

Aboveground and belowground litter have equal contributions to soil CO₂ emission: an evidence from a 4-year measurement in a subtropical forest

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Abstract

Aims Changing quantity and quality of plant C input to soils under global change influences soil C cycling in terrestrial ecosystems. However, how soil CO₂ emission responds to changes in C input remains poorly understood.

Methods A detritus input and removal experiment was conducted in a subtropical forest (*Cunninghamia lanceolata*) to investigate how aboveground and belowground litters affect soil respiration (R_{Soil}). In this

experiment, four treatments with three replicates were included, as follows: control (CK), litter removal (NL), root trenching (NR), and no C-input (i.e. litter removal combined with root trenching, NI). R_{Soil} was measured for 4 years from 2011 to 2014.

Results The mean annual CO₂ effluxes in the NL, NR, and NI plots were lower (23.4%, 24.9%, and 38.8%, respectively) than those in the CK plots, suggesting that decreasing C input significantly decreased R_{Soil} . Soil heterotrophic respiration (soil organic matter decomposed by microorganisms) accounted for 52.1% of R_{Soil} , and aboveground recent litter decomposition and belowground autotrophic respiration (live roots and associated microorganism) contributed to 23.7% and 24.2% of R_{Soil} , respectively. R_{Soil} was significantly and positively correlated with soil temperature, but negatively correlated with volumetric soil moisture. The variation in R_{Soil} was more explained by soil temperature than soil moisture, and the responses of R_{Soil} to soil temperature and moisture were altered by C input manipulation. Annual R_{Soil} rate was strongly and negatively related to the Gram-positive bacteria concentration and the ratio of Gram-positive to -negative bacteria.

Conclusion This study highlighted that aboveground recent leaf litter and belowground live roots and associated microbes had similar contributions to R_{Soil} , and soil temperature and Gram-positive bacteria were the dominant factors controlling R_{Soil} in a subtropical forest.

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Introduction

As the primary pathway of CO₂ emission from terrestrial ecosystems to atmosphere, soil respiration (R_{Soil}) is estimated to be 80–100 Pg C yr⁻¹ (Bond-Lamberty and Thomson 2010), which is approximately ten-fold greater than CO₂ emissions from fossil fuel combustion. Thus, even a small change in R_{Soil} will greatly influence the soil C stock in terrestrial ecosystems and the atmospheric CO₂ concentration. R_{Soil} is controlled by inputs of aboveground (e.g. leaf litter) and belowground (e.g. roots) C into soil (Li et al. 2004; Wang et al. 2013). These C inputs will be changed by increases in atmospheric CO₂ concentration, nitrogen deposition, and extreme climate events (Xia and Wan 2008; Leff et al. 2012; Wu et al. 2014), but it is unclear to which extent this change in plant-C inputs affects R_{Soil} . Therefore, understanding how R_{Soil} responds to changes in C inputs is crucial to accurately predict soil CO₂ efflux under global change.

Aboveground leaf litter and belowground roots with high turnover rates are two of the most dynamic components of C cycling in forest ecosystems (Trumbore 2000). However, there is no consistent conclusion on contributions of them to soil CO₂ efflux. For example, Rey et al. (2002) reported that root respiration (belowground autotrophic respiration, R_{BA}) contributed to 55% of R_{Soil} in a coppiced oak forest, but this contribution was only 17.6% in a *Pinus tabulaeformis* plantation (Fan et al. 2015), and some studies found that the contributions of aboveground leaf litter decomposition (R_{AL}) and R_{BA} to R_{Soil} were similar in a subtropical coniferous forest (Wang et al. 2013). This inconsistency may stem from considerable differences in quantity of leaf litter and roots under various forest types (Allison and Vitousek 2004; Li et al. 2004; Wang et al. 2007). Moreover, different duration of measurement also leads to various values of the contribution of leaf litter and roots to R_{Soil} because their contributions have large inter-annual variations (Sulzman et al. 2005). Therefore, long-term measurement is needed to accurately quantify the roles of leaf litter and roots in regulating R_{Soil} in forest ecosystems, which is also critical to understanding soil C cycling in the global change context.

Besides the quantity of plant-C inputs, changes in soil temperature and moisture induced by C-input manipulation also affect the contributions of leaf litter and root to R_{Soil} because soil temperature and moisture affect R_{Soil} and its components (Sayer and Tanner 2010; Suseela et al. 2012; Wang et al. 2016). However,

the current knowledge on this topic is limited, although Wang et al. (2013) found that C-input manipulation had greater effects on the response of R_{Soil} to temperature during the dormant season than during the growing season in a subtropical forest. This influence is likely related to changes in soil microbial activity and community composition that regulate key processes of soil C cycling (Wan et al. 2014). Soil microbial community composition is affected by litter quantity and quality, and mediates the response of R_{Soil} to litter change (Wang et al. 2014; Creamer et al. 2015). For example, root and litter exclusion altered the bacterial community structure and reduced fungal biomass in mineral soil from old-growth Douglas-fir (*Pseudotsuga menziesii*)—western hemlock (*Tsuga heterophylla*) forest (Brant et al. 2006). In an incubation experiment, Wang et al. (2014) found that litter addition increased bacterial and fungal biomass measured by phospholipid fatty acids (PLFAs), but decreased the ratio of bacteria to fungi. However, the effects of the differences in soil microbial community composition on response of R_{Soil} to C-input manipulation remain poorly quantified.

Detritus Input and Removal Treatment (DIRT) experiments provide an excellent opportunity to examine the contributions of aboveground leaf litter and belowground roots to R_{Soil} through alterations in plant-C inputs to soils, and many efforts have been made (Sulzman et al. 2005; Sayer et al. 2011; Fekete et al. 2014; Huang and Spohn 2015). However, most of the DIRT sites have been located in temperate forests, whereas tropical and subtropical regions are underrepresented. Furthermore, in tropical and subtropical forests soil C cycling is more sensitive to variations in C inputs than in temperate forest ecosystems (Xu et al. 2013). Therefore, we established a DIRT experiment in a subtropical coniferous forest to investigate the influence of C inputs on soil C cycling. This study aimed to: 1) examine how R_{Soil} responds to changes in soil C inputs and quantify the contributions of leaf litter and roots to soil CO₂ efflux through a 4-year experiment, and 2) quantify the relationship between R_{Soil} , soil temperature, moisture and microbial community composition.

Materials and methods

Site description

The study was conducted at the Huitong National Research Station of Forest Ecosystem (26°40'N, 109°26'E)

in Hunan Province, subtropical China. The altitude ranges from 300 to 1000 m above sea level. Soils with 60–80 cm depth were developed from grayish-green slate parent materials with moderate weathering, and belong to Ultisol according to the second edition of the U.S. Soil Taxonomy. The sand, silt, and clay contents were about 11.4%, 44.8%, and 43.8%, respectively. This region is characterized by its humid mid-subtropical monsoon climate (warm, wet season: April–November, and cool, dry season: December–March), with a mean annual temperature of 16.5 °C and precipitation of 1200 mm over 20 years (1992–2012). A mean minimum temperature of 1.9 °C is detected in January and a mean maximum temperature of 29.0 °C occurs in July. *Cunninghamia lanceolata* is the dominant tree species at this site, and the site productivity is approximate 9.18 Mg ha⁻¹ y⁻¹. The dominant understory vegetation species at the site are *Rubus rosifolius*, *Pteridium aquilinum*, *Maesa japonica*, *Parathelypteris chinensis*, and *Microlepia marginata*.

Experiment design

The study site at 450 m above sea level is part of the long-term intersite DIRT project, and the experiment was established according to the design in the H.J. Andrews Experimental Forest, Oregon (Nadelhoffer et al. 2004). The current DIRT experiment was established in April 2010 in a 23-year-old pure *C. lanceolata* stand situated on a 23° south-facing slope, and included four treatments: control (CK), litter removal (NL), root trenching (NR), and no input (NI). Each treated plot was typically 3 m × 3 m, although some plots had small deviations in size because of the presence of spaces or obstacles. The annual amount of litterfall in this forest stand was approximate 4.9 Mg ha⁻¹, which was measured by 20 collection crates with the size of 1 m². The experiment was laid out in three replicate blocks, and each block was divided into four treated plots with the four treatments randomly placed. In each block, the four plots had similar slope and exposure. In the NL and NI plots, the standing litter with different stages of decomposition was removed carefully, but decomposed litter that was potentially unidentifiable and/or too small to remove was left on the soil surface. Furthermore, fresh litter in these plots was excluded with 1 mm mesh screens placed 0.5 m above the ground, and was removed monthly. In the NR and NI plots, the roots were excluded by trenching to 60 cm depth, sufficient to exclude all roots. A 0.35 mm-

thick polyethylene sheet was inserted as root barriers around the trenched plots. The details for treatments have been described in Wang et al. (2013). New vegetation was removed monthly by hand from the NR and NI plots. Therefore, we assumed that no roots grew into the trenched plots during the experiment.

Soil respiration measurement

To measure R_{Soil}, three polyvinyl chloride (PVC) collars, each with an inner diameter of 10.4 cm and height of 8 cm, were permanently installed in each plot in December 2010. The PVC collars were pushed 5 cm into the mineral soil to anchor the R_{Soil} chamber. R_{Soil} was measured using a Li-Cor 8100 infrared gas analyzer (Li-Cor Inc., Lincoln, NE, USA). To minimize the effect of soil disturbance and decomposition of dead fine roots caused by trenching, R_{Soil} measurement began in January 2011 (9 months post-trenching), and lasted for 4 years. For each measurement, the soil respiration chamber was placed on each PVC collar; the measurement was repeated three times for each collar and the average of the three data was as the R_{Soil} for this PVC collar. Annual amount of respired CO₂ was the sum of monthly CO₂ flux from January to December every year, and the monthly CO₂ flux was calculated as multiplying monthly rate of R_{Soil} by the number of days in this month (Fan et al. 2014). During each R_{Soil} measurement, soil temperatures at depths of 5 cm were simultaneously measured using a Li-cor thermocouple. Volumetric soil water content was measured using a moisture probe (Delta-T Devices, Cambridge, UK) near the collars.

Soil sampling and analysis

Mineral soils (0–10 cm) were collected in July 2013 and July 2014 after removing the litter layer. In each 3 m × 3 m plot, nine soil cores (diameter = 25 mm) were randomly taken and then mixed to a composite soil sample. Soil samples were immediately taken to the laboratory and sieved through a 2 mm mesh. Roots and stones were manually removed. One part of the soil samples was air-dried in the laboratory for determining soil chemical properties; one part was stored at 3 °C for the determination of soil microbial biomass C (MBC) and dissolved organic C (DOC). The other parts were freeze-dried for measuring soil microbial community by PLFAs.

Soil organic C and total N contents were measured from the sieved composite sample using an elemental

analyzer (Elementar, Germany). Soil pH was measured with a pH meter (PB-10C, Sartorius, Germany) from soil slurry with 1:2.5 ratio (weight:volume). Soil $\text{NH}_4^+\text{-N}$ was extracted using 2 mol L^{-1} KCl solution and determined by colorimetry. Available soil P was analyzed colorimetrically through molybdate blue method after the soil was extracted with 1 mol L^{-1} NH_4F solution. Soil MBC was determined through fumigation extraction method, and organic C in the K_2SO_4 extracts was analyzed using a total C analyzer (Shimadzu model TOC-500, Kyoto, Japan). MBC was calculated according to Wu et al. (1990): $\text{MBC} = K_{\text{EC}} \times 2.2$, where K_{EC} is the difference between C extracted from fumigated and unfumigated soils. Organic C extracted with 0.5 M K_2SO_4 from soils without chloroform fumigation was termed as DOC.

The soil microbial community composition was assessed using PLFAs. A total of 5.0 g of soil sample was extracted for 2 h by using 19 mL of chloroform–methanol–phosphate buffer (1:2:0.8). The chloroform layer was decanted and dried under N_2 at 30 °C. The extracts were sequentially fractionated into neutral lipids, glycolipids, and phospholipids with chloroform, acetone, and methanol by using silica gel-filled solid-phase extraction cartridges (0.50 g Si). The soil samples were then subjected to mild alkaline methanolysis by dissolution in 1 mL of methanol–toluene mixture (1:1) and 1 mL of 0.2 M L^{-1} KOH and then heating at 37 °C for 15 min. Subsequently, 2 mL of H_2O and 0.3 mL of 1.0 M L^{-1} acetic acid were added. The resulting fatty acid methyl esters were separated, identified, and quantified with an Agilent 7890 gas chromatograph, which is equipped with a flame ionization detector and an Ultra-2 column. The fatty acid methyl ester Mix and BAME (Bacterial Acid Methyl Esters CP Mix, Sigma-Aldrich) controls were used to recognize peaks. Methyl nonadecanoate (19:0) was added as the internal standard for quantifying the PLFAs. The fatty acids used as biomarkers for soil microbial groups are listed in Table 1.

Calculation and statistical analysis

When calculating for the components of R_{Soil} , we assumed that there was no disturbance of trenching and that decomposition of severed roots in the trenched plots had no contribution to soil CO_2 efflux 9 months post-trenching. Soil respiration includes three components: R_{AL} (the CO_2 from recent leaf litter decomposition), R_{BA} (belowground autotrophic respiration, i.e., live roots and associated microorganism), and R_{H} (heterotrophic respiration, i.e., soil

Table 1 Fatty acids used in the analysis of microbial community composition in the study (Hill et al. 2000)

Microbial groups	Diagnostic fatty acids
Bacteria	i14:0; i15:0; a15:0; i16:0; 16:1 ω 7t; i17:0; a17:0; 18:1 ω 7c; cy19:0
Fungi	18:1 ω 9; 18:2 ω 6,9
Gram-positive bacteria	i14:0; i15:0; a15:0; i16:0; i17:0; a17:0
Gram-negative bacteria	16:1 ω 7t; 17:1 ω 8c; 18:1 ω 7c; cy17:0; cy19:0

The prefixes “a” and “i” indicate antiso- and iso-branching, respectively, and “cy” indicates a cyclopropane fatty acid

organic matter decomposed by microorganisms). SOM is generally consisting of not readily identifiable plant residues at various stages of decomposition in soil matrix, cells and tissues of soil organisms, and substances synthesized by soil organisms. The components of R_{Soil} were calculated as follows (Wang et al. 2013):

$$R_{\text{AL}} = \text{Respiration in CK plots} - \text{Respiration in NL plots} \quad (1)$$

$$R_{\text{BA}} = \text{Respiration in CK plots} - \text{Respiration in NR plots} \quad (2)$$

$$R_{\text{H}} = \text{Respiration in CK plots} - R_{\text{AL}} - R_{\text{BA}} \quad (3)$$

A univariate model was used to quantify the relationship between R_{Soil} and soil temperature (Eq. 4). The relationship between R_{Soil} and soil moisture was described by a simple linear regression equation (Eq. 5), as follows:

$$R_{\text{Soil}} = ae^{bT} \quad (4)$$

$$R_{\text{Soil}} = \beta_1 W + \beta_2 \quad (5)$$

The synergistic relationship of R_{Soil} with soil temperature and moisture was described by a bivariate model (Eq. 6), as follows:

$$R_{\text{Soil}} = ae^{bT} W^c \quad (6)$$

where RS ($\mu\text{mol m}^{-2} \text{s}^{-1}$) is soil respiration; T (°C) is soil temperature at 5 cm depth; W (%) is soil moisture; and a , b , and c are model parameters to be estimated. Temperature sensitivity (Q_{10}) of R_{Soil} was calculated using Eq. 7, as follows:

$$Q_{10} = e^{10b} \quad (7)$$

where Q_{10} is the response of R_{Soil} to temperature; and b is the model parameter to be estimated in Eqs. 4 and 6.

The data were statistically analyzed using SPSS version 19.0 for Windows. A repeated measures ANOVA was used to test the effect of C input manipulation on R_{Soil} , R_{AL} , R_{BA} , R_{H} , soil temperature, and moisture from January 2011 to December 2014; followed by the Tukey's HSD test to reveal differences among treatments. One-way ANOVA with Tukey's HSD was used to test the differences among treatments in soil chemical parameters, MBC, DOC, and PLFAs, as well as the annual means of R_{Soil} , R_{AL} , R_{BA} , and R_{H} , and their contributions to R_{Soil} . Univariate models and a bivariate model were used to examine the relationships of R_{Soil} with soil temperature and moisture. Two-way ANOVA was employed to examine the effect of soil temperature and moisture on R_{Soil} . Type III sums of squares were used to assess the significance of main effects and interactions. The relationships among annual R_{Soil} rate, soil chemical variables, and PLFAs were further examined using Pearson linear regression model. Statistically, significant differences were set at P values <0.05 , unless otherwise stated.

Results

Changes in soil biochemical properties

Soil temperature and moisture at 5 cm depth had large seasonal and annual variations from 2011 to 2014 (Fig. 1). The highest soil temperatures occurred in July or August, and lowest in December or January; whereas the lowest soil moisture generally occurred in August or September. The annual soil temperature was highest in 2013 and lowest in 2014; while annual soil moisture was lowest in 2011 and highest in 2014. Soil temperature and moisture were not affected by C-input manipulation. Litter exclusion significantly decreased DOC concentration, and root trenching or no C-input decreased soil $\text{NH}_4^+\text{-N}$ concentration (Table 2).

Soil microbial community was significantly altered by C-input manipulation, but had temporal effects (Table 3). In 2013, bacterial, fungal, and Gram-negative bacterial PLFAs in the NI plots were lower than those in the NR plots, but did not significantly differ from those in the CK plots. In 2014, compared with CK, litter removal increased the abundance of bacteria, fungi, Gram-positive, and Gram-negative bacteria. Lower Gram-negative bacterial PLFAs in the NR and NI plots resulted in a higher

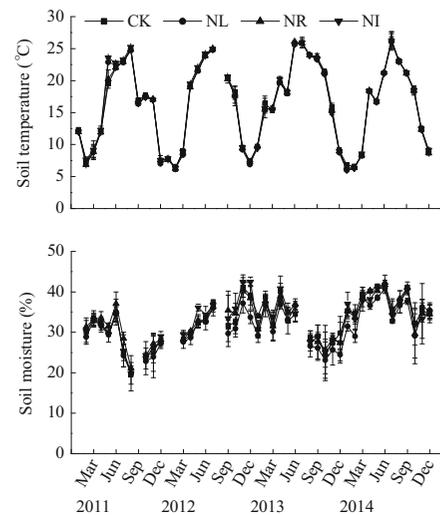


Fig. 1 Seasonal dynamics of soil temperature and moisture in the DIRT experiment from 2011 to 2014. CK, control; NL, leaf litter remove; NR, root trenching; NI, leaf litter remove and root trenching

ratio of Gram-positive to Gram-negative bacteria. In NI plots, decline in bacterial PLFAs also led to reduced ratios of fungi to bacteria.

Soil respiration

R_{Soil} had similar seasonal patterns across the different treatments, with lower values in the cold season (December–March) than in the warm season (April–November) (Fig. 2). The peak values and the mean rate of R_{Soil} in 2012 and 2014 were significantly lower than those in 2011 and 2013. Decreasing plant-C inputs significantly reduced R_{Soil} , but the reduction in R_{Soil} varied across the four years. Compared with the CK plots, the decline of R_{Soil} in the NL plots varied from 21.3% in 2014 to 25.6% in 2013, in the NR plots from 19.5% in 2014 to 32.4% in 2013, and in the NI plots from 35.1% in 2011 to 43.8% in 2013.

The 4-year mean annual amount of respired CO_2 ranged from $291.3 \text{ g C m}^{-2} \text{ y}^{-1}$ in the NI plots to $476.0 \text{ g C m}^{-2} \text{ y}^{-1}$ in the CK plots (Fig. 3). The mean CO_2 efflux was 23.4%, 24.9%, and 38.8% lower in the NL, NR, and NI plots than in the CK plots, respectively, thereby indicating that effects of leaf litter and roots on R_{Soil} was negatively non-additive. The lowest annual CO_2 efflux for each treatment occurred in 2014 and the higher values in 2011 and 2013 indicate that the CO_2 efflux had large inter-annual variations.

R_{AL} and R_{BA} had similar seasonal patterns, but partly differed from those of R_{H} that had large temporal

Table 2 Surface soil (0–10 cm) biochemical properties in 2014

	SOC	Total N	MBC	DOC	pH	NH ₄ ⁺ -N	Available P
CK	15.93 ± 1.26a	1.41 ± 0.09a	166.9 ± 11.8a	436.7 ± 22.9a	4.24 ± 0.14a	9.30 ± 0.49a	1.78 ± 0.30a
NL	15.86 ± 1.33a	1.45 ± 0.23a	183.3 ± 7.5a	379.1 ± 18.9b	4.28 ± 0.15a	7.71 ± 1.09ab	1.40 ± 0.22a
NR	16.21 ± 0.86a	1.53 ± 0.08a	161.4 ± 17.6a	404.9 ± 15.1a	4.31 ± 0.20a	6.67 ± 1.23b	1.69 ± 0.24a
NI	14.91 ± 0.36a	1.39 ± 0.08a	167.0 ± 9.3a	383.3 ± 32.5ab	4.29 ± 0.15a	7.18 ± 1.03b	1.49 ± 0.18a

CK, control; NL, leaf litter removal; NR, root trenching; NI, leaf litter removal and root trenching

fluctuations (Fig. 4). R_H was the major component of R_{Soil} and accounted for 52.1% of R_{Soil} (Table 4). R_{AL} and R_{BA} had similar contributions to R_{Soil} , accounting for 23.7% and 24.2%, respectively. These contributions had large annual variations throughout the 4-year period. The R_{BA} contribution ranged from 19.5% in to 31.8% in 2013, the R_{AL} contribution varied between 19.3% and 26.3%, and the R_H contribution varied from 41.9% in 2013 to 60.0% in 2014.

Relationship between soil respiration and biochemical property

Univariate and bivariate models were used to assess the relationships between R_{Soil} and soil temperature and moisture (Table 5). R_{Soil} was significantly positively correlated with soil temperature, and negatively correlated with soil moisture. Soil temperature explained 37.4%–79.0% of variation in R_{Soil} and soil moisture explained 0.7%–12.8% in the univariate models. The variation explained by soil temperature was greater during the cold season than during the warm season, whereas the opposite was observed for soil moisture. Variations explained by soil temperature were higher in the CK plots, followed by NR, NL, and NI treatments. Both on an annual basis and

during the warm season, variations in R_{Soil} explained by soil moisture were higher in the CK plots and lower in the NL plots. In the bivariate model, soil temperature and moisture together explained 41.6%–85.4% of variation in R_{Soil} , highest in the CK plots and lowest in the NI plots during whole year and warm seasons, while the explained variation was highest in the NR plots and lowest in the NL plots during the cold season.

The correlation analysis demonstrated that annual R_{Soil} rate was strongly and positively related to the content of SOC, DOC, and NH₄⁺-N (Table 6). However, R_{Soil} rate was negatively related to the Gram-positive bacterial concentration and the ratio of Gram-positive to Gram-negative bacteria (Fig. 5). Other soil properties including bacterial and fungal PLFAs were not correlated with the annual R_{Soil} rate.

Discussion

Influence of C-input manipulation on soil respiration

Across the 4-year C-input manipulation in the *C. lanceolata* forest, a significant decrease in R_{Soil} following litter removal and root trenching indicates that

Table 3 Concentrations of microbial PLFAs and two ratios in different groups in the DIRT experiment in July 2013 and 2014

	2013				2014			
	CK	NL	NR	NI	CK	NL	NR	NI
Bacteria	25.93 ± 2.13ab	25.49 ± 1.36ab	19.85 ± 1.03b	30.69 ± 3.91a	28.6 ± 0.9b	39.0 ± 1.2a	27.9 ± 1.2bc	23.7 ± 1.7c
Fungi	7.24 ± 1.05a	6.71 ± 0.63a	4.85 ± 0.35b	8.93 ± 0.93a	8.9 ± 0.4b	13.4 ± 0.5a	8.2 ± 0.3b	8.4 ± 0.9b
Fungi: Bacteria	0.28 ± 0.02a	0.26 ± 0.03a	0.24 ± 0.01a	0.30 ± 0.06a	0.31 ± 0.01bc	0.34 ± 0.02c	0.30 ± 0.003b	0.35 ± 0.01a
G+	6.85 ± 0.48a	7.23 ± 0.55a	7.34 ± 1.00a	8.09 ± 0.78a	11.8 ± 1.0b	15.7 ± 0.8a	12.9 ± 0.5b	10.7 ± 0.5b
G-	10.78 ± 1.76a	10.21 ± 1.11a	6.84 ± 0.45b	13.77 ± 13.78a	9.1 ± 0.2b	11.6 ± 0.2a	7.82 ± 0.29c	5.9 ± 0.54d
G+:G-	0.66 ± 0.15a	0.71 ± 0.05a	1.08 ± 0.28a	0.61 ± 0.14a	1.3 ± 0.1b	1.4 ± 0.04bc	1.7 ± 0.01 ac	1.8 ± 0.1a

Values are expressed as mean ± SD. G+ and G- indicate Gram-positive and Gram-negative bacteria, respectively

Different letters following the data in the same column at the same year denote significance at $P = 0.05$ level

CK, control; NL, leaf litter removal; NR, root trenching; NI, leaf litter removal and root trenching

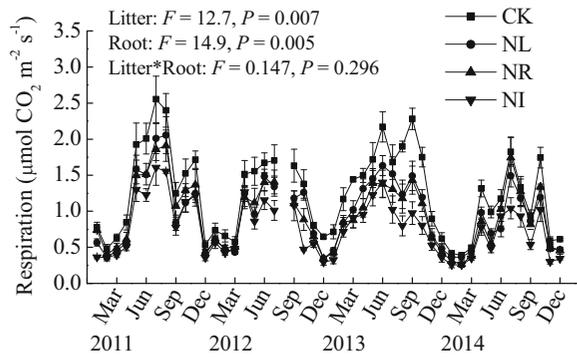


Fig. 2 Seasonal dynamics of soil respiration in the DIRT experiment from 2011 to 2014. CK, control; NL, leaf litter removal; NR, root trenching; NI, leaf litter removal and root trenching

R_{Soil} is sensitive to variations in leaf litter and root inputs in this subtropical forest. Here, we should note that in our DIRT experiment all aboveground litters excluding unidentifiable and small fractions have been removed in NL and NI treatments when conducting this experiment. This may overrate our estimate of the relative importance of R_{AL} for total R_{Soil} in comparison with many previous studies as they only removed fresh litter. Meanwhile, the degree of R_{Soil} reduction induced by litter removal was in agreement with previous observations by Sulzman et al. (2005) Subke et al. (2006), and Sayer and Tanner (2010), and lower than that reported by Li et al. (2004) who found that R_{Soil} decreased by 54% in a *P. caribaea* plantation and 68% in a tropical secondary forest. In a Central-European deciduous forest, however, Fekete et al. (2014) found that annually, detritus treatments had no significant effects on R_{Soil} . The large variations in R_{Soil} reduction among the different studies can be attributed to

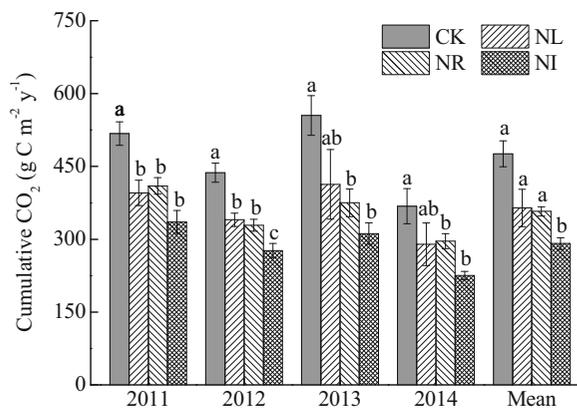


Fig. 3 Annual soil CO_2 efflux and the mean across 4 years of the DIRT experiment

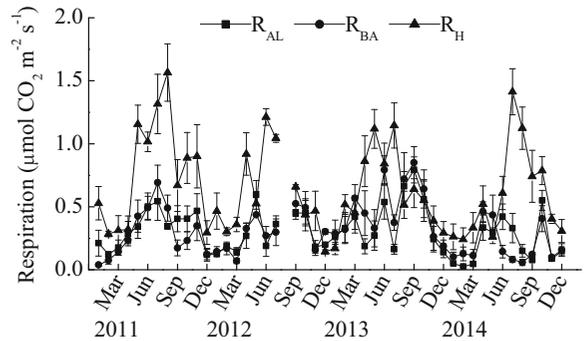


Fig. 4 Seasonal dynamics of aboveground leaf litter decomposition (R_{AL}), belowground autotrophic respiration (live roots and associated microorganism, R_{BA}), and heterotrophic respiration (soil organic matter decomposed by microorganisms, R_H) in the DIRT experiment from 2011 to 2014

differences in production and turnover of leaf litter and roots in forest ecosystems with different species and ages (Allison and Vitousek 2004; Li et al. 2004; Wang et al. 2007). The varying reduction in R_{Soil} can also be partly attributed to the differences in the temporal sampling scheme and time gaps after litter removals due to underlying large temporal variations (Sulzman et al. 2005; Wang et al. 2013) and effects of DIRT treatments on soil C and nutrient availability may vary over time (data not shown). In our experiment, R_{Soil} was measured 9 months after experimental treatments were applied, whereas Li et al. (2004) measured the R_{Soil} 6 years after their treatments. Therefore, influences of plant-C inputs on R_{Soil} are complicated and need to be investigated further in various forest ecosystems.

Similar reductions of R_{Soil} in NL and NR plots suggest that recent leaf litter and live roots have the same importance in regulating R_{Soil} in this subtropical forest.

Table 4 Annual contributions of leaf aboveground litter decomposition (R_{AL}), belowground autotrophic respiration (live roots and associated microorganism, R_{BA}), and heterotrophic respiration (soil organic matter decomposed by microorganisms, R_H) to total soil respiration in the DIRT experiment from 2011 to 2014

	R_{AL}	R_{BA}	R_H
2011 (%)	24.6 ± 4.8a	19.5 ± 3.8b	55.9 ± 6.1ab
2012 (%)	24.8 ± 3.5a	24.8 ± 4.3ab	50.4 ± 5.1ab
2013 (%)	26.3 ± 4.9a	31.8 ± 3.2a	41.9 ± 6.0b
2014 (%)	19.3 ± 3.0a	20.7 ± 3.4b	60.0 ± 5.0a
Mean (%)	23.7 ± 4.9a	24.2 ± 4.8ab	52.1 ± 6.1ab

Values are expressed as mean ± SD. Different letters following the data in the same column denote significance at $P = 0.05$ level

Table 5 Relationships of soil respiration rate with soil temperature and moisture in each treatment in the DIRT experiment

	$R_{\text{soil}} = ae^{bT}$				$R_{\text{soil}} = \beta_1 W + \beta_2$			$R_{\text{soil}} = ae^{bT} W^c$				
	<i>a</i>	<i>b</i>	Q_{10}	R^2	β_1	β_2	R^2	<i>a</i>	<i>b</i>	<i>c</i>	Q_{10}	R^2
Annual												
CK	0.327	0.0743	2.102	0.790	-0.040	2.625	0.128	3.093	0.066	-0.590	1.935	0.780
NL	0.245	0.0747	2.112	0.744	-0.021	1.633	0.045	1.470	0.071	-0.488	2.034	0.671
NR	0.243	0.0747	2.111	0.778	-0.021	1.672	0.057	1.327	0.070	-0.450	2.014	0.744
NI	0.208	0.707	2.028	0.673	-0.021	1.491	0.088	1.202	0.063	-0.448	1.878	0.638
Warm season												
CK	0.413	0.063	1.879	0.503	-0.043	2.965	0.239	4.188	0.056	-0.614	1.751	0.619
NL	0.287	0.067	1.954	0.457	-0.028	2.081	0.119	1.797	0.063	-0.495	1.878	0.462
NR	0.313	0.063	1.874	0.492	-0.027	2.075	0.148	1.828	0.058	-0.470	1.786	0.537
NI	0.242	0.063	1.885	0.374	-0.023	1.714	0.150	1.736	0.052	-0.482	1.682	0.416
Cold season												
CK	0.262	0.097	2.625	0.676	0.0049	0.507	0.0044	1.925	0.114	-0.620	3.127	0.848
NL	0.207	0.092	2.512	0.586	0.0046	0.352	0.0075	1.672	0.111	-0.665	3.034	0.730
NR	0.196	0.097	2.626	0.623	-0.0016	0.524	0.0011	0.465	0.101	-0.274	2.746	0.854
NI	0.170	0.092	2.499	0.531	0.0012	0.375	0.0070	0.472	0.116	-0.361	3.190	0.825

T and *W* represent soil temperature and moisture in the 0–5 cm depth, respectively. *a*, *b*, and *c* are model parameters to be estimated. Cold season: December–March; warm season: April–November

Different letters following the data in the same column denote significance at $P = 0.05$ level

CK, control; NL, leaf litter removal; NR, root trenching; NI, leaf litter removal and root trenching

However, Sulzman et al. (2005) found that root respiration was more important for total R_{Soil} than leaf litter respiration in the H.J. Andrews experimental forest, and Li et al. (2004) observed that roots had a greater effect on R_{Soil} than the leaf litter in a *Pinus caribaea* plantation. The greater importance of root respiration in the previous studies may partly be attributed to an underestimation of respiration from aboveground litter as this included only fresh litter. Together with previous studies, we provide an evidence that effects of leaf litter and roots on R_{Soil} depend on forest types. In addition, the influence of live roots on R_{Soil} was also related to litter quantity because litter quantity affects the R_{AL} rate and R_{Soil} is a sum of R_{H} , R_{AL} and R_{BA} . For example, Sayer and Tanner (2010) found that R_{BA} measured 1 year after trenching constituted 39%, 24%, and 36% in the control, litter addition, and litter removal plots, respectively, in a lowland tropical semi-evergreen forest.

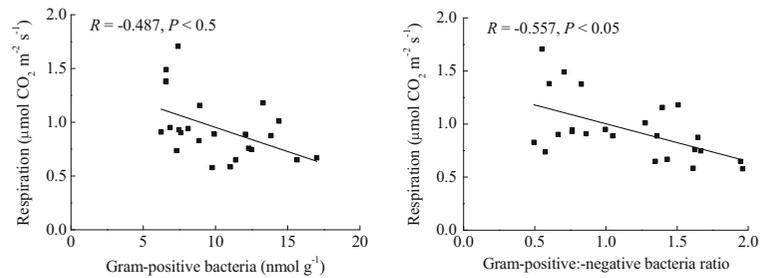
The 4-year mean contributions of R_{AL} and R_{BA} to R_{Soil} were similar in this DIRT experiment. The values were consistent with previous observations for temperate forests (Rey et al. 2002; Sulzman et al. 2005; Kotrocó et al. 2014) and fell within the range reported by Subke et al. (2006) for tropical forests. However, the share of R_{AL} and R_{BA} to total R_{Soil} showed a large variation among the published studies. Such variation can partly be attributed to differences in the amount and decomposition rate of leaf litter and roots in various forest ecosystems (Allison and Vitousek 2004; Wang et al. 2007). Moreover, the inconsistencies in the contributions of R_{AL} and R_{BA} to R_{Soil} among different experiments may stem from the inter-annual variation. In the current experiment, contributions of R_{AL} and R_{BA} had large inter-annual differences. Sulzman et al. (2005) also noted that the annual proportion to total R_{Soil} derived from roots ranged from 49% to 65% in a temperate

Table 6 Relationship between annual soil respiration rate and soil chemical properties across all treatments in the DIRT experiment ($n = 24$)

	SOC	DOC	MBC	Total N	NO ₃ -N	NH ₄ -N	Available P	pH
Annual R_{Soil}	0.666*	0.412*	0.330	0.382	0.143	0.584*	0.139	0.158

*denotes significant correlation at $P = 0.05$

Fig. 5 Relationship between annual soil respiration rate and microbial community across all treatments in the DIRT experiment ($n = 24$)



forest. As above-mentioned, in fact, there is still much uncertainty in what drives the relative differences in different respiratory pathways to the total R_{Soil} , including e.g. site productivity, root:shoot ratio and the chemistry of above- and belowground litter.

In this experiment, it should be noted that R_{Soil} in the trenched plots was likely to be overestimated, although R_{Soil} was measured since 9 months after root trenching. Dead roots of the vegetation in NR and NI plots were likely not completely decomposed and remained in the soil because their decomposition can last for several months to over a year (Subke et al. 2006; Sayer and Tanner 2010; Wang et al. 2013). Thus, the dead root decomposition would likely have affected R_{Soil} in the trenched plots, resulting in the underestimation of the contribution of R_{BA} to R_{Soil} as R_{BA} was calculated as the difference between R_{Soil} in the control plots and that in the root-trenched plots. In old-growth moist lowland tropical forest, Sayer and Tanner (2010) found that the estimates of root-rhizosphere respiration obtained from measurements taken one day after trenching were very similar to the values obtained at the same time of year (May–June) one year after trenching, and speculated that the immediate decrease in R_{Soil} on the first day after trenching provided the most reliable estimate of root-rhizosphere respiration. Although root trenching has some disadvantages to differentiate between autotrophic and heterotrophic respiration (Silver et al. 2005; Subke et al. 2006), it is still to date a simple and useful tool to estimate R_{BA} (Díaz-Pinés et al. 2010).

Effects of soil temperature, moisture and biochemical property on soil respiration

The similar seasonal patterns of R_{Soil} and soil temperature suggested that soil temperature is one of the key controlling factors of the R_{Soil} seasonality in the subtropical forest. Here, we note that other soil processes (e.g. root growth, root C exudation) that are correlated to annual

temperature also affect R_{Soil} seasonality. Numerous studies have documented the dominant role of soil temperature in controlling R_{Soil} (Knorr et al. 2005; Smith and Fang 2010; Yuste et al. 2007; Fekete et al. 2014). Stronger correlations of R_{Soil} with soil temperature than with soil moisture also emphasize that the variation of R_{Soil} is to a higher degree controlled by soil temperature than by soil moisture, in agreement with previous observations in other forest ecosystems (Sulzman et al. 2005; Atarashi-Andoh et al. 2012; Wang et al. 2013). In a temperate forest, Sayer and Tanner (2010) also found that root trenching decreased the degree of R_{Soil} variation explained by soil temperature. Root presence increased the response of R_{Soil} to soil temperature in subtropical forests, which has been previously shown by Boone et al. (1998) in a temperate forest.

A negative correlation between R_{Soil} and volumetric soil moisture content in this study suggested that soil moisture suppressed R_{Soil} , although the overall correlation between R_{Soil} and soil moisture was low (4.5%–23.9%). The weak relationship was perhaps due to optimal water content being bimodal and out of phase with biologically optimal temperature (Sulzman et al. 2005; Suseela et al. 2012). A relative low peak of R_{Soil} occurring in the growing season was attributed to the low soil moisture and suggested that soil moisture becomes important in controlling R_{Soil} when it is extreme. This statement is supported by the negative temperature sensitivity in R_{Soil} during low soil moisture conditions, as reported by Tucker and Reed (2016). These results indicate that soil temperature and moisture has a synergistic effect on R_{Soil} in the warm season, whereas in the cold season, soil temperature is a dominant controller of R_{Soil} . Another important finding was that, when each treatment was considered alone and based on annual means, the correlation coefficients between R_{Soil} and soil moisture in NL, NR, and NI plots were lower than those in the CK plots (Table 5), indicating that C-input manipulation decreased the suppression effect of soil moisture on R_{Soil} .

Role of microbial community composition in controlling soil respiration

To test the importance of microbial community composition for responses in R_{Soil} to plant-C inputs, we analyzed the correlation between the annual R_{Soil} rate and the soil microbial community composition indicators. We found a significantly negative correlation between the annual R_{Soil} rate and the abundance of Gram-positive bacterial PLFAs, suggesting that Gram-positive bacteria is a strong determinant of R_{Soil} responses to altered C inputs in this subtropical forest. This result is consistent with the observation of Wang et al. (2013). Also, Whitaker et al. (2014) found that heterotrophic respiration responses to changing C input were best predicted by soil microbial community composition, and that Gram-positive bacteria, relative to Gram-negative bacteria, explained more of the variance in C substrate mineralization. In a forest dominated by *Pinus contorta*, *Abies lasiocarpa* and *Picea engelmannii*, Monson et al. (2006) reported that soil microbial community composition controlled R_{Soil} in winter, but did not clarify which group of microorganisms that was important for R_{Soil} . Therefore, our results somewhat enhanced our understanding of the importance of soil microbial community composition for soil C cycling.

In conclusion, in a 4-year study with C-input manipulations in a subtropical *C. lanceolata* forest, we highlighted how R_{Soil} responded to changes in C inputs through leaf litter and roots. The similar degree of reduction in R_{Soil} induced by leaf litter removal and root trenching suggests that aboveground leaf litter and belowground live roots with the associated microorganism had similar importance for the total soil respiratory loss (R_{Soil}). Soil temperature was more important in regulating R_{Soil} than soil moisture, but their roles are somewhat altered by C-input manipulation and vary seasonally. These findings suggest that it is essential that plant–soil feedbacks should be taken into account in predictions of the C sequestration potential of subtropical forests under global change. From our study and previous investigations, we know that effects of aboveground and belowground litters on soil C emissions are complicated and controlled by many factors, and to fully understand the share and control of various respiratory pathways that contribute to total R_{Soil} more work needs to be conducted.

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