to soils (Eldhuset et al., 2006; Valverde-Barrantes et al., 2015). Although root biomass accounts for 15-30 % of total tree biomass (Persson and Ahlström, 2002; Zhou et al., 2011), more than 50 % of the C acquired annually by plants is transferred to below ground (Wan et al., 2004).

Environmental factors such as soil temperature and moisture, strongly relevant to climate change, can influence fine root biomass (Gimbel et al., 2015). For example, it was found that elevated temperature increases root length growth and mortality (Gill and Jackson, 2000), but decreases total root biomass of tree seedlings (Wan et al.,

2004). Similarly, decreased root biomass under soil warming was observed in temperate (Zhou et al., 2011) and boreal (Bergner et al., 2004) forests. Contrary to the above findings, a study in an orchard ecosystem found an enhanced fine root biomass caused by the increased temperature (Allen and Vu, 2009). The contradictory responses of fine root biomass to experimental warming among different studies may result from the variations in other environmental factors, such as soil nutrient availability and precipitation. Decreased precipitation can also regulate fine root biomass. For example, decreased fine root biomass under experimental drought has been found in a moist tropical forest (Davidson et al., 2004). In central European mixed stands, however, Leuschner et al. (2001) found that soil water deficits apparently stimulated fine root growth of *Fagus sylvatica*, but had no influence in *Quercus petraea* during the drought period.

Fine root decomposition represents a large and rapid flux of C and nutrient released into belowground system (Lin et al., 2011). Soil moisture and temperature are regarded as the main abiotic factors influencing root decomposition (Silver and Miya, 2001; Chen et al., 2002). On global scale, root decomposition rate is positively correlated with mean annual temperature but negatively correlated with mean annual precipitation (Zhang and Wang, 2015). However, there have been few studies on the response of root decomposition to simultaneously changing precipitation and temperature (King et al., 2005). Although single factor experiments have proven to be useful to improve our understanding of the respective responses of plant root biomass and its decomposition to either decreased precipitation or climate warming (Rustad,

2008). However, there is little known about their combined effects on fine root biomass and decomposition.

Oak forests (*Quercus* spp.) form the largest biome in terms of area and vegetation C storage in China. It covers a total area of 15.5×10^6 ha (15.2%; Fang et al., 1996) and store 835.94 Tg vegetation C (22.4%; Wang et al., 2001). As a representative forest community in central China, the temperate oak forest plays an important role in maintaining the region's C cycling (Liu et al., 2012). The previous studies focused largely on soil respiration and its components in the warm temperate oak chronosequence (Luan et al., 2011; Luan et al., 2012). However, there has been little information on how fine root biomass and decomposition respond to climate change in this forest ecosystem, particularly to the concurrent driving factors, *i.e.*, decreased precipitation and climate warming.

In this study, we established an *in situ* throughfall reduction and soil warming experiment in a warm temperate oak forest in central China, to study the effects of throughfall reduction, soil warming, and their interactions on fine root biomass and decomposition. Soil warming can increase soil N availability (Rustad et al., 2001) and soil microbial activity (Bergner et al., 2004), and thus, we first hypothesized that soil warming should increase fine root biomass and its decomposition. Given that throughfall reduction can partially decrease soil water availability, our second hypothesis is that throughfall reduction will suppress fine root biomass and its decomposition.

2. Materials and methods

2.1 Study site

The study site is located at the Baotianman Nature Reserve (111°47'-112°04' E, 33°20'-33°36' N), Henan Province, China. Mean annual temperature is 15.1 °C, with monthly air temperature ranging from 1.5 °C in January to 27.8 °C in July. Mean annual precipitation is approximately 900 mm with 60 % distributed in summer (from June to August). Upland soils were dominated by Haplic luvisol (Luan et al., 2011). The study area is dominated by natural secondary forest composed of *Quercus aliena* var. *acuteserrata*, which accounts for 66 % of the canopy cover. Other tree species include *Carpinus cordata* BI., *Lindera obtusiloba* BI., *Acer* spp., and *Dendrobenthamia hupehensis* Fang. Stand mean tree diameter at breast height (DBH) is 20.1 ± 4.5 cm with an age of 58 years. The soil bulk density and soil organic C content at 10 cm surface layer are 0.70 ± 0.09 g cm⁻³ and 41.20 ± 1.71 g kg⁻¹, respectively.

2.2 Experimental design

In March 2011, we plotted three blocks with a distance of at least 10 m between each other. Four 3 m × 4 m plots separated by an approximately 3 m wide buffer strip were established in each block, and were randomly assigned to four treatments (control, throughfall reduction, soil warming, and throughfall reduction plus soil warming). We measured soil temperature and moisture in April 20th and 25th before all treatments started. Soil temperature ranged from 10.7 ± 0.6 °C in the control plots to 11.1 ± 0.6 °C in the throughfall reduction plots, with no significant difference among different treatments. Soil moisture ranged from $31.5 \pm 3.8\%$ in the warmed plots to $35.8 \pm 6.6\%$

in the control plots, with no significant difference among different treatments.

Throughfall was reduced by ~50 % by covering 50 % of the plot area with interception roof. A 4 m × 5 m roof covered with eight 5 m × 0.25 m plastic sheets mounted to iron wire frames was installed above each throughfall reduction plot (3 m × 4 m) with 0.5 m distance to plot edge under the forest canopy. Gaps of 0.2 m between adjacent plastic sheets allowed the air to circulate through the roof, minimizing the temperature and humidity accumulation effects. The plastic sheets were transparent and able to transmit 60-70 % of radiation activating photosynthesis. The roof was kept at approximately 10-15 ° slope by setting the two sides at different heights (3 m for one side and 2 m for the other) to ensure quick drainage of rainfall by gravity.

Excluded precipitation from each roof was drained and collected into eight 30 L PVC tanks, and the water was dumped out to the nearest valley 70 m away following each rainfall event. Litters on the plastic sheets were swept into the plots every 4-5 days. Throughfall reduction operated during the snow free season, from early May 2011 to November 2012. The plastic sheets were carefully removed during the snow season to avoid damage of the roof frame from snowpack. They were put back in place before the next rain season starting in late April 2012. To eliminate surface water flow and lateral movement of soil water into plots, a 0.5 m deep trench was excavated and lined with double-layer plastic sheets on uphill side of the experimental site (Heisler-White et al., 2008). The plastic sheet was kept 0.2 m above ground surface, and then the trench was refilled.

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 warming plot at a height of 2.25 m above the ground (Xia et al., 2009; Aguilos et al., 2011). The heating operated 24 hours per day and 365 days per year since May 1, 2011. The effects of infrared radiators on soil temperature were spatially uniform within the warmed plots, assessed using coefficient of variation ($C.V. = 3\%$).

2.3 Soil temperature and moisture

From July 9, 2011, Soil temperature and soil volumetric water content (Vol.%) at the depth of 10 cm of each plot were recorded automatically using 111N temperature probe and FDS-100 moisture probe connected with a Datalogger (HL20 Datalogger, Jauntering International Corp., Taiwan, China) during the experimental period. Measurements were taken in 10-min intervals and averages of the six measurements were stored as the hourly averages. Precipitation data with one hour intervals was collected from a nearby weather station located less than 800 m away from the study sites.

2.4 Fine root biomass

Fine root (< 2 mm diameter) biomass was determined using soil cores method (Ostonen et al., 2005; Valverde-Barrantes et al., 2015). Soil cores were collected three times from April 2011 to July 2012 (25 April 2011, 25 July 2011 and 28 July 2012). For each sampling, three soil cores of 50 mm in diameter were taken along the diagonal direction in each plot to a 20 cm depth to cover the most active fine roots. Each soil core was first sieved by 2 mm mesh, and fresh soil samples were collected

for further analysis in the laboratory. And then the remnant soil samples in the mesh were washed and hand sorted to separate living fine roots from dead roots, stem materials and litter fractions based on the morphology and color (Zhou et al., 2011). Living fine roots biomass was weighted after oven drying at 70 °C for 72 hours.

2.5 Fine root decomposition

The litter bag technique was used to estimate the fine root decomposition rate over 12 months (King et al., 2005; Sariyildiz, 2015). Fresh fine root of 0-20 cm depth was destructively collected from the same stand next to the experiment plots on 9 June 2011. All root samples were washed manually, and fine roots (< 2 mm) were separated from coarse roots and litter fractions. Equivalent quantities of air dried fine roots (3 g) were placed inside 25 cm × 25 cm plastic bags with 1 mm mesh. On 19 June 2011, four bags were buried in soil at a depth of 10 cm and marked on soil surface in each plot. Every three months, one bag containing decomposing root was randomly obtained from each plot, and a total of three duplicate bags for each treatment.

After retrieval, the remaining roots were washed to remove attached soil particles, and then they were oven dried at 70 °C to a constant weight. Fine root decomposition rate was expressed as percentage weight remaining of the initial dry weight (%). The oven dried root samples were milled to powder and analyzed for total C by the dichromate oxidation method (Nelson et al., 1996), and total nitrogen (N) using micro-Kjeldahl technique (Bremner et al., 1996).

2.6 Soil sample analysis

One part of the fresh soil samples sieved with 2 mm mesh was air dried and sieved with 250 μm mesh to measure soil organic C (SOC) (Nelson et al., 1996) and soil total N (TN) (Bremner et al., 1996). Another part of soil samples was stored in iceboxes, and subsequently transferred to the laboratory for microbial biomass analysis. Soil microbial biomass C (MBC) and N (MBN) were measured by the chloroform fumigation-extraction method (Vance et al., 1987). Briefly, for each sample, extractable C and N of both filtered K_2SO_4 -extracts (fumigated sample and unfumigated sample) were analyzed by TOC analyzer (TOC-VCPH Shimadzu Corp., Japan). MBC and MBN were calculated from the differences between extractable C and N contents in the fumigated and the non-fumigated 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).

2.7 Statistical analysis

Annual decomposition constant (k) was calculated with a single exponential model by the equation (Olson, 1963):

$$\text{Ln}(X_t/X_o) = -kt$$

Where X_t is the mass remaining at time t ; X_o is the initial mass value; k is the exponential decay coefficient; t is time in days. Repeated-measurement analysis of variance (ANOVA) was used to examine effects of throughfall reduction, soil warming, and their interaction on soil temperature and soil moisture (Table 1), fine root decomposition rate and chemical characteristic during the decomposition process (Table 3). Two-way ANOVA was used to test the effects of throughfall reduction, soil

warming, and their interaction on the fine root biomass in 2011 and 2012 and fine root decomposition constant (Table 2). Since significant interactions between throughfall reduction and soil warming on fine root biomass and its decomposition constant, one-way ANOVA was further carried out separately to test differences between treatments. Regression with correction for autocorrelation and stepwise multiple linear analysis was used to examine relationships of fine root biomass and decomposition rate with abiotic and biotic factors (Table 4). All statistical analysis was carried out with SPSS software (SPSS 12.0 for WINDOWS).

3. Results

3.1 Soil temperature and moisture

There was substantial inter-annual variation in soil temperature ($P < 0.01$, Table 1), with the lowest value ($-1.02\text{ }^{\circ}\text{C}$) in January and the highest value ($17.90\text{ }^{\circ}\text{C}$) in July in control plots (Fig. 1a). Across the 12 months, soil warming significantly elevated soil temperature by $1.80\text{ }^{\circ}\text{C}$ compared with unheated plots. ($P < 0.01$, Fig. 1b and Table 1). Soil moisture fluctuated greatly across the 12 months ($P < 0.01$, Table 1; Fig. 1c), with the lowest soil moisture in January (16.8 %) and the highest in July (36.3 %) in the control plots (Fig. 1c). Throughfall reduction showed substantial negative effects on soil moisture ($P < 0.05$), with 6.5 % and 4.4 Vol. % reduction under ambient temperature and soil warming, respectively. Soil warming significantly reduced soil moisture by 9.3 Vol. % under ambient precipitation and 7.5 Vol. % (both $P < 0.05$) under throughfall reduction treatment, respectively (Fig. 1d).

Table 1. Results (*P* value) of repeated-measurements ANOVA on the effects of month (M), throughfall reduction (T), soil warming (W), and their interactions on soil temperature and soil moisture (n = 3; Data used was monthly mean values from July 2011 to July 2012).

Source of variation	Soil temperature	Soil moisture
M	<0.001	<0.001
T	0.882	0.025
W	<0.001	0.037
T×W	0.179	0.284
M×T	0.534	0.006
M×W	<0.001	<0.001
M×T×W	0.442	0.065

Fig. 1. Seasonal variations (a, c) and overall means (b, d) of soil temperature and soil moisture of 0-10 cm from July 2011 to July 2012. C, control; T, throughfall reduction; W, soil warming; TW, throughfall reduction plus soil warming. Data of soil moisture from December 2011 to March 2012 was not included when calculated the overall means due to frozen water in the unheated plots. Common lower case letters denote treatments not significantly different at $P < 0.05$ (*LSD* test; mean $\pm$ *S.E.*, n=3).

3.2 Fine root biomass

Fine root biomass ranged from 2.47 t ha⁻¹ to 2.76 t ha⁻¹ for all treatments in April 2011, and the differences between plots were not significant (all $P > 0.05$, Table 2, Fig. 2a). Throughfall reduction significantly interacted with soil warming in affecting fine root biomass in July 2011 and 2012 (both T×W: $P < 0.05$, Table 2), indicating that effects of throughfall reduction on fine root biomass varied with temperature levels. Throughfall reduction substantially increased root biomass by 15.9 % in July 2011 ($P < 0.05$, Fig. 2b) and by 37.7 % in 2012 ($P < 0.05$, Fig. 2c) under ambient temperature, but showed negative effects on fine root biomass under soil warming in both years (-13.6 % and -19.1 %, both $P < 0.05$, Fig 2b, c). Soil warming significantly elevated fine root biomass by 26.3 % in July 2011 and by 30.5 % in 2012 under ambient precipitation, but substantially decreased it by 23.3% under throughfall reduction in

2012 (all $P < 0.05$, Fig 2b, c).

Table 2 Results (P value) of two-way ANOVA on the effects of throughfall reduction (T), soil warming (W), and their interactions on fine root biomass in April 2011, July 2011 and July 2012, and fine root decomposition.

Source of variation	Fine root biomass (t ha^{-1})			decomposition constant
	April 2011	July 2011	July 2012	
T	0.541	0.581	0.546	0.271
W	0.613	0.818	0.307	0.122
T×W	0.423	0.015	0.011	0.001

Fig. 2. Fine root biomass at the soil depths of 0-20 cm before treatments started (April 2011, a) and in July 2011 (b) and 2012 (c) for control (C), throughfall reduction (T), soil warming (W), and throughfall reduction plus soil warming (TW). Common lower case letters denote treatments not significantly different at $P < 0.05$ (LSD test; mean $\pm$ $S.E.$, $n=3$).

3.3 Fine root decomposition and nutrient release

The percentage of mass remaining of fine root ranged from 71.7 % for throughfall reduction plus soil warming plot to 59.7 % for soil warming (Fig. 3a). Soil warming

significantly interacted with throughfall reduction in affecting root decomposition (T×W: $P < 0.01$, Table 2). The remaining percentage of fine root in throughfall reduction plots (62.7 %) was significantly lower than that in control plots (70.7 %), , but the throughfall reduction plus warming treatment had higher remaining mass percentage (71.7 %) than soil warming treatment (59.7 %, Fig. 3a). The remaining percentage of fine root in soil warming plots was substantially lower (59.7 %) than that in control plots (70.7 %), but the combined plot had higher remaining mass percentage (71.7 %) than that in throughfall reduction plots (62.7 %; Fig. 3a). Significant interactive effect of throughfall reduction and soil warming was found on decomposition constant ($P < 0.05$, Table 2). Throughfall reduction tended to increase decay constant by 0.08 year^{-1} under the ambient precipitation, but substantially suppressed it by 0.20 year^{-1} with soil warming ($P < 0.05$, Fig. 3b). Soil warming significantly elevated decay constant by 0.17 year^{-1} under ambient precipitation ($P < 0.05$), but decreased it with throughfall reduction (Fig. 3b).

Fig. 3. Percentage of fine root mass remaining in litter bags (a) and decomposition constant (b) for control (C), throughfall reduction (T), soil warming (W), and throughfall reduction plus soil warming (TW). * represents $P < 0.05$ based on One-way ANOVA. Common lower case letters denote treatments not significantly different at $P < 0.05$ (*LSD* test; mean $\pm$ *S.E.*, $n=3$).

The initial C concentration of the air dried root samples was $58.1 \text{ g } 100 \text{ g}^{-1}$. Sampling date significantly interacted with throughfall reduction and soil warming in affecting decomposing root C concentration ($P < 0.05$, Table 3), with significant differences in the last two stages (Fig. 4a). The C concentration of decomposed root showed gradual reduction over decomposition process, with highest values in combined treatment ($53.8 \text{ g } 100 \text{ g}^{-1}$) and lowest values in the warming treatment ($49.7 \text{ g } 100 \text{ g}^{-1}$, $P < 0.01$, Fig. 4a). Compared with control plots, the C concentration was significantly decreased by $2.2 \text{ g } 100 \text{ g}^{-1}$ under soil warming, but significantly elevated by $2.1 \text{ g } 100 \text{ g}^{-1}$ under combined treatment ($P < 0.05$, Fig. 4a). There was significant interactive effect of throughfall reduction and soil warming on C concentration of decomposing root ($P < 0.05$, Table 3). Soil warming significantly elevated C release by 18.1% under ambient precipitation ($P < 0.05$), but show no effect under throughfall reduction.

Table 3. Results (P value) of repeated-measurement ANOVA on the effects of sampling date (D), throughfall reduction (T), soil warming (W), and their interactions

on fine root chemistry during the decomposition process (n=3).

Source of variation	C %	N %
D	<0.001	<0.001
T	<0.001	0.040
W	<0.001	0.001
T×W	0.006	0.499
D×T	0.001	0.064
D×W	<0.001	0.046
D×T×W	<0.001	0.003

The initial N concentration of dried root samples was $0.61 \text{ g } 100\text{g}^{-1}$. The N concentration of decomposing root increased over time, with 2.1-2.7 times higher than the initial value at the end of the experiment ($P < 0.01$, Table 3; Fig. 4b). Both throughfall reduction and soil warming significantly increased N concentration during decomposition process ($P < 0.05$, Table 3). However, there were no interactive effects of throughfall reduction $\times$ soil warming and sampling date $\times$ throughfall reduction on N concentration (Table 3).

Fig. 4. Changes in total carbon (a) and nitrogen concentrations (b) for remaining root in litter bags for control (C), throughfall reduction (T), soil warming (W), and throughfall reduction plus soil warming (TW) during the decomposition process (mean $\pm$ S.E., n=3).

3.4 Effects of environmental factors on fine root biomass and decomposition rate

Fine root biomass increased linearly with soil temperature ($R^2 = 0.32$, $P < 0.01$, Fig. 5a), but declined linearly with soil moisture ($R^2 = 0.36$, $P < 0.01$, Fig. 5b). Stepwise regression analysis (Table 4) showed that MBN alone explained 88.0 % ($P < 0.01$) of the variation in fine root biomass in 2011, whereas the most important factor influencing fine root biomass in 2012 was soil TN (partial $R^2 = 0.188$, $P < 0.01$). As to the fine root biomass across two years, 38.5 % variation of fine root biomass could be explained by the mean soil temperature in July ($P < 0.05$, Table 4).

Fig. 5. Dependences of fine root biomass upon monthly mean soil temperature (a), and soil moisture (b) in July.

Fine root decomposition constant showed positive linear dependence upon soil temperature ($R^2 = 0.33$, $P < 0.05$, Fig. 6a), but tended to negatively correlated with soil moisture ($R^2 = 0.21$, $0.05 < P < 0.1$, Fig. 6b). MBC accounted for 33.1% ($P < 0.05$) of the variation in decomposition rate of root, and additional 30 % variation in decomposition rate could be explained by soil temperature and MBN.

Table 4. Results of stepwise regression analysis of fine root biomass and decomposition rate as a function of environmental factors (n=3).

	Models	Partial R^2	Model R^2	P
Fine root biomass				
2011	0.002MBN+0.587	0.880	0.880	<0.01
	0.002MBN-0.758TN+0.881	0.012	0.892	<0.01
2012	-3.437TN+4.997	0.188	0.188	<0.05
Total	0.229ST _m -1.175	0.385	0.385	<0.05
Root decomposition rate				
	0.001MBC+0.552	0.331	0.331	<0.05
	0.001MBC-0.034ST _m +1.11	0.140	0.471	<0.05
	0.001MBC-0.047ST _m +0.001MBN+1.288	0.160	0.631	<0.05

MBC, microbial biomass carbon; MBN microbial biomass nitrogen. TN, soil total nitrogen at depth of 0-10 cm in July 2011 and 2012. ST_m, the monthly mean soil temperature at the depth of 0-10 cm in July 2011 and 2012.

Fig. 6. Dependences of decomposition constant of fine root upon annually mean soil temperature (a) and soil moisture (b).

4. Discussion

4.1 Positive effect of soil warming on fine root biomass and its decomposition

Several studies have demonstrated that fine root dynamics are sensitive to rising temperature in most ecosystems (Gill and Jackson, 2000; Wan et al., 2004; Bai et al., 2010). Suppressed fine root production and biomass have been reported in Norway spruce forest (Majdi, 2004), maple tree seedlings (Wan et al., 2004), and Harvard Forest under soil warming (Zhou et al., 2011). In this study, however, soil warming increased fine root biomass of oak forest, which is in accordance with the previous study in an orange tree orchard (Allen and Vu, 2009) and in an alpine tree line ecosystem (Dawes et al., 2015).

The positive effect of soil warming on fine root biomass under ambient precipitation in this study seems to depend on the different effects of soil warming on fine root growth and mortality. It has been reported that higher temperature could extend the length of the growing season, and root production is a function of the length of the

growing season (Fitter et al., 1999), thus soil warming has a potential to stimulate root production. In addition, rates of root mortality also seem to increase with soil temperature (Forbes et al., 1997; King et al., 1999). However, the increases in root growth and mortality under warming are asymmetric. Seasonally elevated soil temperature has been found to increase both the production and the mortality of root length in trembling aspen (*Populus tremuloides*), with greater proportional increases in production resulting in greater net root length production (production-mortality; (King et al., 1999). In addition, numerous studies have shown that fine root biomass is negatively responds to increased soil total N (Nadelhoffer, 2000; Lee et al., 2007; Zhou et al., 2011). Therefore, decrease in soil total N under soil warming in both 2011 and 2012 (unpublished data) can elevate fine root growth and biomass, which was also justified by the results of multiple regression analysis (Table 4).

It has been well known that fine root decomposition is regulated by a number of factors, including the decomposer communities (Van der Krift et al., 2001; Chen et al., 2002), environmental factors (Wildung et al., 1975; Gill and Jackson, 2000), and substrate quality (Silver and Miya, 2001; Lin et al., 2011; Mao et al., 2011). On global scale, decomposition rates of fine roots increase exponentially with mean annual temperature in forests (Gill and Jackson, 2000). Our results showed that elevated temperature stimulated root decomposition rate during the whole period (Fig. 3). The positive response of root decomposition rate and decay constant to soil warming can be accounted for by the stimulated soil microbes. Consistently with previous studies (Rustad et al., 2001; Melillo et al., 2002; Yin et al., 2012), soil warming can stimulate

soil microbial biomass, thus accelerate decomposition of fine root (Table 4). In addition, the enhanced soil temperature can increased soil enzyme activity, thus speed up the rate at which enzymes collide with and breakdown substrates (Brzostek and Finzi, 2011; Zhou et al., 2013). Moreover, mean soil temperature in July and diameter at breast height of the tree nearest to the plot also partly explained the elevated decomposition rate of fine root. As Sánchez-Moreno et al. (2014) and Thakur et al. (2014) reported that soil nematode and faunae greater under larger trees compared with small ones, which showed positive effects on root decomposition.

As one of the active warming approaches, infrared heater has been widely used in manipulative experiments in different ecosystems (Rustad et al., 2001; Wu et al., 2011). It has been proven to be effective in elevating soil temperature with low spatial heterogeneity (Xia et al., 2009). However, one of the deficiencies is the warming negative effect on soil moisture (decreased by 9.3 Vol. % in this study). Similar to most warming experiments (Rustad et al., 2001), soil drying induced by warming treatment has not been considered in experimental design. The aggravated evaporation and evapotranspiration are well known characteristics of climate warming. Decreased soil water content was treated as a reason of changes in soil respiration under soil warming.

4.2 Positive effect of throughfall reduction on fine root biomass and decomposition

Throughfall reduction showed a strong positive effect on fine root biomass, which was in accordance with the previous study (Leuschner et al., 2001). In ecosystem scale, decreased soil water availability to some extent could increase root/shoot ratios

of several tree species by increasing C allocation to the root system (Leuschner et al., 2001; Brando et al., 2008) and elevated tree root growth in absolute terms during water stress (Kramer and Boyer, 1995; Leuschner et al., 2001). In dry conditions, plant root tends to extend to deeper soil levels to reach the deep water resources (Nepstad et al., 1994; Brando et al., 2008). Therefore, elevated root biomass under throughfall reduction is probably related to the change in C allocation. Although our experimental plot does not include trees due to its small area, our finding also suggests that soil water availability is still suitable for plant growth under throughfall reduction.

In the northern Chihuahuan Desert, drought event decreased decay of fine root litter for two shrub species (*Larrea tridentata* and *Prosopis glandulosa*) by about 25% (Kemp et al., 2003). However, throughfall reduction was found to increase fine root decomposition rate in this study (27.2%). The Differences in the responses of fine root decomposition to decreased precipitation may attribute to the following two aspects. First, as a water-unlimited forest ecosystem in this study, soil microbe activity might be increased due to elevated soil temperature under throughfall reduction. Second, throughfall reduction directly leads to the increased oxygen in soil capillary pore that favor soil microbes. Throughfall reduction slowed down the release rate of root C and N content (Fig.4). This could possibly be explained by the weakened leaching process of soluble organic fractions result from reduction of throughfall (Lin et al., 2011).

4.3 Negative responses of fine root biomass and decomposition rate to combined treatments

Throughfall reduction plus soil warming suppressed fine root biomass and decomposition rate suggesting that soil warming offsets the positive effects of throughfall reduction on fine root biomass and decomposition rate. The different responses of fine root biomass to soil warming with and without throughfall reduction indicates that the response of root system to water shortage depends on drought intensity and duration (Meier and Leuschner, 2008). Severe soil water deficit likely occurred under the combined treatments (Fig. 1b), thus induced a physical barrier for root extension due to dried soil. We concluded that there are two possible reasons for the negative effect of soil warming on fine root decomposition. On one hand, decreased soil water availability directly suppressed mass loss of root through leaching (Cleveland et al., 2006; Cleveland et al., 2010). On the other hand, soil moisture under two-factor treated plots was significantly lower than single-factor treated plots, which probably exceeds the optimum range for soil microbe metabolism (Liu et al., 2014), meanwhile reduced substrate utilizability (Hasibeder et al., 2015), and thus decreased fine root decomposition.

The responses of fine root biomass and its decomposition to treatments do not represent a long-term effect in this study. We did not observe a potential root and microbial acclimations to treatments during our first two years experiment. Therefore, further research based on long-term investigation is needed to reveal the long term response of fine root biomass and its decomposition to climate change.

5. Conclusion

In a warm temperate oak forest in Central China, throughfall reduction significantly

interacted with soil warming in affecting fine root biomass and decomposition rate. Throughfall reduction and soil warming stimulates root biomass and decomposition when they occur separately, but the root biomass and decomposition suppression appears when they execute concurrently. The response patterns of fine root biomass were mainly attributed to soil microbial biomass C and soil total N, but root decomposition was largely dependent on microbial biomass C and soil temperature. The study shows that throughfall reduction can weaken the positive effect of soil warming on fine root biomass and root decomposition, suggesting that concurrent decreased precipitation and climate warming may have less impact on fine root turnover in a warm temperate oak forest.

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References

Aguilos M, Takagi K, Liang N, Watanabe Y, Goto S, Takahashi Y, et al. Soil warming

- in a cool-temperate mixed forest with peat soil enhanced heterotrophic and basal respiration rates but Q_{10} remained unchanged. *Biogeosci. Disc.* 2011; 8: 6415-6445.
- Allen LH, Vu JCV. Carbon dioxide and high temperature effects on growth of young orange trees in a humid, subtropical environment. *Agric. For. Meteorol.* 2009; 149: 820-830.
- Bai W, Wan S, Niu S, Liu W, Chen Q, Wang Q, et al. Increased temperature and precipitation interact to affect root production, mortality, and turnover in a temperate steppe: implications for ecosystem C cycling. *Global Change Biol.* 2010; 16: 1306-1316.
- Beier C, Emmett B, Peñuelas J, Schmidt IK, Tietema A, Estiarte M, et al. Carbon and nitrogen cycles in European ecosystems respond differently to global warming. *Sci. Total Envir.* 2008; 407: 692-697.
- Bergner B, Johnstone J, Treseder KK. Experimental warming and burn severity alter soil CO₂ flux and soil functional groups in a recently burned boreal forest. *Global Change Biol.* 2004; 10: 1996-2004.
- Brando PM, Nepstad DC, Davidson EA, Trumbore SE, Ray D, Camargo P. Drought effects on litterfall, wood production and belowground carbon cycling in an Amazon forest: results of a throughfall reduction experiment. *Philos. Trans. R. Soc. Lond. B-Biol. Sci.* 2008; 363: 1839-48.
- Bremner J, Sparks D, Page A, Helmke P, Loeppert R, Soltanpour P, et al. Nitrogen-total. In: Sparks D, editor. *Methods of Soil Analysis*. SSSA Book Ser,

- Madison, 1996, pp. 1085-1121.
- Brookes P, Landman A, Pruden G, Jenkinson D. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. *Soil. Biol. Biochem.* 1985; 17: 837-842.
- Brzostek ER, Finzi AC. Substrate supply, fine roots, and temperature control proteolytic enzyme activity in temperate forest soils. *Ecology* 2011; 92: 892-902.
- Bytnerowicz A, Omasa K, Paoletti E. Integrated effects of air pollution and climate change on forests: a northern hemisphere perspective. *Environ. Pollut.* 2007; 147: 438-445.
- Chen H, Harmon ME, Sexton J, Fasth B. Fine-root decomposition and N dynamics in coniferous forests of the Pacific Northwest, U.S.A. *Can. J. For. Res.* 2002; 32: 320-331.
- Cleveland CC, Reed SC, Townsend AR. Nutrient regulation of organic matter decomposition in a tropical rain forest. *Ecology* 2006; 87: 492-503.
- Cleveland CC, Wieder WR, Reed SC, Townsend AR. Experimental drought in a tropical rain forest increases soil carbon dioxide losses to the atmosphere. *Ecology* 2010; 91: 2313-23.
- Davidson EA, Ishida FY, Nepstad DC. Effects of an experimental drought on soil emissions of carbon dioxide, methane, nitrous oxide, and nitric oxide in a moist tropical forest. *Global Change Biol.* 2004; 10: 718-730.
- Dawes MA, Philipson CD, Fonti P, Bebi P, Hättenschwiler S, Hagedorn F, et al. Soil

- warming and CO₂ enrichment induce biomass shifts in alpine tree line vegetation. *Global Change Biol.* 2015; 21: 2005-2021.
- Easterling DR, Meehl GA, Parmesan C, Changnon SA, Karl TR, Mearns LO. Climate extremes: observations, modeling, and impacts. *Science* 2000; 289: 2068-2074.
- Eldhuset TD, Lange H, De Wit HA. Fine root biomass, necromass and chemistry during seven years of elevated aluminium concentrations in the soil solution of a middle-aged *Picea abies* stand. *Sci. Total Envir.* 2006; 369: 344-356.
- Fang J, Liu G, Songling X. Biomass and net production of forest vegetation in China. *Act. Ecolog. Scinic.* 1996; 16: 497-508.
- Fitter A, Self G, Brown T, Bogie D, Graves J, Benham D, et al. Root production and turnover in an upland grassland subjected to artificial soil warming respond to radiation flux and nutrients, not temperature. *Oecologia* 1999; 120: 575-581.
- Forbes P, Black K, Hooker J. Temperature-induced alteration to root longevity in *Lolium perenne*. *Plant Soil* 1997; 190: 87-90.
- Gill RA, Jackson RB. Global patterns of root turnover for terrestrial ecosystems. *New Phytol.* 2000; 147: 13-31.
- Gimbel K, Felsmann K, Baudis M, Puhlmann H, Gessler A, Bruehlheide H, et al. Drought in forest understory ecosystems—a novel rainfall reduction experiment. *Biogeosciences* 2015; 12: 961-975.
- Hasibeder R, Fuchslueger L, Richter A, Bahn M. Summer drought alters carbon allocation to roots and root respiration in mountain grassland. *New Phytol.*

2015; 205: 1117-1127.

Heisler-White JL, Knapp AK, Kelly EF. Increasing precipitation event size increases aboveground net primary productivity in a semi-arid grassland. *Oecologia* 2008; 158: 129-140.

IPCC. Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. In: Core Writing Team RKPALAM, editor, IPCC, Geneva, Switzerland, 2014, pp. 151.

Kemp PR, Reynolds JF, Virginia RA, Whitford WG. Decomposition of leaf and root litter of Chihuahuan desert shrubs: effects of three years of summer drought. *J. Arid. Environ.* 2003; 53: 21-39.

King JS, Pregitzer KS, Zak DR. Clonal variation in above-and below-ground growth responses of *Populus tremuloides* Michaux: influence of soil warming and nutrient availability. *Plant Soil* 1999; 217: 119-130.

King JS, Pregitzer KS, Zak DR, Holmes WE, Schmidt K. Fine root chemistry and decomposition in model communities of north-temperate tree species show little response to elevated atmospheric CO₂ and varying soil resource availability. *Oecologia* 2005; 146: 318-28.

Kramer PJ, Boyer JS. *Water Relations of Plants and Soil*. New York: Academic Press, 1995.

Lee EH, Tingey DT, Beedlow PA, Johnson MG, Burdick CA. Relating fine root biomass to soil and climate conditions in the Pacific Northwest. *For. Eco.*

- Manag. 2007; 242: 195-208.
- Leuschner C, Backes K, Hertel D, Schipka F, Schmitt U, Terborg O, et al. 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. Eco. Manag. 2001; 149: 33-46.
- Lin C, Yang Y, Guo J, Chen G, Xie J. Fine root decomposition of evergreen broadleaved and coniferous tree species in mid-subtropical China: dynamics of dry mass, nutrient and organic fractions. Plant Soil 2011; 338: 311-327.
- Liu Y, Liu S, Wang J, Zhu X, Zhang Y, Liu X. Variation in soil respiration under the tree canopy in a temperate mixed forest, central China, under different soil water conditions. Eco. Res. 2014; 29: 133-142.
- Liu Y, Zhang Y, Liu S. Aboveground carbon stock evaluation with different restoration approaches using tree ring chronosequences in Southwest China. For. Eco. Manag. 2012; 263: 39-46.
- Luan J, Liu S, Zhu X, Wang J. Soil carbon stocks and fluxes in a warm-temperate oak chronosequence in China. Plant Soil 2011; 347: 243-253.
- Luan J, Liu S, Zhu X, Wang J, Liu K. Roles of biotic and abiotic variables in determining spatial variation of soil respiration in secondary oak and planted pine forests. Soil. Biol. Biochem. 2012; 44: 143-150.
- Majdi H. Interactive effects of soil warming and fertilization on root production, mortality, and longevity in a Norway spruce stand in Northern Sweden. Global Change Biol. 2004; 10: 182-188.

- Mao R, Zeng D-H, Li L-J. Fresh root decomposition pattern of two contrasting tree species from temperate agroforestry systems: effects of root diameter and nitrogen enrichment of soil. *Plant Soil* 2011; 347: 115-123.
- Meier IC, Leuschner C. Belowground drought response of European beech: fine root biomass and carbon partitioning in 14 mature stands across a precipitation gradient. *Global Change Biol.* 2008; 14: 2081-2095.
- Melillo JM, Steudler PA, Aber JD, Newkirk K, Lux H, Bowles FP, et al. Soil warming and carbon-cycle feedbacks to the climate system. *Science* 2002; 298: 2173-2176.
- Nadelhoffer KJ. The potential effects of nitrogen deposition on fine - root production in forest ecosystems. *New Phytol.* 2000; 147: 131-139.
- Nelson DW, Sommers LE, Sparks D, Page A, Helmke P, Loeppert R, et al. Total carbon, organic carbon, and organic matter. In: Sparks D, editor. *Methods of Soil Analysis*. Soil Sci Soc Am Inc., Madison, 1996, pp. 961-1010.
- Nepstad DC, De Carvalho CR, Davidson EA, Jipp PH, Lefebvre PA, Negreiros GH, et al. The role of deep roots in the hydrological and carbon cycles of Amazonian forests and pastures. 1994.
- Olson JS. Energy storage and the balance of producers and decomposers in ecological systems. *Ecology* 1963; 44: 322-331.
- Ostonen I, Lõhmus K, Pajuste K. Fine root biomass, production and its proportion of NPP in a fertile middle-aged Norway spruce forest: comparison of soil core and ingrowth core methods. *For. Eco. Manag.* 2005; 212: 264-277.

- Persson H, Ahlström K. Fine-root response to nitrogen supply in nitrogen manipulated Norway spruce catchment areas. *For. Eco. Manag.* 2002; 168: 29-41.
- Pritchard SG, Rogers HH, Davis MA, Van Santen E, Prior SA, Schlesinger WH. The influence of elevated atmospheric CO₂ on fine root dynamics in an intact temperate forest. *Global Change Biol.* 2001; 7: 829-837.
- Rustad LE. The response of terrestrial ecosystems to global climate change: towards an integrated approach. *Sci. Total Envir.* 2008; 404: 222-235.
- Rustad LE, Campbell JL, Marion GM, Norby RJ, Mitchell MJ, Hartley AE, et al. A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. *Oecologia* 2001; 126: 543-562.
- Sánchez-Moreno S, Castro J, Alonso-Prados E, Alonso-Prados JL, García-Baudín JM, Talavera M, et al. Tillage and herbicide decrease soil biodiversity in olive orchards. *Agron. Sustain. Dev.* 2014; 35: 691-700.
- Sariyildiz T. Effects of tree species and topography on fine and small root decomposition rates of three common tree species (*Alnus glutinosa*, *Picea orientalis* and *Pinus sylvestris*) in Turkey. *For. Eco. Manag.* 2015; 335: 71-86.
- Silver WL, Miya RK. Global patterns in root decomposition: comparisons of climate and litter quality effects. *Oecologia* 2001; 129: 407-419.
- Thakur MP, Reich PB, Fisichelli NA, Stefanski A, Cesarz S, Dobies T, et al. Nematode community shifts in response to experimental warming and canopy conditions are associated with plant community changes in the

- temperate-boreal forest ecotone. *Oecologia* 2014; 175: 713-723.
- Valverde - Barrantes OJ, Smemo KA, Feinstein LM, Kershner MW, Blackwood CB. Aggregated and complementary: symmetric proliferation, overyielding, and mass effects explain fine - root biomass in soil patches in a diverse temperate deciduous forest landscape. *New Phytol.* 2015; 205: 731-742.
- Van der Krift TAJ, Kuikman PJ, Möller F, Berendse F. Plant species and nutritional-mediated control over rhizodeposition and root decomposition. *Plant Soil* 2001; 228: 191-200.
- Vance E, Brookes P, Jenkinson D. An extraction method for measuring soil microbial biomass C. *Soil. Biol. Biochem.* 1987; 19: 703-707.
- Wan S, Norby RJ, Pregitzer KS, Ledford J, O'Neill EG. CO₂ enrichment and warming of the atmosphere enhance both productivity and mortality of maple tree fine roots. *New Phytol.* 2004; 162: 437-446.
- Wang X, Feng Z, Ouyang Z. Vegetation carbon storage and density of forest ecosystems in China. *China J. Appi. Eco.* 2001; 12: 13-16.
- Wildung R, Garland T, Buschbom R. The interdependent effects of soil temperature and water content on soil respiration rate and plant root decomposition in arid grassland soils. *Soil. Biol. Biochem.* 1975; 7: 373-378.
- Wu J, Joergensen R, Pommerening B, Chaussod R, Brookes P. Measurement of soil microbial biomass C by fumigation-extraction-an automated procedure. *Soil. Biol. Biochem.* 1990; 22: 1167-1169.
- Wu Z, Dijkstra P, Koch GW, PeñUelas J, Hungate BA. Responses of terrestrial

- ecosystems to temperature and precipitation change: a meta-analysis of experimental manipulation. *Global Change Biol.* 2011; 17: 927-942.
- Xia J, Chen S, Wan S. Impacts of day versus night warming on soil microclimate: results from a semiarid temperate steppe. *Sci. Total Envir.* 2010; 408: 2807-16.
- Xia J, Niu S, Wan S. Response of ecosystem carbon exchange to warming and nitrogen addition during two hydrologically contrasting growing seasons in a temperate steppe. *Global Change Biol.* 2009; 15: 1544-1556.
- Yin H, Xu Z, Chen Z, Wei Y, Liu Q. Nitrogen transformation in the rhizospheres of two subalpine coniferous species under experimental warming. *Appl. Soil. Ecol.* 2012; 59: 60-67.
- Zhang X, Wang W. The decomposition of fine and coarse roots: their global patterns and controlling factors. *Sci. Rep.* 2015; 5.
- Zhou X, Chen C, Wang Y, Xu Z, Han H, Li L, et al. Warming and increased precipitation have differential effects on soil extracellular enzyme activities in a temperate grassland. *Sci. Total Envir.* 2013; 444: 552-8.
- Zhou Y, Tang J, Melillo JM, Butler S, Mohan JE. Root standing crop and chemistry after six years of soil warming in a temperate forest. *Tree Physiol.* 2011; 31: 707-717.