

RESEARCH ARTICLE

# Effects of Manipulated Above- and Belowground Organic Matter Input on Soil Respiration in a Chinese Pine Plantation

Juan Fan<sup>1</sup>\*, Jinsong Wang<sup>1</sup>\*, Bo Zhao<sup>1</sup>, Lianhai Wu<sup>2</sup>, Chunyu Zhang<sup>1</sup>, Xiuhai Zhao<sup>1</sup>\*, Klaus v. Gadow<sup>3</sup>

**1** Key Laboratory for Silviculture and Conservation of the Ministry of Education, Beijing Forestry University, No. 35 Tsinghua East Road, Haidian District, Beijing 100083, P. R. China, **2** Rothamsted Research, North Wyke, Okehampton, Devon EX20 2SB, United Kingdom, **3** Faculty of Forestry and Forest Ecology, Georg-August-University Göttingen, BÜsgenweg 5, D-37077 Göttingen, Germany

\* These authors contributed equally to this work.

\* [zhaoxh@bjfu.edu.cn](mailto:zhaoxh@bjfu.edu.cn)



OPEN ACCESS

**Citation:** Fan J, Wang J, Zhao B, Wu L, Zhang C, Zhao X, et al. (2015) Effects of Manipulated Above- and Belowground Organic Matter Input on Soil Respiration in a Chinese Pine Plantation. PLoS ONE 10(5): e0126337. doi:10.1371/journal.pone.0126337

**Academic Editor:** Xuhui Zhou, Fudan University, CHINA

**Received:** October 14, 2014

**Accepted:** April 1, 2015

**Published:** May 13, 2015

**Copyright:** © 2015 Fan et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

**Data Availability Statement:** All relevant data necessary to replicate the results of our study are contained within the paper and its tables and figures.

**Funding:** This research was supported by the National Basic Research Program of China (973 Program; 2011CB403203) and the State Key Program of National Natural Science Foundation of China (41330530). Rothamsted Research receives grant aided support from the Biotechnology and Biological Science Research Council (BBSRC), UK. The funders had a role in study design, data collection and analysis, decision to publish, and preparation of the manuscript.

## Abstract

Alteration in the amount of soil organic matter input can have profound effect on carbon dynamics in forest soils. The objective of our research was to determine the response in soil respiration to above- and belowground organic matter manipulation in a Chinese pine (*Pinus tabulaeformis*) plantation. Five organic matter treatments were applied during a 2-year experiment: both litter removal and root trenching (LRRT), only litter removal (LR), control (CK), only root trenching (RT) and litter addition (LA). We found that either aboveground litter removal or root trenching decreased soil respiration. On average, soil respiration rate was significantly decreased in the LRRT treatment, by about 38.93% ± 2.01% compared to the control. Soil respiration rate in the LR treatment was 30.65% ± 1.87% and in the RT treatment 17.65% ± 1.95% lower than in the control. Litter addition significantly increased soil respiration rate by about 25.82% ± 2.44% compared to the control. Soil temperature and soil moisture were the main factors affecting seasonal variation in soil respiration. Up to the 59.7% to 82.9% seasonal variation in soil respiration is explained by integrating soil temperature and soil moisture within each of the various organic matter treatments. The temperature sensitivity parameter,  $Q_{10}$ , was higher in the RT (2.72) and LA (3.19) treatments relative to the control (2.51), but lower in the LRRT (1.52) and LR treatments (1.36). Our data suggest that manipulation of soil organic matter input can not only alter soil CO<sub>2</sub> efflux, but also have profound effect on the temperature sensitivity of organic carbon decomposition in a temperate pine forest.

## Introduction

Globally, it is assumed that soils store more organic carbon than carbon in plants and the atmosphere combined [1]. Temperate forest soils contain more than twice as much carbon as the

**Competing Interests:** The authors have declared that no competing interests exist.

living biomass [2]. As a result, changes in the dynamics of soil carbon of temperate forests could have a profound impact on global carbon cycling. Soil respiration in forest ecosystems is a major pathway for carbon dioxide (CO<sub>2</sub>) returning to the atmosphere. It originates from the decay of organic matter in the soil, the decomposition of aboveground litter and respiration within the rhizosphere, including roots and mycorrhizae.

Soil respiration can be affected by soil microclimatic factors, such as temperature, soil moisture and pH [3]. Increasing evidence also suggests there is a strong linkage between soil respiration and recent photosynthates [4]. As a biological process, soil respiration is closely tied to plant growth and the supply of photosynthetic substrates. Tree girdling experiments [5], shading and clipping experiments [6] and correlations between soil respiration and the supply of substrates among different forest sites [7], have all been cited as evidence for above- and belowground organic matter input being the main driver of soil respiration. Therefore, any factor that impacts the above- and belowground substrate supply is likely to cause changes in soil respiration and corresponding climate-carbon feedbacks.

In the context of global change, alterations in the amount of aboveground litter are becoming more and more likely. It has been reported that elevated atmospheric CO<sub>2</sub> concentrations [8], increased nitrogen deposition [9] and rising temperatures are predicted to raise the amount of aboveground litter via enhanced plant productivity, whereas elevated O<sub>3</sub> [10] and drought stress [11] generally decrease productivity. Some other drastic disturbances, i.e., severe ice storms [12], insect and disease infestation [13] and wildfires [14] can also lead to sudden and dramatic changes in litter input. In addition, human activity such as litter layer removal and understory clearing are common forest management practices in many regions [15,16,17,18], with the aim not only to harvest fuel, but also to eliminate combustible loads to prevent fire. These changes in the amount of aboveground litter can inevitably lead to a direct effect on soil respiration, via changes in microclimate of the litter layer and alterations in the supply of substrates.

Changes in the amount of soil organic matter are closely related to physical, chemical and biological processes in the soil [19], but the underlying mechanisms controlling the effect of soil organic matter input on soil respiration are still not well understood. Organic matter manipulation involving litter removal or addition is a direct way for studying the effect of the amount of litter on soil respiration [20]. However, existing evidence on the effect of soil organic matter on soil respiration may differ greatly among forest ecosystems. It has been reported that litter removal often drives proportional declines in soil respiration [21,22,23,24]. Soil respiration may increase disproportionately in response to litter addition, suggesting that increased litter input may not only release a portion of the newly added carbon, but also accelerate the decomposition of older organic matter through a priming effect [25].

Soil respiration is not only affected by the amount of aboveground litter, but also influenced by the supply of organic matter input to soil through root turnover and root exudates. Roots play an important role in translocating photosynthates from plants to the soil [26]. Microbial activity may increase with the decomposition of root debris [27]. In addition, roots release exudates including carbohydrates, sugars, amino acids, organic acids and phenolic compounds [28]. It has been estimated that approximately 75% of carbon allocated to the roots is respired by soil microorganisms [29]. The contribution of root respiration to total soil respiration can account for as little as 10% to more than 90% worldwide [30]. It has been reported that the absence of roots eliminates the supply of root exudates and may decrease microbial activity, which in turn inhibits soil CO<sub>2</sub> efflux [26]. Although this effect is of ecological importance, changes in belowground roots have usually been ignored in ecological studies of forests.

In this study, we use an *in situ* above- and belowground organic matter manipulation experiment in a Chinese pine (*Pinus tabulaeformis*) plantation in order to test our hypothesis that (1) either aboveground litter removal or root trenching will always decrease soil respiration,

whereas litter addition will increase it; (2) litter removal will have a higher impact than root trenching on soil respiration through our entire observation period because roots might be able to maintain respiration for some time after root trenching; (3) the extent of increase in soil respiration in the litter addition treatment will be larger than that of decrease in soil respiration only in the litter removal treatment because of the priming effect.

## Materials and Methods

### Ethics statement

The research station for this study is owned by Beijing Forestry University. Our study was approved by the Taiyue Mountain Ecosystem Research Station and the Key Laboratory for Silviculture and Conservation of the Ministry of Education.

### Site description

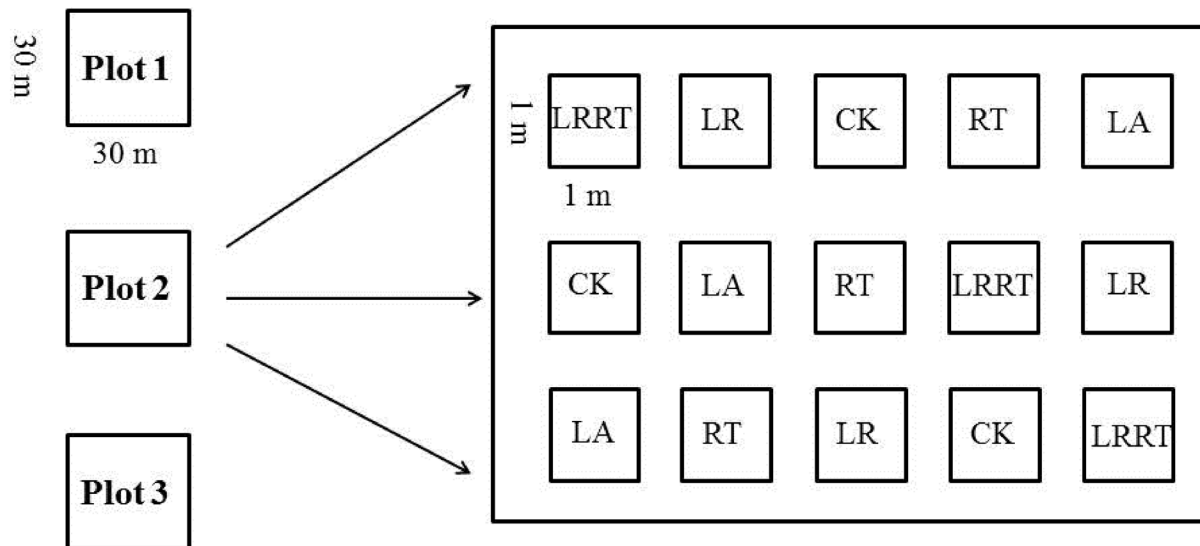
Field work was carried out at the Taiyue Mountain Ecosystem Research Station (36°18' N, 111°45' E, 1560 m a.s.l), located in Shanxi Province, in northern China (the Taiyue Forestry Bureau issued the permission to conduct this study at each location). The region is classified as belonging to a warm-temperate semi-arid continental monsoon-affected climate, with a mean annual temperature of 9.9°C. The highest monthly average temperature of 22.4°C is observed in July while the lowest monthly average temperature of -4.6°C occurs in January. Mean annual precipitation is 548 mm with a mean relative humidity of 65%. The distribution of precipitation over a year is relatively uneven. The wet season is from July to September and accounts for more than 60% of annual precipitation. The soil is a typical brown forest soil of 60–100 cm in thickness. The soil pH ranges from 6.8 to 7.3 and soil organic carbon is 3%–4%.

The study field is an artificial forest dominated by a 38-year *Pinus tabulaeformis* stand that has been protected since the 1990s. Stand density is 2213 stems per hectare. The dominant overstory vegetation in the stand is *P. tabulaeformis* with a mean breast-height diameter of 14.5 cm ± 1.5 cm and a mean height of 16.8 m ± 1.8 m. The understory layer consists mainly of *Ostryopsis davidiana*, *Lespedeza bicolor*, *Hippophae rhamnoides*, *Corylus mandshurica*, *Swida bretschneideri* and *Rosa xanthina*. Mean annual litterfall in the forest is 504 g·m<sup>-2</sup> and the density of fine roots is 192 g·m<sup>-2</sup>.

### Experimental layout

Three 30×30 m plots were established in the plantation in January 2010. All live and dead trees with woody stems exceeding 1 cm in diameter at their breast-height were tagged and identified in each plot. The breast-height diameter, tree height and crown dimension of each tree were measured and recorded. In order to evaluate soil conditions in the plantation, we randomly collected twenty soil samples with a volume of 100 cm<sup>3</sup> in each plot from the top soil (0–20 cm) in March 2010. All plot samples were collected as one common sample and sieved to 2 mm to remove coarse fragments and then air-dried to analyze their bulk density and nutrient contents. The total amount of N was measured using Kjeldahl's digestion with a salicylic acid modification [31], while total amount of phosphorus and potassium were fused using the NaOH method [32]. Soil organic carbon was measured following the method described by Kalembasa and Jenkinson [33].

By the end of May 2010, fifteen 1×1 m subplots were randomly established in each plot for soil respiration measurements. The schematic diagram of the experimental plot layout is shown in Fig 1. The subplots were subjected to five organic matter treatments: (1) both litter removal and root trenching (LRRT for short), (2) only litter removal (LR), (3) control (CK), (4) only



**Fig 1. Schematic diagram of experimental plot layout. Organic matter treatments: both litter removal and root trenching (LRRT), only litter removal (LR), control (CK), only root trenching (RT), and litter addition (LA).**

doi:10.1371/journal.pone.0126337.g001

root trenching (RT), (5) litter addition (LA). Each treatment was replicated three times. In the LRRT and LR treatments, organic layers above the mineral soil were removed and a 1×1 m litter trap was set up 1 m above the ground to intercept fresh foliage. Root trenching was achieved by digging the subplot perimeter. To prevent roots originating from the plants growing outside of a subplot from penetrating into the subplot, a 0.5 mm thick polyethylene sheet along the sides of the trench was inserted before backfilling. The root-free plots were kept free of vegetation by cutting plant regrowth manually throughout the study period, with extra care taken to minimize disturbance to the soil. For the LA treatment, litter was transferred from the LR subplots. In each subplot, we inserted a 10 cm height cylinder with a 20 cm inner diameter into the soil up to 5–6 cm deep. We initiated soil respiration rate ( $R_s$ ) measurements 24 hours after installing the cylinder. The location of the cylinder did not change during the  $R_s$  measurements. We performed the  $R_s$  measurements in the middle and at the end of each month during the two growing seasons 2010 (from June to October) and 2011 (from May to October).

### Soil respiration rate, soil temperature and soil moisture measurements

We measured  $R_s$  from 8:00 to 18:00 on each day of the two measurements every month, using a portable, closed dynamic chamber (LI-8100, LI-COR, Nebraska, USA). Daily respiration rates were averaged for the calculation of accumulative monthly mean soil respiration efflux ( $\text{g C m}^{-2}$ ) for the various organic matter treatments. Accumulative monthly mean soil respiration efflux was calculated as follows:

$$CR_s = [DR_s \times (30/31) \times 24 \times 3600 \times 12] \cdot 10^{-6} \quad (1)$$

where  $CR_s$  is the accumulative monthly mean soil respiration efflux,  $DR_s$  is the daily mean soil respiration rate ( $\mu \text{mol m}^{-2} \text{s}^{-1}$ ), 30/31 are the days of one month, 24 represents the number of hours per day, 3600 the number of seconds per hour and 12 is the molar mass of carbon (C). We summed  $CR_s$  to obtain the accumulative seasonal soil respiration efflux.

We also recorded soil temperature and soil moisture at 5 cm depth with the LI-8100 system simultaneously with the  $R_s$  measurements. In addition, air temperature and precipitation were

measured using a Davis Weather Station (Vantage Pro, Davis Inc. USA) located at the Research Station.

### Modeling soil respiration rate with soil temperature and moisture

We used univariate and bivariate models to describe the relationship between soil respiration rate ( $R_s$ ), soil temperature ( $T$ ) and soil moisture ( $M$ ). The first model shows  $R_s$  as an exponential function with only soil temperature,  $T$  ( $^{\circ}\text{C}$ , referred to as the  $T$  model) as the independent variable:

$$R_s = \beta_0 e^{\beta_1 T} \quad (2)$$

$R_s$  as a function of soil moisture,  $M$  (%) was estimated with a quadratic equation (referred to as the  $M$  model):

$$R_s = \beta_2 + \beta_3 M + \beta_4 M^2 \quad (3)$$

In the third model,  $R_s$  is a function of both soil temperature and soil moisture (referred to as the  $T$  &  $M$  model):

$$R_s = \beta_5 e^{\beta_6 T} M^{\beta_7} \quad (4)$$

where  $R_s$  is the soil respiration rate ( $\mu\text{ mol m}^{-2} \text{ s}^{-1}$ );  $T$  the soil temperature at 5 cm depth ( $^{\circ}\text{C}$ ) and  $M$  is the volumetric water content of soil at a depth of 5 cm (%). As suggested by Lloyd and Taylor [34], soil temperature sensitivity,  $Q_{10}$ , was calculated as follows:  $Q_{10} = e^{10\beta_1}$ , where  $\beta_1$  is taken from the  $T$  model (Eq 2;  $\beta_1$ ) and from the  $T$  &  $M$  model (Eq 4;  $\beta_6$ )

### Soil respiration components

Soil respiration components were calculated according to the method proposed by Rey et al. [35] as follows:

$$R_m = R_1; R_L = R_3 - R_2; R_r = R_3 - R_4$$

where  $R_m$  is the heterotrophic respiration rate derived from the decomposition of soil organic matter in the soil;  $R_L$  the respiration rate from litter decomposition and  $R_r$  the rate of root respiration.  $R_1$ ,  $R_2$ ,  $R_3$  and  $R_4$  are, respectively, the soil respiration rates from the LRRT, LR, CK and RT subplots. To test the precision of our  $R_s$  measurements, the sum of  $R_m$ ,  $R_L$  and  $R_r$  was compared with the soil respiration rate in the CK subplots.

### Microbial biomass carbon measurements in the soil

In order to evaluate microbial biomass carbon in the soil of the various organic matter treatments, we randomly collected three soil cores with a 2.5 cm diameter from the top soil (0–10 cm) in each subplot in November 2010 and then mixed them to form a composite sample. After removing roots and plant residue, these composite samples were immediately sieved through a 2 mm mesh sieve in the field and kept refrigerated after transport to the laboratory. Microbial biomass carbon (MBC) in the soil was measured using a chloroform direct-fumigation extraction method [36] in the laboratory.

### Statistical analysis

The objective of the statistical analysis was to determine differences in  $R_s$  among organic matter treatments by fluctuation, i.e., fluctuation (%) =  $100(A-B)/B$ , where  $A$  represents  $R_s$  in the LRRT, LR, RT and LA treatments and  $B$  the corresponding value in the CK.

The effect of organic matter treatment, temporal (month-to-month) variation and their interactions on  $R_s$ ,  $T$  and  $M$  were analyzed using a two-way analysis of variance (ANOVA). Levene's test was used to test for the homogeneity of variance. Two-way ANOVA was also used to examine the effects of aboveground litter and belowground roots on  $R_s$ ,  $T$  and  $M$  during the entire observation period. In addition, one-way ANOVA was conducted to analyze the significance of the difference in accumulative seasonal soil respiration efflux among the five organic matter treatments. Differences among treatments were compared by a multiple LSD test. The significant level was set at 0.05 and all statistical tests were performed using SPSS (ver. 16.0) and R 2.15.2 (<http://www.R-project.org/>).

## Results

### Effects of organic matter treatments on soil respiration rate

Soil respiration rate exhibited clear seasonal variations in the various organic matter treatments (Fig 2). Maximum  $R_s$  occurred during the summer, due to high temperatures and moisture in the soil, with minimum  $R_s$  in October, when mean monthly precipitation was low.

Soil organic matter treatments significantly affected  $R_s$  throughout the study period ( $p < 0.001$ ) (Table 1). The average  $R_s$  were 1.62, 1.82, 2.73, 2.30 and 3.42  $\mu\text{mol m}^{-2} \text{s}^{-1}$  in the LRRT, LR, CK, RT, and LA treatments, respectively. Either aboveground litter removal or root trenching significantly decreased  $R_s$  when compared to the CK ( $p < 0.001$ ; Table 2).

On average,  $R_s$  was significantly reduced by the LRRT treatment, about  $38.93\% \pm 2.01\%$  lower than in the CK (Fig 3). The corresponding values in the LR and RT treatments were  $30.65\% \pm 1.87\%$  and  $17.65\% \pm 1.95\%$  lower than in the CK. The soil respiration rate in the RT treatment was initially higher than that of the CK for up to 2 months after trenching, with rates, in late June and mid-July 2010, 27.87% and 0.33% higher than that of the CK. Soil

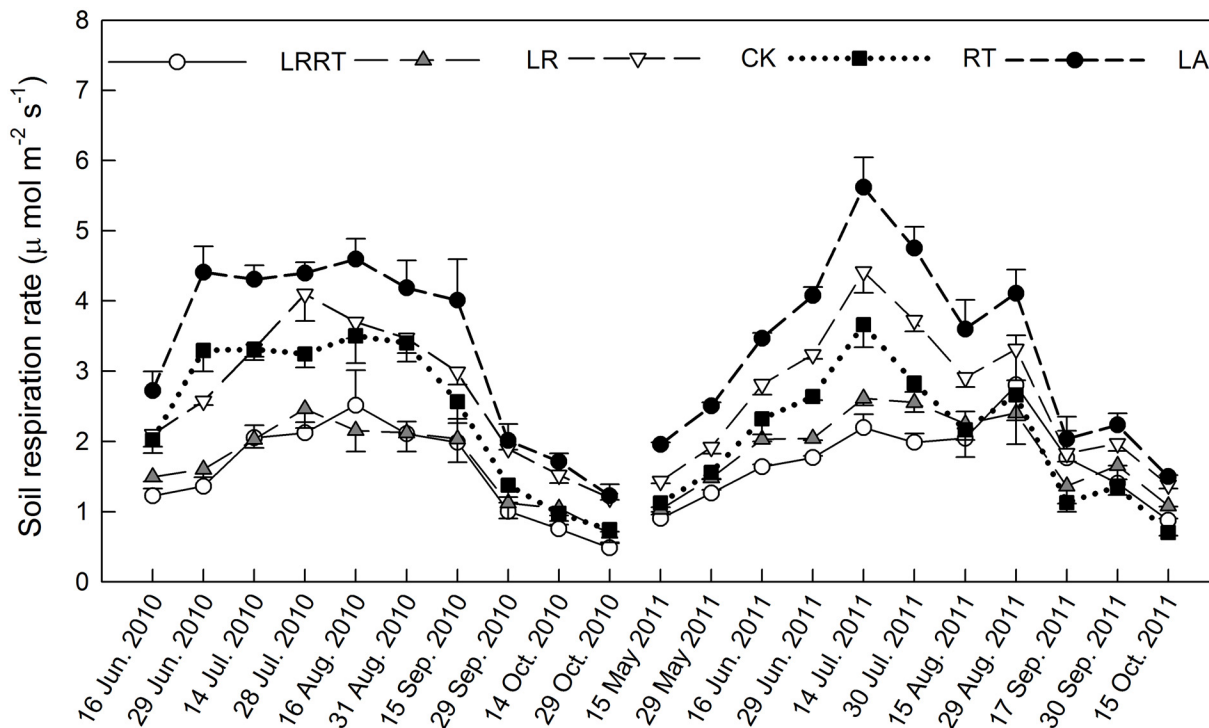


Fig 2. Variations of soil respiration in the various organic matter treatments as a function of time.

doi:10.1371/journal.pone.0126337.g002

**Table 1. Two-way analysis of variance (ANOVA) results of soil respiration rate ( $R_s$ ), soil temperature ( $T$ ) and soil moisture ( $M$ ) at 5 cm depth.**

Source of variation	$R_s$		$T$		$M$	
	$F$	$p$	$F$	$p$	$F$	$p$
Season ( $S$ )	0.009	0.926	1.413	0.235	4.113	0.05
Treatment ( $Tr$ )	54.048	0.000	1.537	0.190	16.706	0.000
$S \times Tr$	1.216	0.303	0.109	0.979	2.049	0.086

doi:10.1371/journal.pone.0126337.t001

**Table 2. Two-way analysis of variance (ANOVA) results of soil respiration rate ( $R_s$ ), soil temperature ( $T$ ) and soil moisture ( $M$ ) at 5 cm depth during the entire observation period (aboveground litter and root trenching being the main factors).**

Source of variation	$R_s$		$T$		$M$	
	$F$	$p$	$F$	$p$	$F$	$p$
Aboveground litter effect ( $ALE$ )	82.879	0.000	2.360	0.125	4.985	0.05
Root trenching effect ( $RTE$ )	13.254	0.000	0.093	0.760	29.826	0.000
$ALE \times RTE$	1.788	0.182	0.995	0.319	3.671	0.056

doi:10.1371/journal.pone.0126337.t002

respiration rate was significantly increased in the LA treatment, about  $25.82\% \pm 2.44\%$  higher than in the CK.

### Contribution of soil respiration components

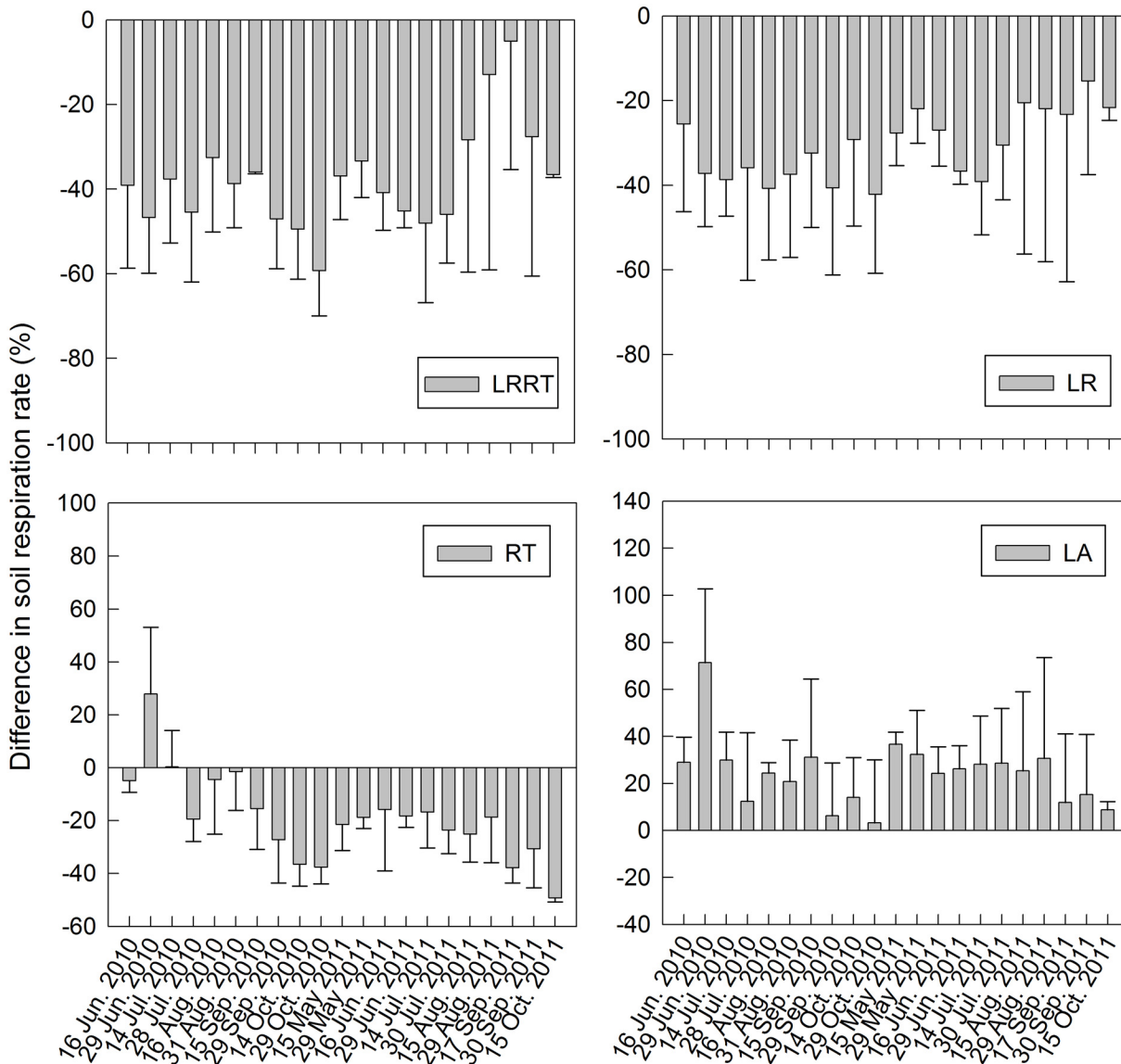
On average,  $R_m$ ,  $R_L$  and  $R_r$  were 1.61, 0.93 and  $0.46 \mu \text{mol m}^{-2} \text{s}^{-1}$  during the entire observation period. The relative contribution of each component to total soil respiration rate was 53.7%, 31.0% and 15.3%. The sum of each component was calculated and compared with the soil respiration rate in the CK. The slope of the fitted equation of the calculated respiration rate as a function of the respiration rate in the control subplots, i.e.  $b_1 = 1.02$  is not significantly different from 1 ( $p = 0.85$ ) (Fig 4).

### Comparison of accumulative seasonal soil respiration efflux

The accumulative seasonal soil respiration efflux varied between 241.0 and  $532.5 \text{ g C m}^{-2}$  in 2010, with the accumulative seasonal soil respiration amounts in 2011 ranging between 300.8 and  $582.4 \text{ g C m}^{-2}$  (Table 3). The accumulative seasonal soil respiration in the LRRT and LR treatments was significantly lower while the value in the LA treatment was significantly higher relative to that of the CK ( $p < 0.05$ ). The RT treatment significantly decreased the accumulative seasonal soil respiration compared to the CK in 2011 ( $p < 0.001$ ); however, there was no significant difference between the accumulative seasonal soil respiration in the RT treatment and that in the CK in 2010 ( $p > 0.05$ ).

### Effects of organic matter treatments on biophysical factors and microbial biomass carbon in the soil

Soil temperature showed distinct seasonal variations in the various organic matter treatments. During the entire observation period, no significant differences in soil temperature were found between the five organic matter treatments ( $p > 0.05$ ; Table 1). The average soil temperature was  $13.4^\circ\text{C}$  in the LRRT,  $14.0^\circ\text{C}$  in the LR,  $12.8^\circ\text{C}$  in the CK,  $13.1^\circ\text{C}$  in the RT and  $12.6^\circ\text{C}$  in the LA treatment throughout the study period.



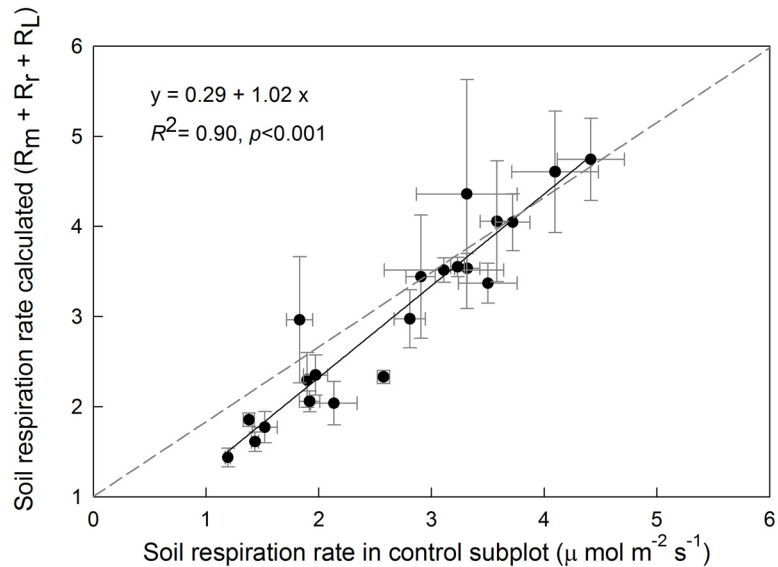
**Fig 3. Changes in soil respiration rates in the various organic matter treatments as a function of time. Bars represent standard errors.**

doi:10.1371/journal.pone.0126337.g003

Soil moisture also experienced clear seasonal variations in the various organic matter treatments. Organic matter treatment significantly affected soil moisture over the course of both growing seasons ( $p < 0.001$ ). The average soil moisture was 29.89% in the LRRT, 23.68% in the LR, 23.41% in the CK, 26.39% in the RT and 20.75% in the LA treatment throughout the study period. Soil moisture in the LRRT treatment was significantly higher than that in the CK ( $p < 0.001$ ). No significant differences in soil moisture were found between the other four treatments ( $p > 0.05$ ).

Organic matter treatment significantly affected microbial biomass carbon (MBC) in the soil ( $p < 0.05$ ). The average MBC was 110.9, 171.1, 240.5, 189.9 and 301.0  $\text{mg kg}^{-1}$  in the LRRT, LR, CK, RT and LA treatment, respectively. MBC in the LA treatment was significantly higher while the values in the LRRT, LR and RT treatments were significantly lower than that of the CK ( $p < 0.05$ ).





**Fig 4. Relationship between soil respiration rate in the control subplots and the calculated value as the sum of the different components. Bars represent standard errors. Dark gray line is 1:1 line and black line is fitting line.**

doi:10.1371/journal.pone.0126337.g004

### Effects of soil temperature and soil moisture on soil respiration

Correlations between soil temperature or soil moisture and soil respiration rate were significant in all treatments during the entire observation period ( $p < 0.001$ ; Table 4), with  $R^2$  values ranging from 0.125 to 0.763. A significant quadratic relation of  $R_s$  to soil moisture was found across all treatments ( $p < 0.05$ ). We found that soil moisture explained 6.3%–17.2% of the variation in  $R_s$  throughout the study period.  $Q_{10}$  values based on the exponential regression of the Eq 2 are higher in the RT (2.72) and LA (3.19) treatments relative to the CK (2.51) and lower in the LRRT (1.52) and LR (1.36) treatments. The non-linear model (Eq 4) including both soil temperature and soil moisture, predict  $R_s$  rather well, with  $R^2$  values ranging from 0.597 to 0.829 (Table 4). With the combined effect of soil temperature and soil moisture, the fitted  $Q_{10}$  values of the Eq 4 were 1.92, 2.04, 2.51, 3.00 and 3.25 for the LRRT, LR, CK, RT and LA treatment, respectively.

## Discussion

### Response of soil respiration to organic matter manipulation

The findings from our manipulative experiment provide an insight into the effects of above- and belowground organic matter on soil respiration in a *P. tabulaeformis* plantation and may

**Table 3. Accumulative seasonal soil respiration efflux (g C m<sup>-2</sup>) among different organic matter treatments during growing seasons (GS) of 2010 and 2011.**

Season	Treatment				
	LRRT	LR	CK	RT	LA
2010 GS	241.0 a	266.1 a	424.2 b	387.3 bc	532.5 d
2011 GS	300.8 a	337.6 a	472.4 b	356.5 a	582.4 c
Average	270.9 a	301.9 a	448.3 b	371.9 c	557.5 d

Different letters within the same row indicate significant difference among organic matter treatments (Two-way ANOVA with LSD test,  $\alpha = 0.05$ )

doi:10.1371/journal.pone.0126337.t003

**Table 4. Parameters of different models of soil respiration rate ( $R_s$ ) as a function of soil temperature ( $T$ ) and soil moisture ( $M$ ) at 5 cm depth.** Data are mean values, with the SE given in parentheses.

Treatment	Parameters					
	T model			$R^2$	$Q_{10}$	
$\beta_0$	$\beta_1$	$\rho$				
LRRT	0.952 (0.122)	0.042 (0.008)	0.000	0.235	1.52	
LR	1.308 (0.172)	0.031 (0.008)	0.000	0.125	1.36	
CK	0.795 (0.083)	0.092 (0.007)	0.000	0.680	2.51	
RT	0.582 (0.070)	0.100 (0.008)	0.000	0.703	2.72	
LA	0.738 (0.078)	0.116 (0.007)	0.000	0.763	3.19	
	M model					
	$\beta_2$	$\beta_3$	$\beta_4$	$\rho$	$R^2$	
LRRT	2.007 (1.105)	-0.081 (0.085)	0.002 (0.002)	0.000	0.172	
LR	1.276 (0.478)	0.029 (0.045)	-0.0002 (0.001)	0.05	0.063	
CK	0.572 (0.552)	0.200 (0.052)	-0.004 (0.001)	0.001	0.141	
RT	-0.695 (0.891)	0.227 (0.074)	-0.004 (0.002)	0.001	0.116	
LA	1.485 (0.722)	0.220 (0.076)	-0.005 (0.002)	0.05	0.082	
	T & M model					
	$\beta_5$	$\beta_6$	$\beta_7$	$\rho$	$R^2$	$Q_{10}$
LRRT	0.085 (0.386)	0.065 (0.006)	0.590 (0.111)	0.000	0.597	1.92
LR	0.165 (0.211)	0.071 (0.005)	0.433 (0.057)	0.000	0.691	2.04
CK	0.477 (0.121)	0.092 (0.005)	0.162 (0.035)	0.000	0.811	2.51
RT	0.322 (0.225)	0.110 (0.006)	0.127 (0.069)	0.000	0.807	3.00
LA	0.786 (0.121)	0.118 (0.005)	-0.032 (0.036)	0.000	0.829	3.25

doi:10.1371/journal.pone.0126337.t004

have significant implications in modeling soil respiration. Organic matter manipulation is expected to affect soil respiration by altering microclimatic conditions [37, 38], carbon chemistry [39] and microbial biomass in the soil [40]. There are many dimensions to organic matter which affect soil respiration, i.e., the type of organic matter, the amount of organic matter and timing of measurement.

Soil respiration includes autotrophic and heterotrophic respiration fluxes [41], showing different responses to organic matter manipulation. Autotrophic respiration is controlled by the root biomass of a specific soil layer, whereas heterotrophic respiration depends on the amounts of aboveground litter and dead organic carbon in the soil [42]. Despite some differences in soil respiration responses to aboveground litter removal and/or root trenching during the first 2 years, we found that either aboveground litter removal or root trenching both decreased soil respiration rate. This is mainly due to aboveground litter removal and root trenching, which leads to a lower carbon and nutrient supply from the aboveground to belowground layers and from roots into the soil, with a consequent lower microbial activity. Our results are also in line with the study conducted by Li et al. [21], who reported that litter removal decreased soil microbial biomass by 67–69% in a tropical pine plantation seven years after the initiation of treatments.

As predicted, litter removal had a higher impact than root trenching on soil respiration throughout the entire observation period. We found that soil respiration rate in the RT treatment did not decrease as expected but increased in late June and mid-July in the first year, which was probably due to the fact that roots are able to maintain respiration for a period of time after trenching. In addition, microbial decomposition of dead roots in the trenching subplots might provide substrates for the growth of microorganisms and stimulate soil respiration rate by increasing heterotrophic respiration [43]. Root trenching also increased soil

temperature and soil moisture when compared to the control subplots. This may in turn stimulate soil respiration rate. After all, maintenance respiration, stimulation of heterotrophic respiration and changes in microclimatic conditions after trenching are possible causes for the initial increase of soil respiration rate in the RT treatment.

Although we did not observe any significant differences in the accumulative seasonal soil respiration between the RT treatment and the CK in 2010, the accumulative seasonal soil respiration in the RT treatment was significantly lower than that in the CK in 2011. This implies that root trenching result in changes in soil respiration that varies over time. According to Lee et al. [44], a short initial increase (of about 2 months) is followed by two years of decrease in soil respiration rate in a cool-temperate deciduous forest. Therefore, the increasing long-term and year-round measurements over time should be given more attention in future studies.

It has been reported that soil respiration rate always increases disproportionately in response to litter addition [45]. Litter addition not only releases a portion of the newly added carbon, but also accelerates the decomposition of older organic matter through the positive priming effect. For example, Prévost-Bouré et al. [45] reported that fresh aboveground litter addition overstimulates soil respiration in a temperate deciduous forest owing to this positive priming effect. Moreover, this priming effect lasted for more than one year in the progressive decomposition of fresh litter. Contrary to our prediction, we did not find such a positive priming effect in the present study. The possible causes may be attributed to two factors. In first instance, litter addition in our study was only applied once at the beginning of the experiment, whereas other studies added fresh litter several times per year [22,25], which made the priming effect to be released gradually. Secondly, the depth of the litter layer in our pine plantation was up to 10 cm thick and needle litter decomposition was much slower than that from most broad-leaved forests [20,46]. Therefore, there were no significant differences between the increase in soil respiration rate in the LA treatment and the decrease in soil respiration rate in the LR treatment.

## Effects of soil temperature and soil moisture on soil respiration

It is well known that seasonal changes in soil respiration rate have been widely reported to be correlated with soil temperature and soil moisture [34,42,47]. Since our manipulative experiment was conducted in a relatively small area and most importantly, crown closure in our plantation did not change, organic matter manipulation did not significantly affect soil temperature in this study. However, the mean soil moisture in the LRRT treatment significantly increased compared to the CK throughout the entire observation period due to the inhibition of water transport between the trenching subplot inside and outside. Such a change would lead to corresponding changes in soil respiration rate.

The variation in soil respiration rate in our organic matter treatments was more sensitive to change in soil temperature than that in soil moisture during both growing seasons. It is probable that much of the soil moisture was in a range suitable for soil respiration, whereas soil temperature, kept at a low level during the study period, became a major factor restricting soil respiration rate. Soil temperature explained 68.0% to 76.3% of the seasonal variation in soil respiration rate, except in the LRRT and LR treatments. Soil temperatures in the LRRT and LR treatments were higher, but the respiration rates lower relative to the CK. This suggests that when aboveground litter is removed, soil temperature is still important, but not as sensitive as before to the seasonal variation in soil respiration rate. Aboveground litter acts as a protective buffer against air temperature. Therefore, the large variation in soil temperature after litter removal is not sufficient in explaining the variation in soil respiration rate.

The sensitivity of soil respiration to soil temperature in the CK was within the reported range ( $Q_{10} = 1.8\text{--}4.1$ ) worldwide [48] and close to the reported median value of 2.4 studied by

Raich and Schlesinger [49]. The  $Q_{10}$  values also varied among organic matter treatments in the present study. It has been reported that higher temperatures increase soil respiration efflux to the atmosphere, thus further aggravating global warming [50]. The highest  $Q_{10}$  value in the LA treatment suggests that litter addition enhances the sensitivity of soil respiration to changes in soil temperature and that soil respiration efflux in the LA treatment could be increased more under climate warming. Soil respiration is not significantly affected by climate warming in the LR treatment. It is caused by that leaf litter decomposition is more sensitive to soil temperature than root litter decomposition. Another reason is that after leaf litter is removed from above-ground, less organic carbon is transferred into soil. In our study, the bivariate model yielded higher  $Q_{10}$  values than the univariate model alone. It is possible that the relationship between soil respiration and soil temperature is confounded by soil moisture, given that we modified the relationship when soil moisture was considered.

## Conclusions

This study is the first report showing the effect of organic matter manipulation on soil respiration in a major pine ecosystem in temperate China. The results of this study will enhance our understanding of the complex impact of above- and belowground organic matter on soil respiration and thus on the ecosystem carbon budget. Either aboveground litter removal or root trenching caused a decrease of soil respiration rate while litter addition increased soil respiration rate over the 2-year period. Litter removal had a higher impact than root trenching on soil respiration throughout the entire observation period. The mean rate of soil respiration in the LR treatment was  $30.65\% \pm 1.87\%$  lower than in the CK, whereas this value in the RT treatment was  $17.65\% \pm 1.95\%$  lower than in the CK. When aboveground litter was removed in the LRRT and LR treatments, soil temperature was not as sensitive as before to the seasonal variation in soil respiration rate. Soil temperature and soil moisture were the main controlling factors of the seasonal variation in soil respiration rate. Up to the 59.7% to 82.9% seasonal variation in soil respiration is explained by integrating soil temperature and soil moisture in the various organic matter treatments.

Our current results suggest that litter addition will increase soil respiration efflux in response to climate warming, while litter removal will decrease the sensitivity of soil respiration to changes in soil temperature. Litter layer removal and understory clearing are common forest management practices in many countries and regions. Appropriate forest management in forest ecosystems emphasizes the promotion of organic carbon turnover and carbon sequestration in the soil. Therefore, our finding is crucial to forest managers in both predicting the consequences of forest management and guiding to manage forest carbon flux in temperate pine plantations. In temperate China, there are also large areas of natural pine forests, where *P. tabulaeformis* grows with a mixture of various broadleaved species. We speculate that soil respiration responses to organic carbon manipulation in natural forests would be different from that in plantations due to variations in the amount and quality of organic carbon and microclimatic conditions. To clarify the differences between natural forests and plantations would be critical to the management of regional carbon fluxes of *P. tabulaeformis* ecosystems. Further studies focusing on natural forests of *P. tabulaeformis* are therefore needed. In addition, as the responses of soil respiration to organic matter manipulation may vary over time, increasing long-term and year-round measurements over time should be given more attention in future studies.

## Acknowledgments

The authors thank all those who provided helpful suggestions and critical comments on the original version of the manuscript and anonymous reviewers. We gratefully acknowledge the

support from the Taiyue Forestry Bureau for field monitoring and sampling. We also thank Huashan Li, Xing Liu, and Na Wang for help with laboratory and field measurements. The English language was revised by Prof. Tom Hazenberg.

## Author Contributions

Conceived and designed the experiments: JF JSW XHZ. Performed the experiments: JF JSW BZ LHW CYZ XHZ KG. Analyzed the data: JF JSW. Contributed reagents/materials/analysis tools: JF JSW LHW CYZ KG. Wrote the paper: JF JSW.

## References

1. Schlesinger WH. Biogeochemistry: an analysis of global change. 2nd ed. San Diego: Academic Press; 1997. pp. 156.
2. Goodale CL, Apps MJ, Birdsey RA, Field CB, Heath LS, Houghton RA, et al. Forest carbon sinks in the northern hemisphere. *Ecol Appl*. 2002; 12: 891–899.
3. Buchmann N. Biotic and abiotic factors controlling soil respiration rates in *Picea abies* stands. *Soil Biol Biochem*. 2000; 32: 1625–1635.
4. Hartley IP, Armstrong AF, Murthy R, Barron-Gafford GA, Ineson P, Atkin OK. The dependence of respiration on photosynthetic substrate supply and temperature: integrating leaf, soil and ecosystem measurements. *Global Change Biol*. 2006; 12: 1954–1968.
5. Scott-Denton LE, Rosenstiel TN, Monson RK. Differential controls by climate and substrate over the heterotrophic and rhizospheric components of soil respiration. *Global Change Biol*. 2006; 12: 205–216.
6. Wan SQ, Luo YQ. Substrate regulation of soil respiration in a tallgrass prairie: Result of a clipping and shading experiment. *Global Biogeochem Cy*. 2003; 17: 1–12.
7. Kuzyakov Y, Gavrichkova O. Time lag between photosynthesis and carbon dioxide efflux from soil: a review. *Global Change Biol*. 2010; 16: 3386–3406.
8. King JS, Kubiske ME, Pregitzer KS, Hendrey GR, McDonald EP, Giardina CP, et al. Tropospheric O<sub>3</sub> compromises net primary production in young stands of trembling aspen, paper birch and sugar maple in response to elevated atmospheric CO<sub>2</sub>. *New Phytol*. 2005; 168: 623–636. PMID: [16313645](#)
9. Xia JY, Wan SQ. Global response patterns of terrestrial plant species to nitrogen addition. *New Phytol*. 2008; 179: 428–439. doi: [10.1111/j.1469-8137.2008.02488.x](#) PMID: [19086179](#)
10. Liu LL, King JS, Giardina CP. Effects of elevated concentrations of atmospheric CO<sub>2</sub> and tropospheric O<sub>3</sub> on leaf litter production and chemistry in trembling aspen and paper birch communities. *Tree Physiol*. 2005; 25: 1511–1522. PMID: [16137937](#)
11. Zhao M, Running SW. Drought-induced reduction in global terrestrial net primary production from 2000 through 2009. *Science*. 2010; 329: 940–943. doi: [10.1126/science.1192666](#) PMID: [20724633](#)
12. Ostertag R, Scatena FN, Silver WL. Forest floor decomposition following hurricane litter inputs in several Puerto Rican forests. *Ecosystems*. 2003; 6: 261–273.
13. Chapman SK, Hart SC, Cobb NS, Whitham TG, Koch GW. Insect herbivory increases litter quality and decomposition: an extension of the acceleration hypothesis. *Ecology*. 2003; 84: 2867–2876.
14. Wardle DA, Hornberg G, Zackrisson O, Kalela-Brundin M, Coomes DA. Long-term effects of wildfire on ecosystem properties across an island area gradient. *Science*. 2003; 300: 972–975. PMID: [12738863](#)
15. Matsushima M, Chang SX. Effects of understory removal, N fertilization, and litter layer removal on soil N cycling in a 13-year-old white spruce plantation infested with Canada bluejoint grass. *Plant Soil*. 2007; 292: 243–258.
16. Xiong YM, Xia HX, Li ZA, Cai XA, Fu SL. Impacts of litter understory removal on soil properties in a subtropical *Acacia mangium* plantation in China. *Plant Soil*. 2008; 304: 179–188.
17. Wang XL, Zhao J, Wu JP, Chen H, Lin YB, Zhou LX, et al. Impacts of understory species removal and/or addition on soil respiration in a mixed forest plantation with native species in southern China. *Forest Ecol Manag*. 2011; 261: 1053–1060.
18. Zhao J, Wang XL, Shao YH, Xu GL, Fu SL. Effects of vegetation removal on soil properties and decomposer organisms. *Soil Biol Biochem*. 2011; 43: 954–960.
19. Xu S, Liu LL, Sayer EJ. Variability of above-ground litter inputs alters soil physicochemical and biological processes: a meta-analysis of litterfall-manipulation experiments. *Biogeosciences*. 2013; 10: 7423–7433.

20. Sayer EJ, Heard MS, Grant HK, Marthews TR, Tanner EVJ. Soil carbon release enhanced by increased tropical forest litterfall. *Nature*. 2011; 1: 304–307.
21. Li YQ, Xu M, Sun OJ, Cui WC. Effects of root and litter exclusion on soil CO<sub>2</sub> efflux and microbial biomass in wet tropical forests. *Soil Biol Biochem*. 2004; 36: 2111–2114.
22. Sulzman EW, Brant JB, Bowden RD, Lajtha K. Contribution of aboveground litter, belowground litter, and rhizosphere respiration to total soil CO<sub>2</sub> efflux in an old growth coniferous forest. *Biogeochemistry*. 2005; 73: 231–256.
23. Sayer EJ, Powers JS, Tanner EVJ. Increased litterfall in tropical forests boosts the transfer of soil CO<sub>2</sub> to the atmosphere. *PLOS ONE*. 2007; 2: e1299. PMID: [18074023](#)
24. Schaefer DA, Feng WT, Zou XM. Plant carbon inputs and environmental factors strongly affect soil respiration in a subtropical forest of southwestern China. *Soil Biol Biochem*. 2009; 41: 1000–1007.
25. Crow SE, Lajtha K, Bowden RD, Yano Y, Brant JB, Caldwell BA, et al. Increased coniferous needle inputs accelerate decomposition of soil carbon in an old-growth forest. *Forest Ecol Manag*. 2009; 258: 2224–2232.
26. Kuzyakov Y. Review: factors affecting rhizosphere priming effects. *J Plant Nutr Soil Sc*. 2002; 165: 382.
27. Gottlicher SG, Steinmann K, Betson NR, Högberg P. The dependence of soil microbial activity on recent photosynthate from trees. *Plant Soil*. 2006; 287: 85–94.
28. Bertin C, Yang XH, Weston LA. The role of root exudates and allelochemicals in the rhizosphere. *Plant Soil*. 2003; 256: 67–83.
29. Högberg P, Nordgren A, Buchmann N, Taylor AFS, Ekblad A, Högberg MN, et al. Large-scale forest girdling shows that current photosynthesis drives soil respiration. *Nature*. 2001; 411: 789–791. PMID: [11459055](#)
30. Hanson PJ, Edwards NT, Graten CT, Andrews JA. Separating root and soil microbial contributions to soil respiration: a review of methods and observations. *Biogeochemistry*. 2000; 48: 115–146.
31. Pruden G, Powlson DS, Jenkinson DS. The measurement of <sup>15</sup>N in soil and plant material. *Nutr Cycl Agroecosys*. 1985; 6: 205–218.
32. Nanjing Agricultural University. *Soil and Agricultural Chemistry Analysis*. Beijing: China Agriculture Press; 1992. pp 29–33.
33. Kalembasa SJ, Jenkinson DS. A comparative study of titrimetric and gravimetric methods for the determination of organic carbon in soil. *J Sci Food Agr*. 1973; 24: 1085–1090.
34. Lloyd J, Taylor JA. On the temperature dependence of soil respiration. *Funct Ecol*. 1994; 8: 315–323.
35. Rey A, Pegoraro E, Tedeschi V, De Parri I, Jarvis PG, Valentini R. Annual variation in soil respiration and its components in a coppice oak forest in central Italy. *Global Change Biol*. 2002; 8: 851–866.
36. Vance ED, Brookes PC, Jenkinson DS. An extraction method for measure microbial biomass. *Soil Biol Biochem*. 1987; 19: 703–707.
37. Ginter DL, Mcleod KW, Sherrod C. Water stress in longleaf pine induced by litter removal. *Forest Ecol Manag*. 1979; 2: 13–20.
38. MacKinney AL. Effects of forest litter on soil temperature and soil freezing in autumn and winter. *Ecology*. 1929; 10: 312–321.
39. Leff JW, Wieder WR, Taylor PG, Townsend AR, Nemergut DR, Grandy AS, et al. Experimental litterfall manipulation drives large and rapid changes in soil carbon cycling in a wet tropical forest. *Global Change Biol*. 2012; 18: 2969–2979. doi: [10.1111/j.1365-2486.2012.02749.x](#) PMID: [24501071](#)
40. Feng WT, Zou XM, Schaefer D. Above- and belowground carbon inputs affect seasonal variations of soil microbial biomass in a subtropical monsoon forest of southwest China. *Soil Biol Biochem*. 2009; 41: 978–983.
41. Kuzyakov Y. Source of CO<sub>2</sub> efflux from soil and review of partitioning methods. *Soil Biol Biochem*. 2006; 38: 425–448.
42. Davidson EA, Belk E, Boone RD. Soil water content and temperature as independent of confounded factors controlling soil respiration in a temperate mixed hardwood forest. *Global Change Biol*. 1998; 4: 217–227.
43. Ohashi M, Gyokusen K, Saito A. Measurement of carbon dioxide evolution from a Japanese cedar (*Cryptomeria japonica* D. Don) forest floor using an open-flow chamber method. *Forest Ecol Manag*. 1999; 123: 105–114.
44. Lee MS, Nakane K, Nakatsubo T, Koizumi H. Seasonal changes in the contribution of root respiration to total soil respiration in a cool-temperate deciduous forest. *Plant Soil*. 2003; 255: 31–318.

45. Prévost-Bouré NC, Soudani K, Damesin C, Berveiller D, Lata J-C, Dufrêne E. Increase in aboveground fresh litter quantity over-stimulates soil respiration in a temperate deciduous forest. *Appl Soil Ecol.* 2011; 46: 26–34.
46. Sayer EJ. Using experimental manipulation to assess the roles of leaf litter in the functioning of forest ecosystems. *Biol Rev Camb Phil.* 2006; 81: 1–31.
47. Yu XX, Zha TS, Pang Z, Wu B, Wang XP, Chen GP, et al. Response of soil respiration to soil temperature and moisture in a 50-year-old oriental arborvitae plantation in China. *PLOS ONE.* 2011; 12: 1–7.
48. Bååth E, Wallander H. Soil and rhizosphere microorganisms have the same  $Q_{10}$  for respiration in a model system. *Global Change Biol.* 2003; 9: 1788–1791.
49. Raich JW, Schlesinger WH. The global carbon-dioxide flux in soil respiration and its relationship to vegetation and climate. *Tellus B.* 1992; 44: 81–99.
50. Knorr W, Prentice IC, House JI, Holland EA. Long-term sensitivity of soil carbon turnover to warming. *Nature.* 2005; 433: 298–301. PMID: [15662420](https://pubmed.ncbi.nlm.nih.gov/15662420/)