



## Effects of litter manipulation on soil respiration under short-term nitrogen addition in a subtropical evergreen forest

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### ARTICLE INFO

#### Keywords:

Nitrogen deposition  
Litter alteration  
Subtropical forest  
Carbon sequestration

### ABSTRACT

Nitrogen (N) availability is rapidly increasing in subtropical ecosystems, where litterfall is also accelerating and may substantially affect belowground carbon (C) storage and soil respiration ( $R_s$ ). This study aims to detect how litter inputs affect  $R_s$  under N addition in a subtropical forest. We conducted a two-factor experiment (N addition and litter manipulation) in a subtropical *Schima superba* evergreen broad-leaved forest in eastern China. Three levels of N addition included low-N ( $50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ), high-N ( $100 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ) and ambient N ( $0 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ), and three levels of litter manipulation consisting of litter removal (NL), litter addition (DL) and Control litter input were conducted. Our results showed NL decreased  $R_s$  by 41% and 38% under low- and high-N additions, respectively, compared to Control litter input. DL decreased  $R_s$  by 24% compared to Control litter input under high-N addition. Furthermore, low- and high-N additions decreased the effect size of NL on  $R_s$ , whereas high-N addition decreased the effect size of DL on  $R_s$  in the study period, in both rainy and dry seasons. The effect of litter input alteration on  $R_s$  under N addition decreased, compared to  $R_s$  in Control litter input under N addition. Thus, the increase in  $R_s$  under N addition was alleviated, suggesting that soil organic C sequestration may benefit from increasing N deposition in the future. Further study is needed to clarify the effects of litter input alteration on  $R_s$  and soil C cycling under long-term N addition in subtropical forests.

### 1. Introduction

Globally, soil is currently the largest carbon (C) pool in terrestrial ecosystems, and stores more organic C than plants and the atmosphere (IPCC, 2007). Soil respiration ( $R_s$ ), as the second largest carbon dioxide ( $\text{CO}_2$ ) efflux from terrestrial ecosystems to the atmosphere (Luo and Zhou, 2006; Wang and Yang, 2007), is the primary pathway for soil  $\text{CO}_2$  emission from terrestrial ecosystems to the atmosphere (Bond-Lamberty and Thomson, 2010a). Meanwhile,  $R_s$  plays an important role in regulating C sequestration in soils and C cycling in terrestrial ecosystems (Lal, 2004), and even a subtle change in  $R_s$  altered by global change could significantly affect the global C cycle and the consequent feedbacks to global changes (Davidson and Janssens, 2006). Therefore, understanding the responses of  $R_s$  to global changes is urgently needed for accurately evaluating the C balance and climate-C feedbacks.

Human activity (e.g., fossil fuel burning, deforestation, and fertilizer consumption) has doubled reactive nitrogen (N) deposition since the

industrial and agricultural revolution (Galloway et al., 2008; Gruber and Galloway, 2008). And, the widespread N deposition is continuing to alter global and regional environments and has affected  $R_s$  in forest ecosystems (Aerts and Chapin, 1999; Tian et al., 2017). However, the response of  $R_s$  to N addition is inconsistent (Tian et al., 2017), with increases (Craine et al., 2001; Hasselquist et al., 2012), decreases (Phillips and Fahey, 2007; Janssens et al., 2010), and no change (Lee and Jose, 2003; Samuelson et al., 2009) being reported previously. Although there is an emerging consensus that N addition reduces  $R_s$  in temperate forests (Janssens et al., 2010), the responses of  $R_s$  to N addition in subtropical forests are still not fully understood (Bond-Lamberty and Thomson, 2010b; Fan et al., 2014; Yan et al., 2017). There were conflicting results of N addition on  $R_s$  in subtropical forests. For instance, previous studies have reported negative response of  $R_s$  to N fertilization in N saturated subtropical forests (Mo et al., 2008; Yan et al., 2017), whereas positive response (Tu et al., 2013) and no response (Koehler et al., 2009) also have been found in subtropical

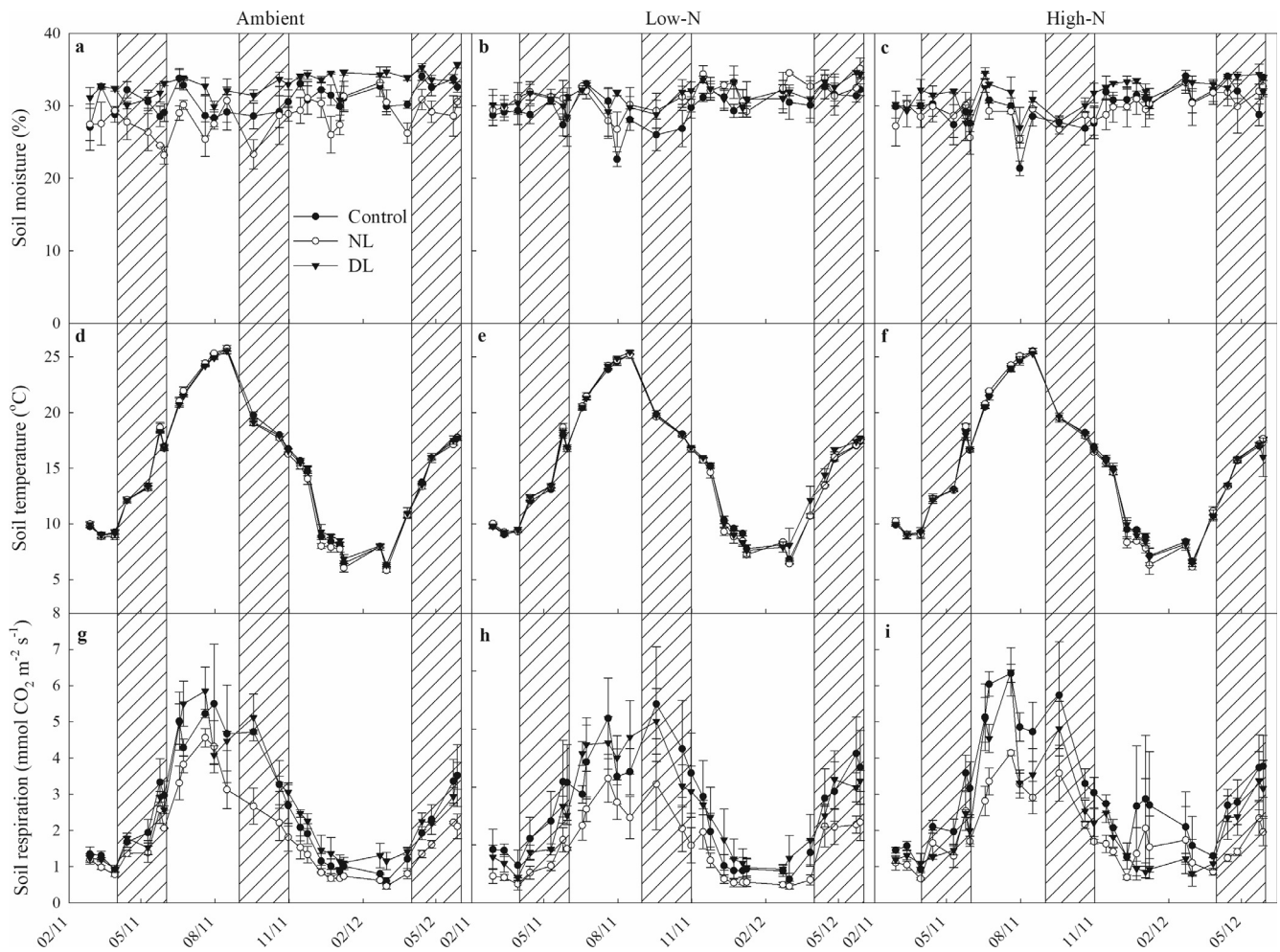
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<https://doi.org/10.1016/j.foreco.2018.06.037>

Received 6 June 2018; Received in revised form 24 June 2018; Accepted 26 June 2018  
Available online 11 July 2018

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**Fig. 1.** Seasonal dynamics of average soil temperature (a, b, c), soil moisture (d, e, f) and  $R_s$  (g, h, i) under different N additions and litter manipulation in a subtropical *Schima superba* forest in eastern China. N additions included ambient N addition of  $0 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ , low-N addition of  $50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ , and high-N addition of  $100 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ . Litter manipulation included Control litter input, NL (no-litter) and DL (double litter) in each N addition plot. Hatched areas correspond to the rainy season (April-June and September – November). Data point represents the mean  $\pm$  SE ( $N = 3$ ).

**Table 1**

Statistical results of three-way ANOVA followed by *post hoc* tests of N addition, litter manipulation and season on soil temperature, soil moisture and  $R_s$  in a subtropical *Schima superba* forest in eastern China. Bold indicates that  $P < 0.05$ .

	$R_s$ ( $\mu\text{mol m}^{-2} \text{ s}^{-1}$ )		Soil moisture (%)		Soil temperature ( $^{\circ}\text{C}$ )	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
N addition (N)	2.252	0.107	0.857	0.426	0.035	0.966
Season (S)	35.734	<b>&lt; 0.001</b>	3.727	0.055	42.713	<b>&lt; 0.001</b>
Litter (L)	13.703	<b>&lt; 0.001</b>	26.262	<b>&lt; 0.001</b>	0.023	0.977
N $\times$ S	1.769	0.173	1.318	0.270	0.016	0.984
N $\times$ L	1.142	0.337	9.630	<b>&lt; 0.001</b>	0.007	1.000
S $\times$ L	1.314	0.271	0.565	0.569	0.010	0.990
N $\times$ S $\times$ L	0.165	0.956	0.453	0.770	0.003	1.000

forests.

Meanwhile, the increase in N availability, combining with increasing atmospheric  $\text{CO}_2$ , usually stimulate forest aboveground net primary production (NPP) and change litter inputs to soils (LeBauer and Treseder, 2008; Xia and Wan, 2008), therefore potentially impacting  $R_s$  through directly altering litter quality and quantity (Liu et al., 2005), changing physiochemical and biological properties of litter layer (Xu et al., 2013), and indirectly affecting both root activity and microbial communities (Ryan and Law, 2005; Sayer, 2006). Although litter manipulation experiments have been widely conducted (Xu et al., 2013; Chen and Chen, 2018) to examine the potential effects of changes in

plant-derived C inputs on belowground C cycling (e.g., Lajtha et al., 2005; Vincent et al., 2010; Feng et al., 2011; Leff et al., 2012), there still remains a knowledge gap on the magnitude and direction of  $R_s$  to litter input alteration among different forest ecosystems (Xu et al., 2013; Chen and Chen, 2018). Especially, the effects in subtropical forests could be quite different from those in temperate forests (Sayer et al., 2007; Leff et al., 2012; Fang et al., 2015; Han et al., 2015). Numerous studies suggest that litter input alteration generally exerts nonlinear effects on  $R_s$  (Sayer et al., 2011; van Groenigen et al., 2014), with disproportionate enhances in  $R_s$  under litter addition due to the priming effects (Nottingham et al., 2009; Kuzyakov, 2010) and decreases in  $R_s$

**Table 2**

Soil temperature and moisture within litter manipulation (NL, DL and Control) under N additions and between seasons in a subtropical *Schima superba* forest in eastern China. Ambient N addition of 0 kg N ha<sup>-1</sup> yr<sup>-1</sup>, low-N addition of 50 kg N ha<sup>-1</sup> yr<sup>-1</sup>, and high-N addition of 100 kg N ha<sup>-1</sup> yr<sup>-1</sup>, respectively. Lowercase letters represent significant differences under the same N addition and varied litter manipulation. Asterisk show significant differences between rainy and dry seasons under the same N addition and litter manipulation. Data point represents the mean ± SE (N = 3).

Litter	N	Soil temperature (°C)			Soil moisture (%)		
		Rainy season	Dry season	Whole period	Rainy season	Dry season	Whole period
NL	Ambient	16.7 ± 0.7 <sup>ab</sup>	12.1 ± 2.1 <sup>ab</sup>	14.6 ± 1.1 <sup>a</sup>	28.12 ± 0.67 <sup>c</sup>	28.70 ± 0.66 <sup>c</sup>	28.39 ± 0.47 <sup>c</sup>
	Low-N	16.8 ± 0.7 <sup>ab</sup>	12.5 ± 2.0 <sup>ab</sup>	14.8 ± 1.0 <sup>a</sup>	31.86 ± 0.46 <sup>a</sup>	30.83 ± 0.63 <sup>a</sup>	31.38 ± 0.39 <sup>a</sup>
	High-N	16.8 ± 0.7 <sup>ab</sup>	12.3 ± 2.0 <sup>ab</sup>	14.7 ± 1.1 <sup>a</sup>	29.70 ± 0.55 <sup>b</sup>	29.72 ± 0.54 <sup>b</sup>	29.71 ± 0.38 <sup>b</sup>
DL	Ambient	16.8 ± 0.7 <sup>ab</sup>	12.4 ± 2.0 <sup>ab</sup>	14.8 ± 1.1 <sup>a</sup>	33.21 ± 0.40 <sup>a</sup>	32.88 ± 0.44 <sup>a</sup>	33.06 ± 0.29 <sup>a</sup>
	Low-N	17.0 ± 0.6 <sup>ab</sup>	12.8 ± 1.9 <sup>ab</sup>	15.0 ± 1.0 <sup>a</sup>	32.02 ± 0.47 <sup>ab</sup>	30.78 ± 0.30 <sup>ab</sup>	31.44 ± 0.31 <sup>a</sup>
	High-N	16.6 ± 0.7 <sup>ab</sup>	12.5 ± 2.0 <sup>ab</sup>	14.7 ± 1.0 <sup>a</sup>	31.76 ± 0.56 <sup>a</sup>	31.62 ± 0.53 <sup>a</sup>	31.70 ± 0.38 <sup>a</sup>
Control	Ambient	16.9 ± 0.7 <sup>ab</sup>	12.3 ± 2.0 <sup>ab</sup>	14.8 ± 1.1 <sup>a</sup>	31.49 ± 0.51 <sup>ab</sup>	30.17 ± 0.49 <sup>ab</sup>	30.88 ± 0.37 <sup>b</sup>
	Low-N	16.7 ± 0.7 <sup>ab</sup>	12.6 ± 1.9 <sup>ab</sup>	14.8 ± 1.0 <sup>a</sup>	30.49 ± 0.57 <sup>b</sup>	29.47 ± 0.65 <sup>a</sup>	30.02 ± 0.43 <sup>b</sup>
	High-N	16.8 ± 0.7 <sup>ab</sup>	12.6 ± 1.9 <sup>ab</sup>	14.8 ± 1.0 <sup>a</sup>	30.13 ± 0.63 <sup>b</sup>	30.01 ± 0.10 <sup>b</sup>	30.08 ± 0.50 <sup>b</sup>

under litter removal predominantly due to the reduction in easily decomposable substrate for microbes (Wang et al., 2013). However, in subtropical forests, the previous study has shown that R<sub>s</sub> presents no response under litter addition in either a subtropical Camphor forest or a Masson pine forest in central south China (Yan et al., 2013).

East Asian subtropical forests account for nearly 10% of the global forest net ecosystem production (Yu et al., 2014). Moreover, according to the trend in anthropogenic N deposition, N supply in these systems is projected to be exacerbated in the coming decades (Reay et al., 2008; Bala et al., 2013). Previously, we have shown the non-linear responses of R<sub>s</sub> and its components to N addition in an N-limited subtropical evergreen forest of *Schima superba* in eastern China (Gao et al., 2014; Gao et al., 2018). Meanwhile, we found that litter removal decreased R<sub>s</sub>, while litter addition did not affect R<sub>s</sub> under ambient N addition (Gao et al., 2015). However, we still have limited information on whether and how R<sub>s</sub> would respond to the changes in litter inputs under different N addition conditions (Wang et al., 2016a). This study build on our previous findings and was conducted to examine the effect of litter manipulation on R<sub>s</sub> under N addition and to clarify the controlling factors in our studied subtropical evergreen forest.

The specific objectives were: (1) to assess the direction and magnitude of litter manipulation on R<sub>s</sub> under N addition; and (2) to evaluate variations of the combined effect size of litter manipulation and N addition on R<sub>s</sub> between rainy and dry seasons. We hypothesized that: (1) combined effects of litter addition and N addition would stimulate R<sub>s</sub>, whereas combined effects of litter removal and N addition would decrease R<sub>s</sub>; (2) treatments of litter addition and N addition would have larger effect size on R<sub>s</sub> than the combination of litter removal and N addition.

## 2. Materials and methods

### 2.1. Site description

The study was conducted in a 50-year-old *Schima superba* forest at Tiantong National Forest Park (29°52'N, 121°39'E, 200 m a.s.l.), in Zhejiang province, eastern China. This forest is characterized by canopy trees of *Schima superba*, *Lithocarpus glaber* and *Symplocos sumuntia* with heights of 12–20 m. The soil type at the site is Acrisol (Gao et al., 2014), and soil texture is mainly medium-heavy loam (Song and Wang, 1995; Yan et al., 2008a), with the thickness of organic soil layer roughly at 5 cm (Gao et al., 2014). Soil pH ranged between 4.4 and 5.1, and the soil overlies a parent material consisting of mesozoic sediments and acidic intrusive rocks, including quartzite and granite (Song and Wang, 1995). The site has a mean annual temperature of 16.2 °C, and average annual precipitation of 1374.7 mm. In general, the area is characterized by a subtropical monsoon climate with a rainy season (April to June, September to November), and a dry season (July to August, December

to March) (Gao et al., 2014).

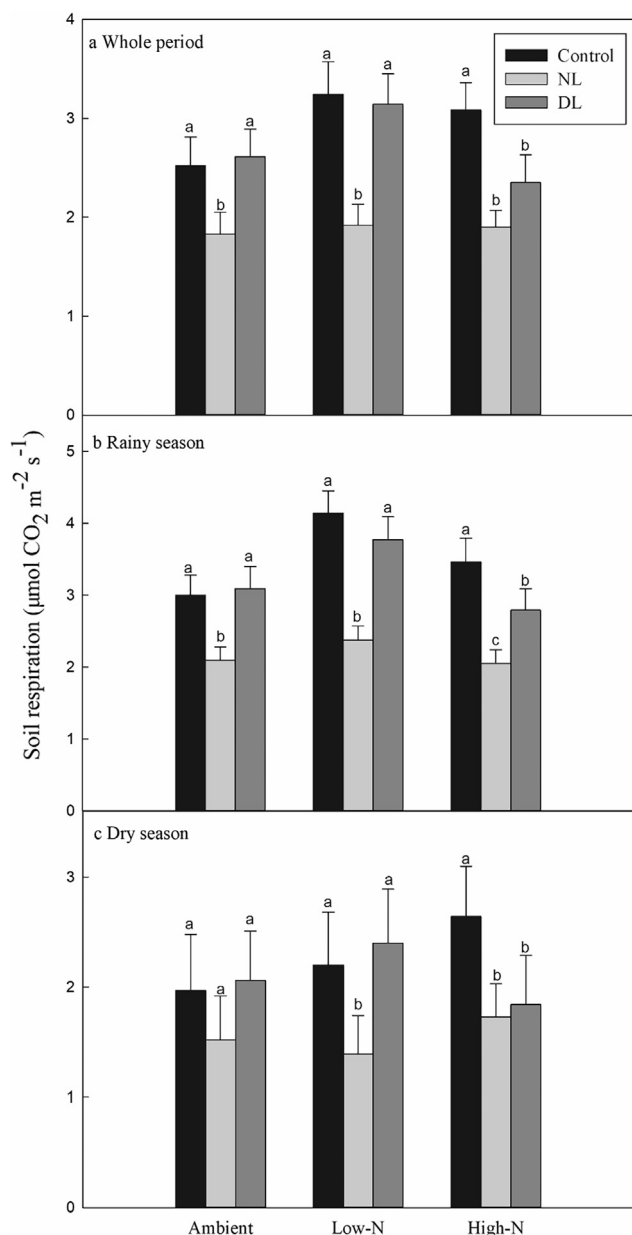
### 2.2. Experimental design

Experimental plots were established in January 2011. Nine 20 × 20 m plots were established in a randomized block design. Plots were selected so as to minimize heterogeneity in stand structure and function. All plots are located within a buffer zone at least 10 cm. We built concrete and plastic barriers around each plot to the depth of the parent rock while establishing the experimental plots, avoiding roots growing outside the treatment area. Treatments consisted of low (50 kg N ha<sup>-1</sup> yr<sup>-1</sup>) and high (100 kg N ha<sup>-1</sup> yr<sup>-1</sup>) N additions, as well as an ambient control (0 kg N ha<sup>-1</sup> yr<sup>-1</sup>). Nitrogen fertilizer was applied monthly since January 2011 using a backpack sprayer that contained NH<sub>4</sub>NO<sub>3</sub> dissolved in 20 L of water. Control plots received 20 L of deionized water. The dose in the low-N addition treatment were based on the present atmospheric N deposition rates in this region and mimic their expected increase in the future (Galloway et al., 2004; Xie et al., 2008; Zhao et al., 2009). In contrast, the amount of N added in the high-N addition treatment is typical of many scientific fertilization experiments (see Hasselquist et al., 2012). Considering the potentially cumulative effects of low-N addition, high-N addition may be useful to predict the long-term effects of low-N deposition.

Litter manipulation consisted of three treatments in each N addition plot: litter removal (no-litter, NL) and litter addition (litter doubling, DL) and Control litter input. For the NL, all litter materials on soil surface were removed at the beginning of the study. We then used a 1 × 1 m nylon mesh trap just above the soil collar in each plot to prevent the input of fresh fallen litter, with a mesh size of 1 mm, located 0.8 m above the soil surface. The litter in the trap was removed at monthly intervals in all NL treatments. The aboveground litter was transferred and the Control litter input was kept the natural status of litter on soil surface and received the normal input process of aboveground litter during the whole study period.

### 2.3. Measurement of soil respiration, soil temperature, and moisture

Starting February 2011, R<sub>s</sub> was measured once or twice monthly using a portable, closed chamber technique (LI-8100 Automated Soil CO<sub>2</sub> Flux System, Li-Cor Inc, Lincoln, NE, USA). Under each litter manipulation and N addition treatment, we randomly inserted PVC soil collars (20 cm diameter and 10 cm in height) for R<sub>s</sub> measurements, which were left *in situ* for the entire study period. Soil collars were inserted ~ 3 cm into the soil surface and allowed to equilibrate for at least 24 h before taking the first measurement. Aboveground biomass within each collar was removed by hand at least one day prior to the measurements to eliminate the contribution of aboveground respiration. In each plot, R<sub>s</sub> was measured three times between 9:00–17:00 h

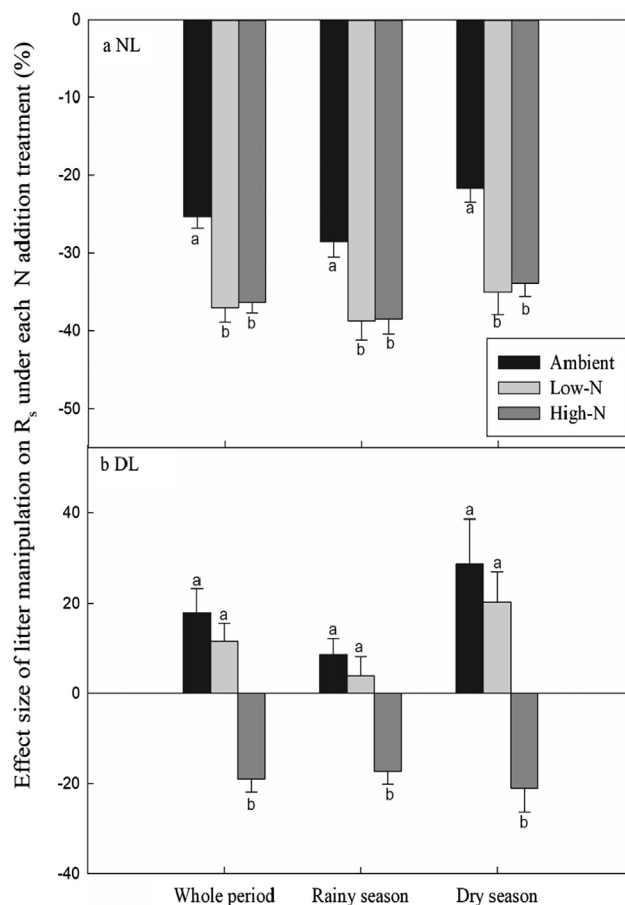


**Fig. 2.** Soil respiration within the different litter manipulation (NL, DL and Control) under N additions in study period (a), rainy (b) and dry (c) seasons in a subtropical *Schima superba* forest in eastern China. Ambient N addition of 0 kg N ha<sup>-1</sup> yr<sup>-1</sup>, low-N addition of 50 kg N ha<sup>-1</sup> yr<sup>-1</sup>, and high-N addition of 100 kg N ha<sup>-1</sup> yr<sup>-1</sup>, respectively. Lowercase letters represent significant differences under the same N addition and varied litter manipulation. Asterisk show significant differences between rainy and dry seasons under the same N addition and litter manipulation. Data point represents the mean ± SE (N = 3).

on each sampling date. The average value of the three measurements under the same treatment was used for data analysis. At the same time as R<sub>s</sub> measurements were taken, we also measured soil temperature at 10 cm depth using the Li-Cor 8100 soil temperature probe. Soil volumetric water moisture in the top 5 cm was also measured adjacent to each soil collar using soil moisture probes (Echo EC-5, Decagon Devices Inc, Pullman, WA, USA).

**2.4. Data analysis**

We calculated the effect size of litter manipulation on R<sub>s</sub>, compared



**Fig. 3.** Effect size of NL (no-litter, a) and DL (double litter, b) treatments on R<sub>s</sub> under N additions in the whole study period, rainy and dry seasons in a subtropical *Schima superba* forest in eastern China. Ambient N addition of 0 kg N ha<sup>-1</sup> yr<sup>-1</sup>, low-N addition of 50 kg N ha<sup>-1</sup> yr<sup>-1</sup>, and high-N addition of 100 kg N ha<sup>-1</sup> yr<sup>-1</sup>, respectively. We calculated the effect size of litter manipulation on R<sub>s</sub>, compared to the R<sub>s</sub> in control litter input under each N addition treatment using the equation: Effect size (%) = (R<sub>NL(DL)</sub> - R<sub>Control</sub>) / R<sub>Control</sub> × 100, where R<sub>NL</sub>, R<sub>DL</sub> and R<sub>Control</sub> are the mean R<sub>s</sub> in NL and DL under each N addition. Data point represents the mean ± SE (N = 3).

to the R<sub>s</sub> under control litter input under each N addition treatment using the following equation:

$$\text{Effect size (\%)} = (R_{NL(DL)} - R_{Control}) / R_{Control} \times 100,$$

where R<sub>NL</sub>, R<sub>DL</sub> and R<sub>Control</sub> are the mean R<sub>s</sub> in litter manipulation under each N addition.

Prior to statistical analysis, R<sub>s</sub>, soil temperature and moisture from the three plots in each litter manipulation and N addition treatment were averaged for each sampling date. All data sets were tested for normal distribution and homogeneous variance using Shapiro-Wilkson test and Levene statistics, respectively. For each R<sub>s</sub>, soil temperature and soil moisture, a three-way ANOVA followed by *post hoc* LSD tests was used to detect their differences among N-additions, litter manipulation and seasons. Two-way ANOVA followed by *post hoc* LSD tests was applied to diagnose the differences in R<sub>s</sub>, soil temperature and soil moisture among N-addition and litter manipulation plots. One-way ANOVA followed by *post hoc* LSD tests was performed to inspect the differences in R<sub>s</sub>, soil temperature and soil moisture among N additions or litter manipulation in rainy and dry seasons and the whole study period, respectively. R<sub>s</sub>, soil temperature and moisture under different combined treatments were divided into discrete periods as rainy and dry seasons: rainy season (April to June, September to November), and dry season (July to August, December to March). Student's *t*-test was



conducted to compare the seasonal variations between rainy and dry seasons on the differences in  $R_s$ , soil temperature and soil moisture among combined treatments of N addition and litter manipulation in rainy and dry seasons and the whole study period, respectively.

One-way ANOVA followed by *post hoc* LSD tests was conducted to compare both the difference in the effect size of  $R_s$  in NL and DL to total  $R_s$  in each N addition in rainy and dry seasons and the whole study period, respectively. All statistical analyses were performed using SPSS 23.0 for Windows (IBM Corp., Armonk, NY, USA). Graphic illustrations were generated using SigmaPlot 12.5 (Systat Software Inc., USA). Mean values  $\pm 1$  standard error were reported in the text. Statistically significant differences were identified when  $P < 0.05$ , unless otherwise stated. Mean values  $\pm 1$  standard error are reported in the text.

### 3. Results

#### 3.1. Variations in soil temperature and moisture

During the study period, soil temperature showed strong seasonal variations with higher soil temperatures in July–August among treatments, compared to the rest of the year (Fig. 1). Moreover, soil temperature was higher in the rainy seasons compared to the dry seasons ( $P < 0.001$ , Tables 1 and 2). In contrast, we observed very little variation in soil moisture during the observation period (25–35% (v/v)) for all treatments (Fig. 1). However, under low-N addition, both NL ( $P = 0.004$ ) and DL ( $P = 0.002$ ) increased soil moisture by ca. 5% compared to the Control litter input in the dry season, and the similar patterns also existed in the rainy season (Table 2). Under high-N addition, DL increased soil moisture by ca. 5% ( $P = 0.001$ ), compared to the Control litter input, and the similar responses occurred in both rainy and dry seasons (Table 2).

Meanwhile, under the Control litter input, we just found ca. 4% increase in soil moisture under high-N addition in the rainy season, compared to that under the ambient N addition ( $P = 0.028$ , Table 2). DL under low- and high-N additions decreased soil moisture by ca. 5 and 4%, respectively ( $P < 0.001$  and  $P = 0.001$ ), compared to the ambient N addition during the study period, which was consistent with the trends in the rainy and dry seasons (Table 2).

#### 3.2. Variability of $R_s$ under N addition and litter manipulation

There were strong seasonal variations in  $R_s$  under N addition and litter manipulation during the study period (Fig. 1), with higher  $R_s$  in the rainy season compared to the dry season for all treatments ( $P < 0.001$ , Fig. 2), except the  $R_s$  under NL and high-N addition. Under low-N addition, NL decreased  $R_s$  by ca. 41% ( $P < 0.001$ ), and the decrease was ca. 43% and 37% in rainy and dry seasons, respectively ( $P < 0.001$  and  $P = 0.049$ , respectively, Fig. 2). In addition, under high-N addition, both NL and DL decreased  $R_s$  by ca. 38% and 24% ( $P < 0.001$  and  $P = 0.002$ , Fig. 2) during the study period, where the decrease was ca. 41% and 19% in the rainy season ( $P < 0.001$  and  $P = 0.014$ , respectively, Fig. 2), and was ca. 34% and 30% in the dry season ( $P = 0.010$  and 0.046, Fig. 2), respectively.

#### 3.3. Effect size of N addition and litter manipulation on $R_s$

Following the N addition gradients, compared to NL under ambient N addition, the effect size of NL ( $-25.32 \pm 1.51$ ) decreased under low-N ( $-37.00 \pm 1.89$ ,  $P < 0.001$ ) and high-N additions ( $-36.32 \pm 1.37$ ,  $P < 0.001$ ), respectively. And the effect size of DL ( $17.86 \pm 5.25$ ) to  $R_s$  also decreased under low-N ( $11.47 \pm 4.08$ ,  $P < 0.001$ ) and high-N additions ( $-19.06 \pm 2.91$ ,  $P < 0.001$ ), respectively (Fig. 3).

Meanwhile, the similar trends were also found in rainy and dry seasons (Fig. 3). In rainy season, the effect size of NL on  $R_s$  decreased under low-N ( $-38.72 \pm 2.50\%$ ,  $P = 0.002$ ) and high-N additions

( $-38.47 \pm 1.96\%$ ,  $P = 0.002$ ) (Fig. 3), compared to that under ambient N addition ( $-28.53 \pm 2.03\%$ ). The effect size of DL on  $R_s$  under ambient N addition ( $30.65 \pm 3.57\%$ ) was higher than that under high-N addition ( $-17.34 \pm 2.89\%$ ,  $P = 0.002$ ) (Fig. 3). In dry season, the effect size of NL on  $R_s$  under ambient N addition ( $-21.61 \pm 1.84\%$ ) was higher than that under low-N ( $-35.01 \pm 2.87\%$ ,  $P < 0.001$ ) and high-N additions ( $-33.84 \pm 1.72\%$ ,  $P < 0.001$ ) (Fig. 3). The effect size of DL on  $R_s$  under ambient N addition ( $28.66 \pm 9.9\%$ ) was higher than that under high-N addition ( $-21.04 \pm 5.4\%$ ,  $P < 0.001$ ) (Fig. 3).

## 4. Discussion

### 4.1. Effect of litter removal on $R_s$ under N addition

Our previous study showed that NL decreased  $R_s$  under ambient N addition mainly due to the reduction of available substrates (Gao et al., 2015), which was consistent with other studies in subtropical forests (Sayer et al., 2011; Leff et al., 2012; Wang et al., 2013; Fang et al., 2015; Liu et al., 2017). Meanwhile, we have found a non-linear response of total  $R_s$  to increasing N availability in this subtropical evergreen forest (Gao et al., 2014). However, contrary to our hypothesis (1) and (2), the  $R_s$  and effect size of NL decreased under low- and high-N additions, with slightly higher decreases under low-N addition than that under high-N addition, which means N addition aggravated the decrease of  $R_s$  under NL in this study, compared to the  $R_s$  under Control litter input and ambient N addition.

It has been shown in our previous study that component fluxes of  $R_s$  (heterotrophic and autotrophic respiration) responded differently to N additions, and the higher  $R_s$  under short-term low-N addition was mainly attributed to enhanced root respiration and litter respiration in this subtropical evergreen forest (Gao et al., 2018). Additionally, microbial respiration reduced under high-N addition rates due to soil acidification (Ochoa-Hueso et al., 2014; Luo et al., 2016), which could lead to exhausted base cations, elevated exchangeable  $H^+$  and  $Al^{3+}$  mobilization and pronounced toxicity to soil biota (Högberg et al., 2006; Lu et al., 2014). Meanwhile, the similarity in the amount of microbial biomass between low- and high-N additions, suggests that, in our N-limited experimental stand (Yan et al., 2008b), the annual addition of  $50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  may exceed the threshold for optimal N input for soil microbes (Aber et al., 1989; Lu et al., 2014). Thus, the similar effect size of NL on  $R_s$  between low- and high-N additions. Even in an N-saturated subtropical forest, Yan et al. (2017) also found similar responses of  $R_s$  between low- and high- N additions. Furthermore, litter possesses an important microbial community (Sayer, 2006), and NL treatment not only decreases the amount of labile C available, but also decreases the amount of decomposers. In hence, under combination of NL and low-N addition, the microbial respiration decreased more, compared to NL under ambient N addition, and thus resulting in the lower effect size. On the other hand, NL reduced the soil moisture under high-N addition (Fig. 1, Table 2), which could affect root growth and the diffusion of oxygen (Skopp et al., 1990), reduce microbial activities and limit the metabolism of heterotrophs, thus reducing  $R_s$  (Leff et al., 2012; Fekete et al., 2014).

### 4.2. Effect of litter addition on $R_s$ under N addition

Inconsistent with our hypothesis (1),  $R_s$  decreased under the combined effects of DL and high-N addition. Meanwhile, inconsistent with our hypothesis (2), we just found high-N addition decreased the effect size of DL on  $R_s$ .

Our previous study showed that DL did not affect  $R_s$  (Gao et al., 2015), opposite to the common dogma that DL would enhance  $R_s$  (Kuzayakov, 2010). This was probably because that the lack of priming effect and the increase in new organic C from litter input may still increase soil organic C, despite the higher release of older soil organic C

in the short time period (Hofmöckel et al., 2011; Kuzyakov, 2011; Leff et al., 2012). In this study, we found  $R_s$  decreased under DL and high-N addition, which might be because that the higher soil moisture may limit the soil oxygen availability, thus decreasing the priming effect on  $R_s$  (Luo et al., 2016; Wang et al., 2016b). Furthermore, the litter decomposition could be inhibited by higher soil moisture (García-Pausas et al., 2004), resulting in the reduction of easily decomposable substrates to microbes and thus decreasing  $R_s$  (Crow et al., 2009). In this study, although soil moisture under DL increased under both low- and high -N additions (Fig. 1, Table 2), there were no distinct response of  $R_s$  under the combined effects of DL and low-N addition. This might be because that the decrease in  $R_s$  under the combined effects of DL and low-N addition offsets the increased contribution of  $R_s$  in other dryer locations (Chen and Chen, 2018) or balances the increase in  $R_s$  under low-N addition.

As we know,  $R_s$  may change due to altered microclimate factors in litter manipulation experiments, such as soil temperature and soil moisture (Sayer et al., 2011; Vogel et al., 2013). Although it has been shown that litter respiration is positively correlated with temperature (Bothwell et al., 2014; Xiao et al., 2014), there was no difference in soil temperature among treatments of N addition and litter manipulation in this study (Table 1), which may be due to the relative stability of crown closure during the study period, maintaining stable solar radiation into the study plots (Lowman and Schowalter, 2012).

In addition, either the effects of experimental N addition or the litter input alteration on  $R_s$  depended on the experimental duration, and long-term responses of  $R_s$  in litter input under N addition may differ from the short-term responses (Janssens et al., 2010; Zhou et al., 2014; Chen and Chen, 2018). N addition often stimulates plant growth and hence increases the root respiration and  $R_s$  in the beginning (Högberg et al., 2006), whereas N addition gradually decreases  $R_s$  (Nohrstedt, 2001), due to the subsequent changes of microbial community (Treseder, 2008). Meanwhile, Morrissey et al. (2017) found changes in soil microbial community composition could be in line with temporal changes in its C use. Soil microorganisms prefer to utilize the fresh litter under DL in the beginning, but switch to utilize the native soil C later due to the inhibition of enzyme activities under the long-term litter input (Morrissey et al., 2017). Therefore, the long-term N addition and litter manipulation experiments are needed to resolve the effect of litter input alteration on  $R_s$  under N addition in the future.

## 5. Conclusions

The study enhanced our understanding on how litter inputs affect  $R_s$  under N addition in a subtropical forest where anthropogenic N deposition is projected to increase in the future. We found N addition decreased the effects of NL on  $R_s$ , mainly due to the decrease in microbial respiration. High-N addition somewhat decreased  $R_s$  and the effect size when litter input was doubled compared to  $R_s$  under high-N addition. These can be ascribed to the high soil moisture in DL under high-N addition which reduced the priming effects on  $R_s$ . The inhibition of litter decomposition from DL under high-N addition could also be a potential mechanism. In conclusion, our study showed that when N addition alters the input process from litter into soil,  $R_s$  may respond differently from the response under N addition or litter manipulation alone. Long-term effects of N addition via litter input alteration on  $R_s$  and the contributions of aboveground litter inputs to the soil organic C sequestration should be further studied in subtropical forest ecosystems.

## Acknowledgements

We thank anonymous reviewers for their insightful comments and suggestions. We also thank the staff who provided assistance for sampling and investigations from Tiantong National Station for Forest Ecosystem Research, Chinese National Ecosystem Observation and

Research Network. This research was financially supported by National Natural Science Foundation of China (31000231, 31722009 and 31600343) and Thousand Young Talents Program in China.

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