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Short communication

Short-term response of soil respiration to nitrogen fertilization in a subtropical evergreen forest

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A B S T R A C T

Little is known about the effects of nitrogen (N) additions on soil respiration (Rs) in tropical and subtropical forests. We therefore conducted an N-fertilization experiment in a subtropical evergreen forest in eastern China to better understand the short-term response of Rs to increased N availability. N additions stimulated Rs compared to control plots, yet the magnitude of the increase depended on the amount of N added, with Rs being greater in the low-N treatment (50 kg N ha^{-1} yr^{-1}) than the high-N treatment (100 kg N ha^{-1} yr^{-1}). Differences in Rs among treatments correlated with changes in fine root biomass, suggesting increases in Rs reflect those in autotrophic respiration. Our findings challenge the dogma that N fertilization often reduces soil respiration and highlights the need to better understand the effects of low N additions, so as to reliably predict how projected climate change scenarios may affect the cycling of soil carbon (C) in tropical and subtropical forests.

Changes in soil nitrogen (N) availability has the potential to drastically alter the global carbon (C) cycle (Cox et al., 2000; Cramer et al., 2001) and, in particular, strongly affect pools and fluxes of soil C (Johnson and Curtis, 2001). Although there is an emerging consensus that N fertilization reduces soil respiration (Rs) (see Janssens et al. (2010)), it is based on studies mostly conducted in temperate and boreal forests (Bond-Lamberty and Thomson, 2010). Few studies have examined the response of Rs to N addition in subtropical and tropical forests where N deposition rates are projected to increase as a result of rapid expansion of industrial and agricultural activities (Reay et al., 2008). Focusing on a subtropical evergreen forest in eastern China we examined the short-term response of Rs, and of factors contributing to it, to two levels of N additions.

The study was conducted in a subtropical evergreen forest in Tiantong National Forest Park (29°52′N, 121°39′E, 200 m a.s.l.). The area is characterized by a subtropical monsoon climate (warm, rainy season, and cool, dry season) with annual mean temperature of 16 °C, and precipitation of 1374 mm (Fig. S1). The stand was harvested in the 1960s and has since undergone natural reforestation. Average stand density is 4178 ± 206 trees ha^{-1} with the canopy composed mostly of *Schima superba*, *Lithocarpus glaber* and *Symplocos sumuntia*. The soil type at the site is an Acrisol (Shen et al., 2012), with a medium-heavy loam texture and an organic layer roughly 5 cm thick (Song and Wang, 1995). More details of soil properties are given in Table 1.

At the site, nine 20 × 20 m plots were randomly selected and subjected to three treatments ($N = 3$): low and high N additions (50 and 100 kg N ha^{-1} yr^{-1}, respectively) and an unfertilized control. Starting in January 2011, N fertilizer (NH_4NO_3 in 20 L of water) was applied monthly. Control plots received 20 L of water. Rs was measured monthly for 16 months using a portable, closed chamber technique (LI-8100, Li-Cor Inc., Lincoln, NE, USA). Soil collars (20 cm in diameter and 10 cm in height) were inserted ~3 cm into the soil surface with litter layer left in place and allowed to equilibrate for at least 24 h before the first measurement. At each sampling date and plot, Rs was measured between 9:00 and 17:00 h and rates were averaged for data analysis. Rs measurement was accompanied by recordings of soil temperature at 10 cm depth (Li-8100) and volumetric soil water content in the top 5 cm of the soil profile (Echo EC-5, Decagon Devices Inc, Pullman, WA, USA).

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Table 1
Comparison of soil properties (0–10 cm depth) among N treatment plots in a subtropical evergreen forest in eastern China. Treatments included an unfertilized control, a low-N addition of 50 kg N ha^{-1} yr^{-1}, and a high-N addition of 100 kg N ha^{-1} yr^{-1}, respectively. Values shown are average ±SE ($N = 3$). Significant differences among N treatment plots are indicated by different letters, based on a one-way ANOVA followed by *post hoc* tests.

Soil properties	Nitrogen treatment plots		
	Control	Low-N	High-N
pH	4.88 ± 0.32	4.96 ± 0.29	4.20 ± 0.05
Bulk density (g cm^{-3})	1.07 ± 0.06	1.13 ± 0.05	1.06 ± 0.12
Total N (%)	0.18 ± 0.02	0.19 ± 0.01	0.19 ± 0.01
Total P (%)	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.01
SOM (%)	4.78 ± 0.44	4.83 ± 0.54	5.55 ± 0.98
C:N ratio	15.79 ± 1.85	14.79 ± 1.28	16.46 ± 2.49
Fine root (g m^{-2})	71.98 ± 18.46	171.42 ± 40.38	121.88 ± 40.51
N in fine root (mg g^{-1})	6.67 ± 0.08^{a}	9.24 ± 0.20^{b}	8.77 ± 0.30^{b}
Litterfall : Fine root ratio	12.85 ± 4.51	4.99 ± 1.15	8.75 ± 3.99
Total N in fine roots (g m^{-2})	0.48 ± 0.12^{a}	1.60 ± 0.41^{b}	$1.08 \pm 0.38^{a,b}$
Microbial biomass (mg g^{-1})	1.75 ± 0.14	1.58 ± 0.25	1.52 ± 0.19

Litter was collected monthly in each plot using four 1 × 1 m nylon mesh traps. In September 2011, a composite sample (three samples, 0–10 cm depth) in each plot was collected for determination of fine root biomass (diameter ≤ 2 mm), pH, and soil organic matter (SOM). Fine roots were powderized and analyzed for N concentration using the flow injection auto-analyzer (Skalar San ++, Netherlands). Soil pH, bulk density, and total N and phosphorus concentrations were determined using standard methods (Sun et al., 2007), and SOM content using the oil bath-K_2CrO_4 titration method (Nelson and Sommers, 1975). Two composite samples (five soil cores) were collected from each plot at the end of the experiment to determine soil microbial biomass using the fumigation extraction technique (Vance et al., 1987).

All datasets were tested for normality and homogeneous variance (Shapiro-Wilkson and Levene statistics). We used two-way ANOVA to test for differences in soil temperature, soil moisture, Rs, and litterfall among N treatments and seasons (rainy and dry), one-way ANOVA to test for differences in soil properties among N treatments, and least-square regression for relationships among measured variables.

Nitrogen additions stimulated Rs during the first 16 months of fertilization. This response was non-linear with mean Rs being 40% and 20% greater in the low- and high-N treatments compared to the

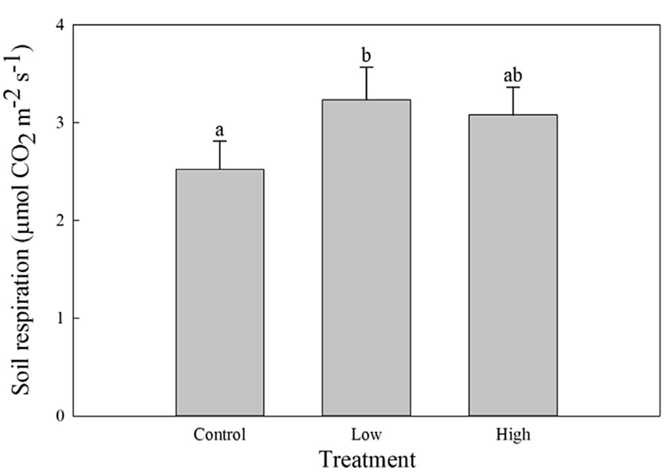

Fig. 1. Overall mean (±SE) soil respiration rate among N treatments during the first 16 months of N fertilization. Significant differences ($P < 0.05$) among N treatments are indicated by different letters.

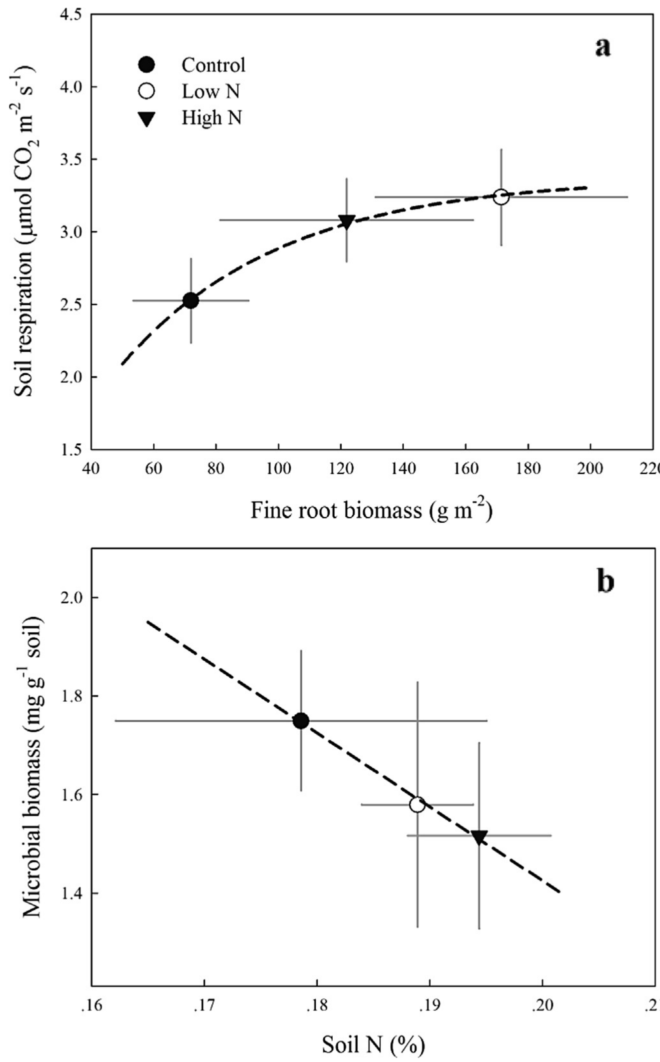

Fig. 2. Correlations between treatment means of fine root biomass and soil respiration (a) ($y = 3.37 \times (1 - e^{(-0.02(x)})$; $P = 0.04$) and soil N and microbial biomass (b) ($y = -15(x) + 4.42$; $P = 0.05$). Data point represents the mean ± SE ($N = 3$).

control, respectively ($P = 0.04$; Fig. 1). This finding is in contrast to the majority of studies showing a reduction in Rs with N additions (see Janssens et al., 2010). However, other studies have shown that N additions can stimulate Rs (Craine et al., 2001; Cleveland and Townsend, 2006; Yoshitake et al., 2007; Hasselquist et al., 2012). These discrepancies in the observed responses may reflect differences in site quality, with Rs decreasing with N additions in N-rich environments (Cusack et al., 2011) and increasing in N-limited forests (Hyvönen et al., 2007), like our experimental stand (Yan et al., 2008).

The major biotic contributors to Rs are autotrophic and heterotrophic respiration (Doff sotta et al., 2004). In our study, fine root biomass was 140% and 70% greater in the low- and high-N treatment compared to the control ($P = 0.09$ and 0.35, respectively). This resulted in a significant relationship between the treatment means of Rs and fine root biomass (Fig. 2a), similar to previous studies (Lee and Jose, 2003; Davidson et al., 2004). In contrast, we found a decreasing trend in microbial biomass with increasing soil N (Fig. 2b). Also the amount of substrate for heterotrophic respiration, SOM and litterfall, were similar among N treatments ($P = 0.95$ and $P = 0.91$, respectively; Table S2), which in part could be explained by the fact that leaf longevity of the dominant canopy trees is

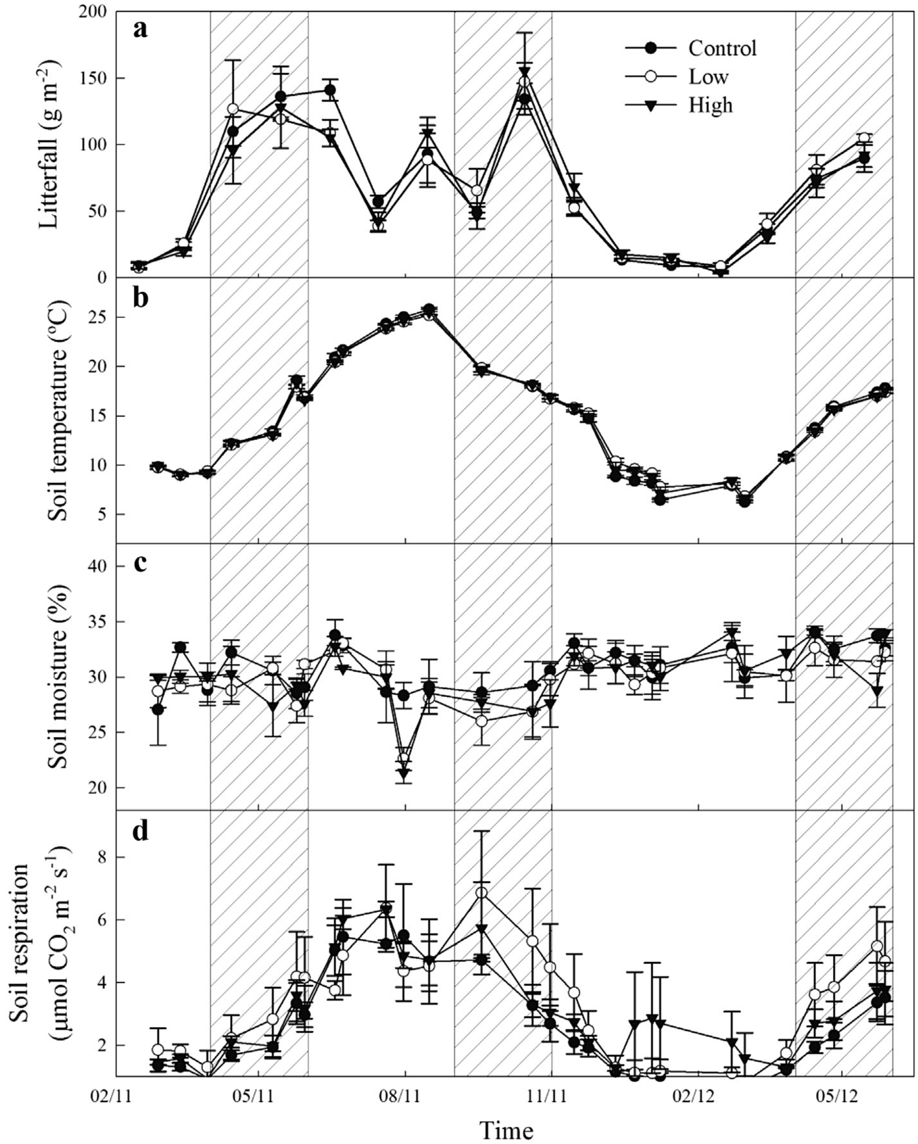

Fig. 3. Seasonal dynamics of litterfall (a), soil temperature at 10 cm depth (b), soil moisture within the top 5 cm of the soil profile (c), and soil respiration rate among the N treatments (d) in a subtropical evergreen forest in eastern China. Hatched areas correspond to the rainy season (April–June and September–November). Data points represent the mean ± SE ($N = 3$).

longer than the duration of this study (Huang et al., 2007). While seasonality of Rs mirrored that of litterfall, seasonal variation in Rs appeared to be mainly driven by changes in soil temperature ($R^2 = 0.72$; $P < 0.001$; Fig. 3). Taken together, our results suggest that greater Rs in response to N fertilization likely reflected an increase in autotrophic respiration. However, further studies using component integration, root exclusion and/or isotopic approaches are needed to better quantify the response of heterotrophic and autotrophic respiration to N additions in N-limited subtropical forests.

In conclusion, these results illustrate a short-term increase in Rs in response to N additions in a subtropical evergreen forest. The response was non-linear and depended on the amount of N added. This finding is in contrast to the common dogma that assumes a linear negative relationship between Rs and N fertilization and highlights the need to better understand how low rates of N addition affect Rs, so as to reliably predict the effects of N deposition on soil carbon pools and fluxes in forests where N deposition rates are projected to increase.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2014.04.020.

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