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# Soil respiration is driven by fine root biomass along a forest chronosequence in subtropical China

*Chao Wang*<sup>1</sup>, *Yinlei Ma*<sup>1</sup>, *Stefan Trogisch*<sup>2,3</sup>, *Yuanyuan Huang*<sup>1</sup>, *Yan Geng*<sup>4</sup>, *Michael Scherer-Lorenzen*<sup>5</sup> and *Jin-Sheng He*<sup>1,\*</sup>

<sup>1</sup> Department of Ecology, College of Urban and Environmental Sciences, and Key Laboratory for Earth Surface Processes of the Ministry of Education, Peking University, 5 Yiheyuan Road, Beijing 100871, China

<sup>2</sup> Department for Geobotany, Institute of Biology/Geobotany and Botanical Garden, Martin-Luther-University Halle-

Wittenberg, Am Kirchtor 1, Halle(Saale) D-06108, Germany

<sup>3</sup> German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Deutscher Platz 5e, Leipzig D-04103, Germany

- <sup>4</sup> Institute of Environment and Sustainable Development in Agriculture, Chinese Academy of Agricultural Sciences, 12 Zhongguancun South Street, Beijing 100081, China
- <sup>5</sup> Faculty of Biology/Geobotany, University of Freiburg, 79104 Freiburg, Germany

\*Correspondence address. Department of Ecology, College of Urban and Environmental Sciences, and Key Laboratory for Earth Surface Processes of the Ministry of Education, Peking University, 5 Yiheyuan Road, Beijing 100871, China. Tel: +86-10-6275-4404; Fax: +86-10-6275-8684; E-mail: jshe@pku.edu.cn

## Abstract

#### Aims

Soil respiration (Rs) is a major process controlling soil carbon loss in forest ecosystems. However, the underlying mechanisms leading to variation in Rs along forest successional gradients are not well understood. In this study, we investigated the effects of biotic and abiotic factors on Rs along a forest successional gradient in southeast China.

#### Methods

We selected 16 plots stratified by forest age, ranging from 20 to 120 years. In each plot, six shallow collars and six deep collars were permanently inserted into the soil. Shallow and deep collars were used to measure Rs and heterotrophic respiration (Rh), respectively. Autotrophic soil respiration (Ra) was estimated as the difference between Rs and Rh. Litter layer respiration (R<sub>L</sub>) was calculated by subtracting soil respiration measured in collars without leaf litter layer (R<sub>NL</sub>) from Rs. Rs was measured every 2 months, and soil temperature (ST) and soil volumetric water content (SVWC) were recorded every hour for 19 months. We calculated daily Rs using an exponential model dependent on ST. Daily Rs was summed to

obtain cumulative annual Rs estimates. Structural equation modelling (SEM) was applied to identify the drivers of Rs during forest succession.

#### **Important Findings**

Rs showed significant differences among three successive stages, and it was the highest in the young stage. Ra was higher in the young stage than in the medium stage. Cumulative annual Rs and Ra peaked in the young and old stages, respectively. Cumulative annual Rh and respiration measured from soil organic matter ( $R_{SOM}$ ) decreased, whereas  $R_L$  increased with forest age. The SEM revealed that cumulative annual Rs was influenced by fine root biomass and SVWC. Our results indicated that the dominant force regulating Rs on a seasonal scale is ST; however, on a successional scale, below-ground carbon emerges as the dominant influential factor.

*Keywords:* forest succession, soil respiration, fine root biomass, soil temperature

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## INTRODUCTION

Soil respiration (Rs) is a key component of the carbon cycle and releases 78–95 PgC globally into the atmosphere each year

(Bond-Lamberty and Thomson 2010a; Hashimoto *et al.* 2015). After carbon assimilation by photosynthesis, Rs is the second largest driver of C flux between terrestrial ecosystems and the atmosphere (Raich and Potter 1995). Forests cover ~30% of the

world's land surface, store ~45% of terrestrial carbon (Bonan 2008) and provide a total sink of 2.4±0.4 Pg of C per year in the global carbon cycle (Pan *et al.* 2011). Rs in forests represents 40–90% of total ecosystem respiration and is the primary path for returning CO<sub>2</sub> to the atmosphere (Granier *et al.* 2000; Schlesinger and Andrews 2000). Therefore, even small changes in forest Rs may greatly affect the global carbon cycle.

The main contributing factors driving Rs have been shown to vary greatly with time (Bond-Lamberty and Thomson 2010b). Although the belowground carbon supply is relatively stable over short periods, there are large seasonal and interannual fluctuations in soil temperature (ST) and soil volumetric water content (SVWC), which are important drivers of Rs (Davidson et al. 1998; Irvine and Law 2002). However, during forest succession, forest biogeochemical cycles are modified (Bakker et al. 1997), which leads to an increase in biomass stocks, improvements in soil fertility (De Kovel et al. 2000; Feldpausch et al. 2004; Li et al. 2017) and soil microbes (Shi et al. 2017; Zhang et al. 2017), and changes microclimatic conditions (Yan et al. 2007). The capacity of forests to accumulate belowground carbon in soil organic matter (SOM) and root biomass may emerge as an important factor influencing Rs during forest development (Campbell and Law 2005). Hence, the factors closely associated with Rs on a seasonal scale and on a successional scale may not be identical. Although many studies have investigated the seasonal variation in Rs and its associated driving factors (Gough et al. 2005; Khomik et al. 2010; Tang et al. 2006; Tedeschi et al. 2006; Wang et al. 2011), there are limited data identifying of the major Rs determinants along forest successional gradients.

Rs varies with forest succession (Chapin et al. 2011), and many studies have analysed forest Rs at different successional stages (Gough et al. 2005; Khomik et al. 2010; Tang et al. 2006; Tedeschi et al. 2006). Most studies have focused on temperate (Campbell and Law 2005; Yan et al. 2015) and boreal forests (Bond-Lamberty et al. 2004; Irvine and Law 2002; Saiz et al. 2006), as well as on the seasonal variation in Rs, whereas forest age-related changes in Rs in other forest biomes, such as the warm-humid subtropics, are largely unknown (Yan et al. 2009a; Yi et al. 2007). Distributed mainly in southeast China, the subtropical broad-leaved forest is recognized as one of the most important biomes on earth (Hu and Yu 2008; Wu 1980), and it plays an important role in regional and global carbon balance (Piao et al. 2009). Annual soil CO<sub>2</sub> production from evergreen broadleaved forests is larger than that from any other vegetation type on earth (Raich et al. 2002). Thus, knowing how Rs is altered and what is the key driver of Rs along a forest successional gradient in a subtropical region will help us understand forest age-related changes in carbon fluxes in evergreen broad-leaved forests.

The present study was performed in 16 plots spanning a forest age gradient from 20 to 120 years. We investigated the effects of the successional stage on Rs, Ra and Rh in an evergreen subtropical broad-leaved forest. We hypothesize that:

- (1) After disturbance, highly labile carbon input from dead roots and aboveground litter creates favourable environment conditions. Thus, Rh is the highest in the young stage, and it declines with forest age (Chapin *et al.* 2011).
- (2) Ra has the same pattern as Net Primary Production (Ryan *et al.* 1997), and it is the highest in the medium stage (Fig. 1).
- (3) Combining Rh and Ra, Rs is characterized by a humped shape (Fig. 1).
- (4) Since ST is not a limiting factor in warm subtropical forests, biotic factors such as substrate and ecosystem productivity may emerge as important elements influencing Rs with forest succession (Campbell and Law 2005). Therefore, we hypothesized that Rs is likely to be driven by soil organic carbon (SOC) and root biomass along a successional gradient.

## MATERIALS AND METHODS

#### Study site

This study was conducted in the Gutianshan National Nature Reserve (NNR), which is located in the western Zhejiang Province in south-eastern China (29°8'18"-29°17'29"N, 118°2′14″–118°11′12″E) (Bruelheide *et al.* 2011). The reserve was found in 1975 to prohibit logging and forest destruction, and the plantations were manually clear-cut every 20-25 years. It covers 81 km<sup>2</sup> of mixed evergreen broad-leaved forest. Approximately 57% of the reserve is a natural forest (Legendre et al. 2009). In total, 1426 seed plant species belonging to 684 genera and 149 families are found in the reserve (Lou and Jin 2000), where Castanopsis eyrei (Fagaceae), Daphniphyllum oldhamii (Daphniphyllaceae) and Schima superba (Theaceae) were the dominant species (Bruelheide et al. 2011). The region is characterized by a subtropical monsoon climate with a warm, humid summer and a cold, dry winter. The annual mean temperature is 15.1°C,



**Figure 1:** idealized patterns of change in fluxes (Rs, Ra and Rh) during secondary succession, all redrawn or calculated from Chapin *et al.* 2011's Fig. 12.16.

and the annual mean precipitation is 2000 mm (Yu *et al.* 2001). The typical soil type is sandy-loamy to silty-loamy textured acidic cambisols, with a pH of 4.56. The prevailing soil parent rock is granite or deeply weathered granite (Geißler *et al.* 2010).

#### Study design

As trees were harvested at different times, the NNR represents a wide range of forest ages (Hu and Yu 2008). The study was carried out in 16 plots (each  $30 \times 30$  m) differing in woody plant diversity (25–69 species), which were randomly selected and stratified by successional age in 2008 (Bruelheide *et al.* 2011). Plots have been assigned into three successional stages that were distinguished according to the age of the fifth largest individual trees in each plot (Bruelheide *et al.* 2011) as follows: young (<40 years), medium (40–80 years), and old (≥80 years). The number of plots per successional stage was 6, 5 and 5. Areas of the NNR showing stand heterogeneity (streams, rocks, clearings) or inaccessibility (inclination > 55°) were excluded for plot selection. Important edaphic and topographic characteristics of successional stages are given in Table 1.

#### **Rs** measurements

Rs was measured using a closed chamber connected to a portable infrared gas analyzer (LI-8100; Li-Cor Inc., Lincoln, NE, USA) from April 2009 to March 2012. In each plot, six shallow polyvinyl chloride (PVC) collars (20 cm interior diameter and 8 cm height) were permanently installed along a transect with 1-m intervals between collars, to depth of 3 cm, thus protruding 5 cm above the ground. Six deep collars (20 cm interior diameter and 70 cm height) were installed to a soil depth of 65 cm to exclude plant roots and organic matter input from decomposing roots (>90% of the

 Table 1: measured environmental factors along the successional gradient

	Young		Medium		Old	
Parameters	Mean	SE	Mean	SE	Mean	SE
SVWC (%)	17.01c	0.19	20.08b	0.16	20.97a	0.15
ST (°C)	16.26a	0.24	15.80a	0.40	16.26a	0.34
FRB (g $m^{-2}$ )	550.8a	45.34	363.5b	53.51	394.9b	46.2
Litter stock (g m <sup>-2</sup> )	676.8a	51.30	834.6ab	52.40	894.8b	98.8
STN (%)	0.23b	0.01	0.31ab	0.05	0.36a	0.05
SOC (%)	4.15b	0.12	5.56ab	0.78	6.39a	0.69
pН	4.76b	0.08	4.72b	0.09	4.43a	0.06
Elevation (m)	375a	53.3	471b	40.0	558c	42.4
Slope (°)	34.33a	1.87	38.20a	3.20	29.40a	3.37

Different letters (a, b and c) within the same row indicate significant differences among successional stages (one-way ANOVA, *post hoc* LSD test). Data shown are means  $\pm$  SE (n = 5-6). Abbreviations: Elevation = mean elevation of successional stages, Slope = mean slope of successional stages, STN = soil total nitrogen content at top soil (0–5 cm).

belowground biomass is distributed in the top 30 cm of soil) (Yan *et al.* 2009b; Yi *et al.* 2007). The shallow and deep collars were used to measure Rs and Rh, respectively. The difference between Rs and Rh was used to estimate Ra. Litter was removed from half of the respiration collars to separate Rs into litter and mineral soil components, and the collars were covered with nylon mesh with a 2 mm pore size to exclude plant litterfall. These three collars were used to measure Rs without litter ( $R_{NL}$ ) (Yi *et al.* 2007). The difference between Rs and  $R_{NL}$  was used to estimate  $R_L$  (respiration from above-ground litter), and the difference between Rh and  $R_L$  was used to estimate  $R_{SOM}$  (respiration from soil organic matter). Both Rs and Rh were measured manually twice with an interval of 1 hour every 2 months.

#### Soil and air temperature and moisture

Soil temperature at a depth of 5 cm was recorded simultaneously with the Rs using a soil temperature probe connected to the portable soil respiration system. From April 2009 to August 2010, the SVWC of the upper 10 cm was measured gravimetrically. In August 2010, we added two EM50 data loggers (Decagon Devices, Inc., Pullman, WA, USA) with an air temperature and humidity sensor and an ECH<sub>2</sub>O soil temperature and moisture probe in the middle of each successional stage. The air sensor and ECH<sub>2</sub>O were used to measure air temperature and humidity at 1.50 m aboveground and ST and SVWC, respectively, at 30-min intervals (Ma *et al.* 2013).

#### Fine root biomass and litter stock

Fine root biomass (FRB; roots < 2 mm in diameter) was determined in each plot by obtaining eight replicate soil cores (10 cm diameter, 45 cm deep) close to the respiration collars in September 2011. The samples were divided into three sections according to depth (0–15, 15–30 and 30–45 cm). Soil samples were soaked to loosen attached soil particles, and live fine roots were extracted from soil by sieving and washing in the laboratory. Fine roots were dried at 65°C for 2 days and then weighed.

To determine the forest floor litter stock, four sample cores were collected using a PVC tube (diameter 19 cm) in undisturbed litter patches located in each plot in the spring, summer, autumn and winter in 2009, respectively. Litter from the four sample cores was pooled, dried at 65°C until constant weight, and litter stocks were averaged across seasons.

#### Soil chemical analyses

Nine replicate soil cores were collected around the respiration collars in each plot. Samples were bulked from the nine cores to obtain one sample per depth increment (0–5, 5–10, 10–20, 20–30, 30–40 and 40–50 cm), yielding six soil samples per plot in total. All analyses were carried out on air-dried and sieved samples (<2 mm). Soil samples for C and N analyses were ground using a ball mill. Then,  $pH_{H20}$  was determined potentiometrically in a 1:2.5 solution (soil to water). Total C and total N were measured by a CN-element analyzer (PE 2400 II CHN elemental analyzer; Perkin-Elmer, Boston, MA, USA).

#### Statistical analysis

Ordinary linear regression was used to investigate the relationship between forest age and ST, SVWC, Rh,  $R_{SOM}$  and  $R_L$ , and the relationship between FRB and Rs, Rh and Ra. The impact of successional stage on Rs and environment parameters was examined by one-way analysis of variance (ANOVA), and a least square deviation (LSD) test was used to compare the mean differences of Rs and environment parameters among successional stages. These analyses were conducted with SPSS 16.0 software (SPSS Inc., USA).

Considering the strong colinearity among the variables, we further used structural equation modelling (SEM) to explicitly evaluate the causal relationships among these interacting variables (Geng *et al.* 2012). We applied SEM to partition the total effect of variables on Rs into direct and indirect effects, both of which were calculated using standardized path coefficients. A path model was developed to relate the cumulative annual Rs to cumulative annual Ra, cumulative annual Rh, ST, SVWC, FRB, litter stock, and SOC based on theoretical knowledge at ecosystem level (Luo *et al.* 2006; Wei *et al.* 2015). Overall, a model with a Fisher's *C* statistic *P* > 0.05, GFI > 0.9, CFI > 0.95 and NFI > 0.95 was considered to be appropriate. Using our model, the Fisher's *C* statistic for *P*, GFI, CFI and NFI was 0.99, 0.99, 1.0 and 1.0,

respectively. SEM was performed using the *sem* package in R 3.2.2 (R Core Team 2015).

#### Modelled soil respiration

In order to estimate cumulative annual Rs, the exponential equation given by Lloyd and Taylor (1994) was used to calculate modelled Rs (Rsm),

$$Rsm = R_{10}e^{E_0\left(\frac{1}{10-T_0} - \frac{1}{T-T_0}\right)} = R_{10}e^{E_0\left(\frac{1}{56.02} - \frac{1}{T+46.02}\right)}$$
(1)

where  $R_{10}$  is Rsm at 10°C, and  $T_0$  and  $E_0$  are two parameters that define the temperature dependency of Rs. To facilitate the convergence of the non-linear regression procedure that was used to fit  $R_{10}$ ,  $T_0$  and  $E_0$ , we estimated  $R_{10}$  and  $E_0$ while fixing  $T_0$  to -46.02°C, as proposed by Lloyd and Taylor (1994). The model fits were then based on ST recorded by the soil temperature probe at a depth of 5 cm. Hourly Rsm values were modelled over the temperature range that was calibrated according to Equation (1). Daily Rsm values were calculated using the hourly Rsm for each day, and cumulative annual Rs was estimated for each plot by calculating the daily sums of Rsm using Equation (1).

The relative temperature sensitivity (RTS) of Rsm was calculated as described by Lloyd and Taylor (1994) as follows:



**Figure 2:** seasonal variations of (**a**) AT, (**b**) ST at 5 cm depth and (**c**) SVWC at 10 cm depth. (**d**) Inset in Panel (**c**) shows the changes of mean annual ST at 5 cm depth (triangle), and mean annual SVWC at 10 cm depth (blue circle) along the successional gradient. Abbreviations: AT = air temperature, MST = mean annual ST at 5 cm depth, MSVWC = mean annual SVWC at 10 cm depth. Vertical bars represent the standard error of the mean (n = 42-48). See color figure in Online.

$$RTS = \left(\frac{1}{f_{(T)}} \times \frac{df_{(T)}}{dT}\right) = \frac{E_0}{\left(T - T_0\right)^2}$$
(2)

For each plot, RTS was also calculated separately.

All graphs were prepared using Sigmaplot (version 12.0). Differences were considered to be statistically significant at  $P \le 0.05$ .

### RESULTS

#### Microclimate and Rs

Over the study period, air temperature and ST showed no significant differences among the three successional stages, whereas SVWC showed significant differences among the three stages, ranging from 17.0% in the young stage to 21.0% in the old stage (Table 1). ST decreased (marginally, P < 0.1, Fig. 2d) and SVWC increased (P < 0.05, Fig. 2d) with forest age.

In all stages, Rs had a distinct seasonal pattern with a minimum value in January or February and a maximum typically occurring during mid-summer (Fig. 3). At the seasonal and yearly scales, Rs was significantly correlated with ST (P < 0.01for all stages, with R<sup>2</sup> ranging from 0.53 to 0.76; Table 2), indicating that ST is the main factor determining the seasonal pattern of Rs. Estimated mean Rs rate was significantly different among stages. It was lowest in the medium stage  $(1.26\pm0.04 \ \mu\text{mol m}^{-2} \text{ s}^{-1})$  and highest in the young stage  $(1.61\pm0.05 \ \mu\text{mol m}^{-2} \text{ s}^{-1})$ . The estimated mean Ra rate in the young stage  $(0.53\pm0.05 \ \mu\text{mol m}^{-2} \text{ s}^{-1})$  was significantly higher than at the medium stage  $(0.28\pm0.06 \ \mu\text{mol m}^{-2} \text{ s}^{-1}; \text{Fig. 3d})$ , and contributed 32.3%, 21.4% and 28.3% to Rs in the young, medium and old stages, respectively. Estimated mean Rh rate was similar in all forest stages.

## Patterns of cumulative annual Rs and RTS during succession

Two peaks of cumulative annual Rs appeared along the forest age gradient (Fig. 4a), specifically in the young and old stages. Cumulative annual Ra had the same pattern as Rs (Fig. 4b). Cumulative annual Rs and Ra were highest in the young and old stages and lowest in the medium stage. Cumulative annual Rh (P < 0.01, Fig. 4c) and R<sub>SOM</sub> (P < 0.01, Fig. 5a) decreased, but R<sub>L</sub> increased (P < 0.01, Fig. 5b) with forest age. RTS for Rh decreased with forest age (P = 0.02, Fig. 5d).

## Effects of biotic and abiotic factors on cumulative annual Rs

SEM demonstrated that Rs was mediated by FRB and SVWC (Fig. 6). FRB indirectly influenced Rs through Ra ( $\beta = 0.87$ ) and Rh ( $\beta = 0.63$ ), which was the most significant parameter



**Figure 3:** seasonal variations of (**a**) total Rs, (**b**) Rh and (**c**) root respiration, and (**d**) Inset in panel (**c**) shows annual mean respiration. Vertical bars represent the standard error of the mean (n = 5-6). Different letters in panel (**d**) denote significant differences (P < 0.05) between the three successional stages.

affecting Rs. The influence of SVWC on Rs was mediated through Rh ( $\beta = -0.56$ ). SVWC affected Ra directly ( $\beta = 0.37$ ) and indirectly through FRB ( $\beta = -0.70$ ) (Table 3). We found that ST ( $\beta = -0.30$ ) also influenced Ra through FRB and litter stock ( $\beta = -0.38$ ) affected Rh directly. The ordinary linear regression results showed that FRB had a significantly positive relationship with Rs, Rh and Ra, while SVWC had no relationship with Rs (Fig. 7).

As evident from the SEM (Fig. 6), SOC could be well predicted from ST and litter stock and explained 81% of the variation in SOC. While SOC was negatively related to ST ( $\beta = -0.71$ ), the litter stock was a weaker positive relationship with SOC ( $\beta = 0.16$ ).

### DISCUSSION

### Rh declines during forest succession

The pattern of cumulative annual Rh supports *Chapin's* hypothesis (Fig. 1), since Rh decreased with forest age (the regression coefficient -0.95). Since our forest age gradient did not cover very early or old growth forest stands, we observed a linear decline in Rh instead of a curved relationship with stand age. Li *et al.* (2013) also found that Rh had a negative exponential relationship with forest age across China. After timber harvest or deforestation, a favourable environment for decomposition is normally created with relatively high nutrient availability and ST, which promote Rh. In our study,

Table 2: parameters of Rs as the exponential function of ST for different successional stages

	Rs				Rh	Rh			
Stage	R <sub>10</sub>	$E_0$	$R^2$	Р	R <sub>10</sub>	$E_0$	$R^2$	Р	
Young	0.65	273.1	0.74	<0.01	0.66	281.9	0.76	< 0.01	
Medium	0.74	276.2	0.62	< 0.01	0.75	180.7	0.53	< 0.01	
Old	0.85	271.6	0.61	< 0.01	0.65	273.1	0.64	< 0.01	

The  $R_{10}$  and  $E_0$  values are reported as means. *P*-values are for overall model fit.



**Figure 4:** the pattern of cumulative annual (**a**) total Rs, (**b**) Ra and (**c**) Rh along the forest age gradient. The different colours (green, red and blue) represent the successional stages (young, medium and old). Panels show the mean cumulative annual respiration of each stage. Vertical bars represent the standard error of the mean.



**Figure 5:** the relationship between (**a**) cumulative annual  $R_{SOM}$ , (**b**) respiration from aboveground litter ( $R_L$ ), (**c**) RTS for Rs and (**d**) RTS for Rh and forest age. The different colours (green, red and blue) represent the successional stages (young, medium and old). Vertical bars represent the standard error of the means.



**Figure 6:** the final SEM for cumulative annual Rs ( $\chi^2 = 7.31$ , df = 7, *P* = 0.99). Measured exogenous and endogenous variables are represented by boxes. Numbers beside arrows are standardized path coefficients. Width of arrows indicates the strength of the relationships. Solid red arrows represent positive paths (*P* < 0.05), solid black arrows represent negative paths (*P* < 0.05) and dashed grey arrows represent non-significant paths (*P* > 0.05). *R*<sup>2</sup> for Rs, Ra, Rh and SOC are 1, 0.55, 0.65 and 0.81, respectively. Abbreviations: MST = annual mean ST at 5cm depth, MSVWC = annual mean SVWC at 10cm depth.



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**Figure 7:** the relationship between (a) Rs and SVWC, and the relationship between FRB and Rs (b), Rh (c) and Ra (d). The different colours (green, red and blue) represent the successional stages (young, medium and old). Vertical bars and horizontal bars represent the standard error of the means. See color figure in Online.

 Table 3: total, direct and indirect effects of environmental parameters on components of Rs as identified by SEM

	Direct effect	Indirect effect	Total
Rs			
Ra	0.80	_	0.80
Rh	0.38	_	0.38
FRB	_	0.69	0.69
SOC	_	0.23 (ns)	0.23 (ns)
Litter stock	_	-0.06 (ns)	-0.06 (ns)
SVWC	_	-0.31	-0.31
ST	_	-0.36	-0.36
Ra			
FRB	0.87	—	0.87
SOC	-0.01 (ns)	—	-0.01 (ns)
Litter stock	-0.04 (ns)	—	-0.04 (ns)
SVWC	0.37	-0.49	-0.12 (ns)
ST	-0.08 (ns)	—	-0.08 (ns)
Rh			
FRB	0.63	-0.16 (ns)	0.47
SOC	0.03 (ns)	_	0.03 (ns)
Litter stock	-0.16 (ns)	0.01 (ns)	-0.15 (ns)
SVWC	-0.56	-0.01 (ns)	-0.55
ST	-0.21 (ns)	-0.02 (ns)	-0.23 ((ns))

Effects are calculated using standardized path coefficients. Abbreviation: ns = non-significant effects.

ST decreased, whereas SVWC increased significantly during (Fig. 2) forest succession

The SEM results showed that Rh was driven by FRB and SVWC (Fig. 6). As Lohila et al (2003) reported that more FRB leads to more root exudates and root litter, thereby promoting Rh by influencing the growth and activity of microbial. We know from laboratory studies and theory that SVWC modifies the physical transportation of gas in the soil (Drever 1994), whereas water content may not be a limiting factor for microbial processes in humid subtropical forests, high SVWC can impede the diffusion of O<sub>2</sub>, and thus reduce decomposition and CO<sub>2</sub> production (Skopp *et al.* 1990). This might have caused a decline in SOC turnover and consequently a reduction in R<sub>SOM</sub> during forest succession (Fig. 5a). The SEM also showed that the forest floor litter stock had a negative relationship with Rh. As litter stock accumulated with forest stand age (Huang et al. 2017; Table 1), cumulative annual R<sub>L</sub> also significantly increased during succession (Fig. 5b). However, the absolute value of the regression coefficient of  $R_{SOM}$  (2.69) was larger than that of  $R_{L}$  (1.48). Thus, the decline of Rh with forest stage was predominantly driven by R<sub>SOM</sub>.

## Rs was higher in the young and old stages of forest succession

Cumulative annual Rs peaked in the young and old stages (Fig. 4a). Our results do not support our third hypothesis stating that cumulative annual Rs shows a humped-shaped pattern

along the forest age gradient. Our results also differed from those that were previously reported. For instance, Campbell and Law (2005) found that the cumulative annual Rs was highest in the young and old stages in regions with high precipitation, but highest in the medium stage in regions with low and moderate precipitation. Furthermore, Tang et al. (2006) and Yan et al. (2009a) found that cumulative annual Rs increased with successional stage. The SEM showed that FRB affected Rs through Ra (Fig. 6). At our site, the young forest was characterized by small trees with high stem density (Chi et al. 2017; Lin et al. 2015), and trees needed abundant fine roots to obtain water and nutrients to satisfy their fast growth (Bu et al. 2017; Sun et al. 2017; Table 1). The old forest had large total living biomass with the same stem density as the medium stage (Lin et al. 2015) and needed more fine roots to maintain the large quantity of living biomass (Table 1). Fine roots have very strong physiological functions, such as high metabolic activity, high rates of nutrient uptake and high growth respiration (Chen et al. 2009; Pregitzer 2002; Wells and Eissenstat 2001). Thus, greater amounts of FRB might have led to a higher Rs, which is similar to the results from previous studies (Gong et al. 2012; Wiseman and Seiler 2004; Yi et al. 2007).

## Drivers of Rs at the seasonal scale and during forest succession

Our results support the second hypothesis that Rs depends strongly on ST during the course of the year, whereas along the successional gradient, Rs is driven by FRB. ST and substrate affect Rs at different time scales (Yvon-Durocher *et al.* 2012). On a seasonal scale, respiration strongly depends on seasonal temperature (Yvon-Durocher *et al.* 2012). For example, ST ranged from 0.15°C to 28.70°C (Fig. 2) at our study site. However, mean annual ST did not differ significantly between successional stages and ranged from 15.8°C to 16.3°C (Table 1). ST is the dominant factor in Rs on the seasonal scale, not the successional scale (Table 2), as a result of the large fluctuations in ST in a year (Fig. 2).

Whereas previous studies focused primarily on the effects of ST, only a few have mentioned the importance of the substrate supply for Rs along forest successional gradients. For example, Campbell and Law (2005) speculated that belowground carbon allocation might surpass soil environment in shaping patterns of cumulative annual Rs. Yan *et al.* (2009a) suggested that cumulative annual Rs increased with forest succession due to changes in soil biology and SOC content. This larger data set thus confirmed the previous findings that revealed that FRB was significantly positively correlated with Rs (Gong *et al.* 2012; Wiseman and Seiler 2004; Yi *et al.* 2007). Taken together, FRB as an important part of belowground substrate supply (Ostonen *et al.* 2005) differed significantly among successional stages (Table 1) and was the most important driver of Rs along the successional gradient (Fig. 6).

#### Limitations of the current study

Spatial distribution of roots and distance between respiration collars and trees may have contributed to within-plot variability of Rs. Moreover, the driver of heterogeneity in Rs may change along time scales (Reichstein and Beer 2008), especially in subtropical and tropical forest, thus more replicates are needed in the future. Owing to the large fluctuations in environmental conditions in the subtropical forest, utilizing an Rs measured only every 2 months might have affected data quality. Cumulative annual Rs was estimated by Equation (1), and it depended on the measurement of Rs. Thus, the continuous measurement of Rs is necessary and important for future studies. Our study provided detailed insights into carbon effluxes from soil; nevertheless, more data on important carbon fluxes and pools are needed to quantify the carbon sequestration potential of subtropical evergreen broad-leaved forests.

## CONCLUSIONS

Cumulative annual Rs was high in the young and old successional stages, and Rh decreased with forest age in subtropical forest. ST was the main factor influencing Rs rates at the intra-annual scale, but FRB emerged as the driver along the successional gradient. Our work has shown that Rh decreases during succession and has identified drivers of Rs in a subtropical forest. This finding demonstrates that the dominant drivers of Rs might vary at different time scales. On the diurnal and seasonal timescales, ST is the main environmental factor controlling Rs, whereas during succession, substrate supply has been identified as key driver for Rs. Because aboveground and belowground carbon fluxes vary during succession, we suggest that research efforts should be focused on the combination of belowground and aboveground carbon processes in the future when evaluating the carbon balance of forests.

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