Nitrogen addition reduces soil respiration in a mature tropical forest in southern China

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Abstract

Response of soil respiration (CO_2 emission) to simulated nitrogen (N) deposition in a mature tropical forest in southern China was studied from October 2005 to September 2006. The objective was to test the hypothesis that N addition would reduce soil respiration in N saturated tropical forests. Static chamber and gas chromatography techniques were used to quantify the soil respiration, following four-levels of N treatments (Control, no N addition; Low-N, $5\,g\,N\,m^{-2}\,yr^{-1}$; Medium-N, $10\,g\,N\,m^{-2}\,yr^{-1}$; and High-N, $15\,g\,N\,m^{-2}\,yr^{-1}$ experimental inputs), which had been applied for 26 months before and continued throughout the respiration measurement period. Results showed that soil respiration exhibited a strong seasonal pattern, with the highest rates found in the warm and wet growing season (April–September) and the lowest rates in the dry dormant season (December–February). Soil respiration rates showed a significant positive exponential relationship with soil temperature, whereas soil moisture only affect soil respiration at dry conditions in the dormant season. Annual accumulative soil respiration was $601 \pm 30\,g\,CO_2\text{-}C\,m^{-2}\,yr^{-1}$ in the Controls. Annual mean soil respiration rate in the Control, Low-N and Medium-N treatments (69 ± 3, 72 ± 3 and $63 \pm 1\,mg\,CO_2\text{-}C\,m^{-2}\,h^{-1}$, respectively) did not differ significantly, whereas it was 14% lower in the High-N treatment ($58 \pm 3\,mg\,CO_2\text{-}C\,m^{-2}\,h^{-1}$) compared with the Control treatment, also the temperature sensitivity of respiration, Q_{10} was reduced from 2.6 in the Control with 2.2 in the High-N treatment. The decrease in soil respiration occurred in the warm and wet growing season and were correlated with a decrease in soil microbial activities and in fine root biomass in the N-treated plots. Our results suggest that response of soil respiration to atmospheric N deposition in tropical forests is a decline, but it may vary depending on the rate of N deposition.

Keywords: China, C sequestration, N deposition, N saturation, soil respiration, tropical forest

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Introduction

Human activities such as fossil fuel burning, forest disturbance, and land conversion have elevated the atmospheric concentration of carbon dioxide (CO_2) and increased the atmospheric deposition of nitrogen (N) (Matson *et al.*, 2002). Industrial development and agricultural intensification are projected to increase in the humid tropics over the next few decades, causing extensive changes to natural ecosystem in many tropical regions, especially in Asia (Galloway *et al.*, 2003). Over 40% of all N fertilizers are now used in the tropics and subtropics and over 60% will be used there by 2020 (Galloway *et al.*, 2003). At the same time, fossil fuel usage is expected to increase by several 100% in many areas of the tropics over the coming decades (Hall & Matson, 1999; Galloway *et al.*, 2003). Forest soil is an important source and sink of CO_2 (e.g. Bowden *et al.*, 2004). N additions to forest soils have shown variable effects on soil respiration (CO_2 emission), including increases, decreases, or unchanged rates after additional N inputs (Bowden *et al.*, 2000, 2004; Burton *et al.*, 2004; Micks *et al.*, 2004; Cleveland & Townsend, 2006). However, most studies of the N deposition effect

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on CO_2 flux have been performed in temperate forest ecosystems, which are often N-limited under natural conditions. Tropical forests differ from temperate forests not only in climate but also are more phosphorus (P) limited than N limited and the soils are often highly acidic with low base cation concentrations (Hall & Matson, 1999, 2003). So far, there have been very few studies of soil respiration responses to N deposition in subtropical and tropical forests (Cleveland & Townsend, 2006).

In Asia, the use and emission of reactive N increased from $14 \, Tg \, N \, yr^{-1}$ in 1961 to $68 \, Tg \, N \, yr^{-1}$ in 2000 and is expected to reach $105 \, Tg \, N \, yr^{-1}$ in 2030 (Zheng *et al.*, 2002). Currently this leads to high atmospheric N deposition (NH_4^+-N + NO_3^--N) in precipitation in some forests of southern China ($30–73 \, kg \, N \, ha^{-1} \, yr^{-1}$) (Ma, 1989; Ren *et al.*, 2000; Xu *et al.*, 2001). In forests of the Dinghushan Biosphere Reserve (DHSBR), N deposition was measured at $36 \, kg \, N \, ha^{-1} \, yr^{-1}$ in 1990 and $38 \, kg \, N \, ha^{-1} \, yr^{-1}$ in 1999 (Huang *et al.*, 1994; Zhou & Yan, 2001). Such high rates of N deposition are comparable with the highest levels of N deposition occurring in Europe (MacDonald *et al.*, 2002), which have been causing N saturation in forest ecosystems. The potential effects of N saturation may include significant changes in the fluxes of CO_2 (Bowden *et al.*, 2004; Micks *et al.*, 2004). It is, therefore, critical to address the effects of increasing deposition of N on the fluxes of CO_2 in the forests of China, especially in southern China where industry and agriculture have been increasing rapidly recently (Zheng *et al.*, 2002).

It has been hypothesized that chronic N additions to N-limited forest soil would initially stimulate soil microbial activity (and increase soil respiration), but over time would result in a carbon-limited state after combined plant and microbial demand for N was satisfied (Aber *et al.*, 1989, 1998). We have reported previously that mature tropical forests in southern China are N saturated and that N addition significantly decreased litter decomposition (Mo *et al.*, 2006; Fang *et al.*, 2007). The objective of this paper is to experimentally examine the effects of different levels of N deposition on soil respiration in a mature tropical forest in southern China. We hypothesize that N addition would reduce soil respiration in these N-saturated tropical forests through combined negative effects on plant and microbial activities.

Materials and methods

Site description

This study was conducted in the DHSBR, an UNESCO/ MAB site located in the middle Guangdong Province in southern China (112°10′E, 23°10′N). In the reserve, there are three major forest types an old-growth monsoon evergreen broadleaf forest (named mature forest hereafter), a mixed pine and broadleaf forest and a pine forest (the later two developed from a 1930 plantation effort). We have established research sites in all three types of forests but only report the data from the mature forest here. The mature forest, at about 250–300 m above sea level, occupies 20% of the reserve (Mo *et al.*, 2003), and is the representative forest of the lower subtropics in China (Wang *et al.*, 1982). These evergreen broadleaf forests have been protected from human impacts for more than 400 years (Zhou *et al.*, 2006).

The region has a monsoon climate and is located in a subtropical/tropical moist forest life zone (*sensu* Holdridge, 1967). The mean annual rainfall of 1927 mm has a distinct seasonal pattern, with 75% of it falling from March to August and only 6% from December to February (Huang & Fan, 1982). Annual mean relative humidity is 80%. Mean annual temperature is 21.0 °C, with an average coldest (January) and warmest (July) temperature of 12.6 and 28.0 °C, respectively. N deposition measured in 2004 and 2005 were 34 and $32 \, kg \, N \, ha^{-1} \, yr^{-1}$, respectively, with roughly 1:1 NH_4^+ to NO_3^- molar ratio (Fang *et al.*, 2007). A survey conducted in June 2003 (before the start of N addition) showed that the major species in the forest was dominated by *Castanopsis chinensis* Hance, *Schima superba* Chardn. & Champ., *Cryptocarya chinensis* (Hance) Hemsl., *Cryptocarya concinna* Hance, *Machilus chinensis* (Champ. Ex Benth.) Hemsl., *Syzygium rehderianum* Merr. & Perry in the canopy and sub-canopy layers, with a standing forest floor litter biomass of $8.9 \, Mg \, ha^{-1}$ (Fang *et al.*, 2006). Stem density, tree height and diameter at the breast height in this forest are given in Table 1. The soil in the study site is lateritic red earth formed from sandstone (oxisols) with a soil depth deeper than 60 cm (Mo *et al.*, 2003). Soil properties in the study site were measured using the samples collected in the Control plots (0–10 cm depth) in July 2004, and showed that soil pH, total C, total N, C/N ratio, available P and bulk density were 3.76 ± 0.01, 32 ± 3, $2.5 \pm 0.2 \, mg \, g^{-1}$, 13 ± 2, $5.0 \pm 0.2 \, mg \, kg^{-1}$ and $0.98 \pm 0.06 \, g \, cm^{-3}$, respectively (Mo *et al.*, 2006).

Experimental treatments

N addition experiments were initiated in 2003. Four N addition treatments (in three replicates) were established: Control (without N added), Low-N ($5 \, g \, N \, m^{-2} \, yr^{-1}$), Medium-N ($10 \, g \, N \, m^{-2} \, yr^{-1}$) and High-N ($15 \, g \, N \, m^{-2} \, yr^{-1}$). Twelve plots, each with $20 \, m \times 10 \, m$ dimensions were established, surrounded by a 10 m wide buffer strip. All plots and treatments were laid out randomly. Ammonium nitrate solution

Table 1 Indices of the forest structure in a mature tropical forest in southern China*

Species	Stem density (tree ha^{-1})	Mean height (m)	Mean DHB (cm)	Basal area (m^2 ha^{-1})	Relative basal area (%)
Castanopsis chinensis	83	12.7	23.5	9.6	37
Machilus chinensis	208	7.1	8.6	4.1	15.8
Schima superba	183	7.7	10.3	3.8	14.5
Cryptocarya chinensis	113	11.5	20.6	2.3	8.9
Syzygium rehderianum	129	11.1	29.4	1.6	6.2
Other trees	1013	5.4	5.8	4.6	17.6
Total	1729			26.0	100

*Data are from Fang *et al.* (2006).

was sprayed monthly as 12 equal applications over the entire year. During each application, fertilizer was weighed, mixed with 20 L of water, and applied to each plot below the canopy using a backpack sprayer. Two passes were made across each plot to ensure an even distribution of fertilizer. The Control plot received 20 L water without N.

Field sampling and measurements

Soil respiration measurements began 26 months after the initial experimental N application. Soil respiration was measured using the static chamber and gas chromatography techniques. Two static chambers were established in each plot at the start of the experiment (September 15, 2005) and the data from two chambers were pooled for each plot. Each chamber was a 25-cm-diameter ring anchored 5 cm into the soil permanently. During flux measurements, a 30-cm-high chamber top was attached to the ring. A fan (about 8 cm in diameter) was installed on the top wall of each chamber to ensure good mixing of the air when collected. Air was sampled from each chamber from 09:00 to 10:00 hours at each sampling date. Diurnal studies in an adjacent site (approximately 0.5 km from the current study site) showed that fluxes of soil CO_2 measured from 09:00 to 10:00 hours (121 ± 16 mg CO_2-C m^{-2} h^{-1}, $N = 10$) were close to daily means (117 ± 17 mg CO_2-C m^{-2} h^{-1}, $N = 10$) in the monsoon evergreen broadleaf forest (Tang *et al.*, 2006). Soil respiration was measured once a week during the growing season (April–September) and fortnightly other times. Gas samples were collected with 100 mL plastic syringes at 0, 10, 20 and 30 min after the chamber closure and analyzed for CO_2 within 24 h using gas chromatography (Agilent 4890D, Agilent Co., Santa Clara, CA, USA). Gas flux was calculated from the linear regression of concentration vs. time using the data points from each chamber to minimize the negative effect of chamber closure on CO_2 production (Keller & Reiners, 1994; Magill *et al.*, 1997; Tang *et al.*, 2006).

Coefficients of determination (r^2) for all linear regressions were >0.98. The static chamber technique is known to underestimate CO_2 production by about 10–15%, because of the rising concentration within the chamber headspace, reduce the diffusion gradient within the soil (Pumpanen *et al.*, 2004). Because we here focus on the comparison between treatments this underestimation is of minor importance.

Soil temperature and moisture at 5 cm below soil surface were monitored at each chamber when gas samples were collected. Soil temperature was measured using a digital thermometer. Volumetric soil moisture was measured simultaneously using a PMKit (Tang *et al.*, 2006).

Two litter traps (1 m × 1 m) with a mesh size of 1 mm were placed randomly in each plot about 0.5 m above the ground surface. The traps were emptied once every month during the year. Litterfall was separated into three components: leaf, small woody material (branches and bark), and miscellaneous (mainly reproductive parts).

Soil samples (0–10 cm depth) were collected in September 2005, using a 6.8 cm diameter stainless-steel corer to measure the fine root biomass (diameter ≤2 mm). Four composite samples of three cores were randomly collected from each plot. Roots were separated by washing and sieving, dried at 60 °C for 48 h and weighed (Cleveland & Townsend, 2006).

Soil samples (0–10 cm depth) were collected on March 30, 2006 to determine soil microbial biomass C (MBC) and extractable dissolved organic C (DOC). Two composite samples of eight cores (2.5 cm in diameter) to 10 cm deep were randomly collected from each plot. The composite samples were gently mixed and stored at 4 °C until processing. After removing large roots, wood and litter, samples were passed through a 2-mm-mesh sieve. Dissolved organic C were extracted with 0.5 M K_2SO_4 from soils with or without chloroform fumigation; and the MBC was calculated using DOC difference and a K_c factor of 0.33 (Jenkinson, 1987; Vance *et al.*,

1987). Extractable DOC in the K_2SO_4 extracts was analyzed using a total carbon analyzer (Shimadzu model TOC-500, Kyoto, Japan).

Statistical analysis

Repeated measures ANOVA [using PROC MIXED from SAS (SAS Institute, 2003)] was performed to examine the soil respiration rate, soil temperature and soil moisture among treatments for the period between October 2005 and September 2006. Soil respiration was square root transformed to improve the homogeneity of the variance. In this analysis we used each measured chamber as a replicate ($N = 6$ for each treatment). Out 816 observations seven were identified as outliers (probably due to chamber leaks) and deleted from the analysis. Subsets of data with a narrow range of either temperature or moisture were explored to investigate soil temperature and soil moisture interactions.

One-way ANOVA with Tukey's HSD test (SPSS 10.0 for windows) was used to test the difference among treatments in fine root biomass, soil microbial biomass C and soil extractable DOC as well as for monthly and yearly litterfall quantity and respiration. The relationship between soil respiration and litterfall was examined with linear regression with a lagging procedure (Cleveland & Townsend, 2006). We pooled the replicate data from each plot, yielding a sample size of three for each N treatment in this part of the analysis.

The relationship between soil respiration rates, soil temperature and soil moisture were further examined by linear and nonlinear regression models (Tang *et al.*, 2006). We fitted measured soil temperature (T) and respiration rate (R) to the exponential equation ($R = \alpha e^{\beta T}$) and obtained the Q_{10}-value from the β coefficient ($Q_{10} = e^{10\beta}$) (Lloyd & Taylor, 1994). One-way ANCOVA test was used to compare the regression slopes among treatments.

Statistical significant differences were set at P values <0.05 unless otherwise stated. Mean values in the text are given ± 1 standard error.

Results

Soil temperatures and moisture

Soil temperatures and moisture exhibited clear seasonal patterns in all plots (Fig. 1a and b). Soil was warm and wet from April to September (growing season) and became cool and dry from December to February (dormant season). There was no treatment effect on soil temperature ($P = 0.2$) and moisture ($P = 0.8$) (Fig. 1a and b), and due to the seasonal pattern soil temperatures were positively correlated with soil moistures

($P < 0.0001$, with $R^2 = 0.59$). The mean soil temperature was $21.3 \pm 0.1\,°C$ and mean soil moisture $24.5 \pm 0.3\,cm^3H_2O\,cm^{-3}$ soil in the measurement year across all plots.
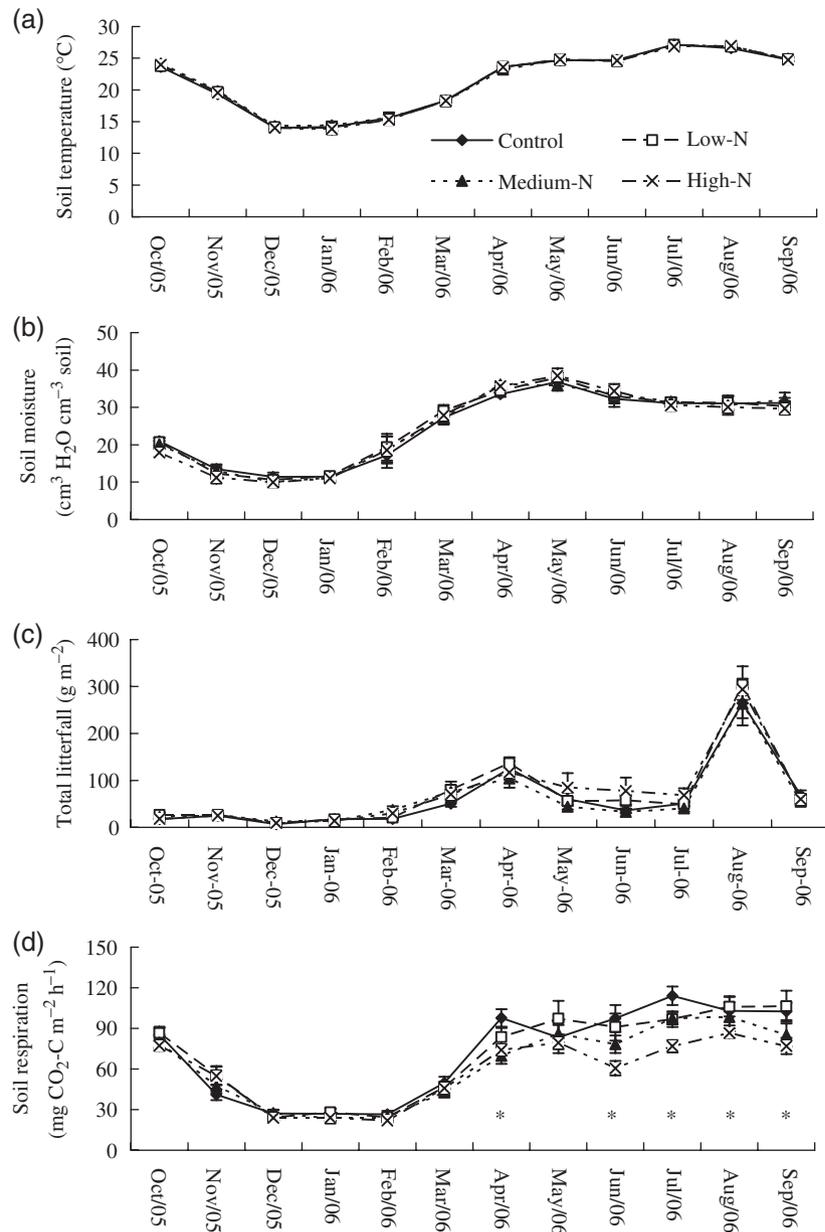
Litterfall

The mass of total litterfall in all plots showed a strong seasonal pattern, with a peak flux observed in August and the lowest input in the dormant season (Fig. 1c). The annual total litterfalls in the Control, Low-N, Medium-N and High-N plots were: 842 ± 164, 742 ± 140, 728 ± 146 and $868 \pm 157\,g\,m^{-2}\,yr^{-1}$, respectively, and were not significantly different among treatments ($P = 0.7$).

Soil respiration

Soil respiration followed a clear seasonal pattern with the highest rates in the growing season and the lowest rates in winter (Fig. 1d). In the Control plots, the mean soil respiration rate was two times higher in the growing season ($95 \pm 6\,mg\,CO_2$-$C\,m^{-2}\,h^{-1}$) than in winter ($43 \pm 1\,mg\,CO_2$-$C\,m^{-2}\,h^{-1}$). The annual mean soil respiration rate was $69 \pm 3\,mg\,CO_2$-$C\,m^{-2}\,h^{-1}$ during the study period, equivalent to an annual C flux of $601 \pm 30\,g\,CO_2$-$C\,m^{-2}$. A positive correlation between monthly soil respiration and litterfall in the Control plots was marginally significant ($P = 0.068$, $R^2 = 0.30$). The relationship became significant when litterfall data were compared with respiration fluxes lagging 1 month behind ($P = 0.035$, $R^2 = 0.37$) (Fig. 1c and d), suggesting a delayed effect of litterfall on soil respiration.

The repeated measures ANOVA showed that N addition significantly reduced soil respiration ($P < 0.001$). The treatment effect was evident in the warm and wet growing season (Fig. 1d), but not significant in the dry season ($P = 0.87$). Soil respiration rates were decreased significantly in the High-N treatment ($P < 0.005$) and marginally significant in the Medium-N treatment ($P = 0.067$) relative to the Control.

The annual mean soil respiration rates were thus also affected by the N treatment ($P = 0.013$), but the Low-N and Medium-N treatments (72 ± 3 and $63 \pm 1\,mg\,CO_2$-$C\,m^{-2}\,h^{-1}$, respectively) did not differ from the Control ($69 \pm 3\,mg\,CO_2$-$C\,m^{-2}\,h^{-1}$). Only the High-N treatment had a mean soil respiration ($59 \pm 1\,mg\,CO_2$-$C\,m^{-2}\,h^{-1}$) significantly lower (14%) than the Control (Fig. 2a). The effect of N addition on mean soil respiration rate in the warm season was similar to that of the whole year but more pronounced with a 20% lower rate in the High-N treatment ($76 \pm 2\,mg\,CO_2$-$C\,m^{-2}\,h^{-1}$) than in the Control ($95 \pm 6\,mg\,CO_2$-$C\,m^{-2}\,h^{-1}$).

Fig. 1 Seasonal variations of soil temperature, soil moisture, total litterfall and soil respiration rate in a mature tropical forest in southern China from October 2005 to September 2006. Plots received three levels of N inputs, in addition to the control. Bars indicate ± 1 SE, $N = 3$. (a) Soil temperature at 5 cm below surface; (b) volumetric soil moisture in the 0–5 cm soil layer; (c) total litterfall; (d) soil respiration rate, asterisk (*) indicates significant difference between Control and at least one level of experimental N inputs at $P < 0.05$. Monthly applications of NH_4NO_3 began in July 2003.

Soil temperature and moisture effects on soil respiration

The repeated measures ANOVA showed that soil respiration was influenced by soil temperature ($P < 0.0001$) and soil moisture ($P < 0.0001$), as well as by their interaction ($P = 0.0005$). The equal strong effects indicated for soil temperature and soil moisture may be due to their high intercorrelation at the site. A regression of residuals from a model with soil temperature alone against soil moisture did not reveal an influence of soil moisture. However, by analyzing subsets of data with low, medium and high soil moisture content we found a significant effect of soil moisture on respiration ($P = 0.016$) at dry conditions and a threshold for the influence was identified at 12 cm^3 H_2O cm^{-3} soil. Below this threshold the best-fitted regression model was a positive linear
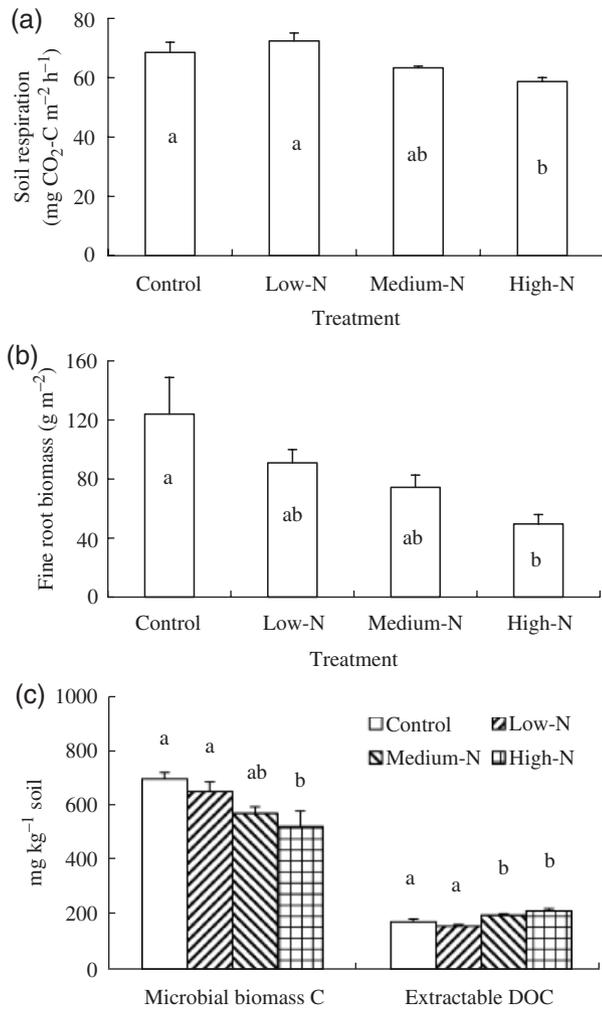
Fig. 2 Comparisons of mean soil respiration rate, fine root biomass, soil microbial biomass C and soil extractable DOC among N treatments in a mature tropical forest in southern China. Bars indicate ± 1 SE, $N = 3$. Soil respiration was measured from October 2005 to September 2006; fine root biomass (0–10 cm) was sampled in September 2005. Soil microbial biomass C and soil extractable DOC were sampled in March 2006 (from 0 to 10 cm soil). Different letters denote significant difference ($P < 0.05$) between treatments.

relationship of soil respiration with both temperature and moisture (Table 2a). Temperature was still the most important factor influencing soil respiration ($P < 0.0001$) below the threshold and adding moisture to a model with temperature only increased R^2 from 0.39 to 0.43.

Because the Low-N and the Control treatments did not differ we pooled the data from those plots to further analyse the effect of soil temperature and treatments on soil respiration. We used a subset of the data with moisture $> 12 \, cm^3 \, H_2O \, cm^{-3}$ soil to exclude the effect of dry conditions. Soil respiration rates exhibited significant positive exponential relationships with soil

temperatures for each of the treatments analysed (Fig. 3). The fitted exponential curves did not differ for Control + Low-N and the Medium-N treatment (Table 2b) and the mean temperature coefficient (Q_{10}) was 2.6 for both treatments. In the High-N plots respiration was reduced compared to the Controls which was reflected as a significantly lower β for the exponential curve and, thus a lower Q_{10} (2.2) for the High-N treatment (Table 2b, Fig. 3).

Fine root biomass, microbial biomass carbon (C) and extractable DOC

Fine root biomass decreased with increasing levels of N addition (Fig. 2b) and the difference between the Control and the High-N plots was significant ($P < 0.05$). Mean fine root biomass were 124 ± 25, 91 ± 9, 74 ± 8 and $50 \pm 6 \, g \, cm^{-2}$, respectively in the Control, Low-N, Medium-N and High-N plots. Similarly, soil microbial biomass C decreased with increasing levels of N addition (Fig. 2c). Soil extractable DOC, however, exhibited opposite pattern in response to N additions, with N additions significantly increased DOC concentrations (Fig. 2c).

Discussion

Soil respiration in all treatments followed a similar seasonal pattern, with the highest rates observed in the warm and wet growing season and the lowest rates in winter (Fig. 1d). This is consistent with many results reported in temperate forests (Dong et al., 1996; Zhang et al., 2001; Bowden et al., 2004). In some of these studies, the seasonality of soil respiration was interpreted as an effect of temperature only, with no effect of soil moisture (Dong et al., 1996; Zhang et al., 2001). In warm and moist forests positive exponential relationships with soil temperatures, as well as positive linear relations with soil moistures have been found in a tropical forest in the central Amazon (Sotta et al., 2004), a lowland tropical rain forest in southwest Costa Rica (Cleveland & Townsend, 2006) and in forests adjacent to ours (Tang et al., 2006). We also found significant effects of both soil temperature and moisture on soil respiration but this was due to a strong correlation between temperature and moisture ($P < 0.0001$, $R^2 = 0.59$). The intercorrelation reflects the monsoon tropical climate of the region, with a distinct separation of a warm and wet season and a cool and dry season (Fig. 1a and b). An effect of soil moisture on soil respiration could only be identified at dry conditions $< 12 \, cm^3 \, H_2O \, cm^{-3}$ soil. Below this threshold our data indicate that drought decrease soil respiration (Table 2a, positive relation between soil moisture and respiration). Drought effects on soil re-

Table 2 Models for the relationship between the soil CO_2 emissions (mg CO_2-C m^{-2} h^{-1}), soil temperature ($T\,°C$) and soil moisture content (M, cm^3 H$_2$O cm^{-3} soil)

Treatment	a	b	c	N	P-value	R^2
(a) Soil moisture $<12\,cm^3\,H_2O\,cm^{-3}$ soil: $Fco_2 = a \times T + b \times M - c$						
All	3.6 (0.3)	1.5 (0.5)	41 (6)	200	<0.00001	0.43
Treatment	α	β	Q_{10}	N	P-value	R^2
(b) Soil moisture $\geq 12\,cm^3\,H_2O\,cm^{-3}$ soil: $Fco_2 = \alpha \times e^{\,(\beta \times T)}$						
Control + Low-N	8.6 (0.9)	0.0942 (0.004)A	2.6	307	<0.00001	0.61
Medium-N	7.8 (0.9)	0.0940 (0.005)A	2.6	153	<0.00001	0.68
High-N	9.6 (1.2)	0.0807 (0.006)B	2.2	145	<0.00001	0.59

The β slopes with different letters are significantly different ($P<0.05$). Values in parentheses are standard errors. The Q_{10} value is obtained from β ($Q_{10} = e^{10\beta}$).
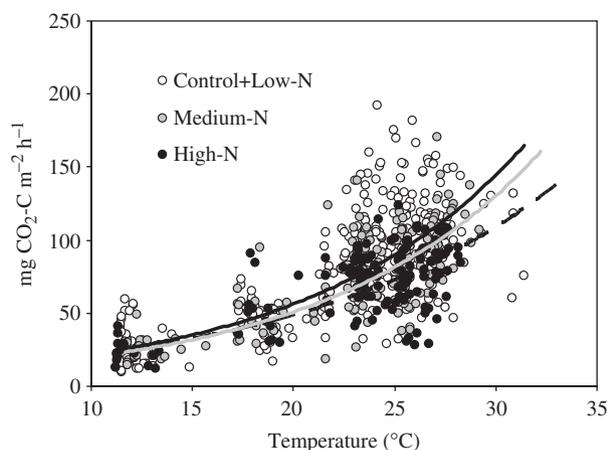
Fig. 3 Relationships between soil respiration rates and soil temperatures at 5 cm below surface in a mature tropical forest in southern China from October 2005 to September 2006. Fitted exponential lines represent Control + Low-N (solid black), Medium-N (solid gray) and High-N (dashed black), equation constants and statistics are in Table 2b.

spiration are known to occur below a certain threshold (e.g. Inclan et al., 2007) that may depend on soil texture (Dilustro et al., 2005).

Temperature was the dominant control on soil CO_2 flux with $R^2>0.59$ for the fitted exponential relationships (Fig. 3, Table 2b). The mean temperature coefficient (Q_{10}) for the respiration rate in the Control treatment of our study was 2.6. This value is higher than that in a tropical forest (2.1 ± 0.03, $N = 3$, Bekku et al., 2003), a subtropical forest in China (1.75–2.55, Huang et al., 1999), and the median value reported in a worldwide literature summary (2.4, Raich & Schlesinger, 1992), but lower than that in a temperate forest (2.9 ± 0.3, $N = 3$) (Bekku et al., 2003) and in a temperate mixed hardwood forest (3.9, Davidson et al., 1998). The annual mean soil respiration rate in the Control plots was 69 ± 3 mg CO_2-C m^{-2} h^{-1} during the monitoring period. This is lower than the data reported in an evergreen tropical forest on the island of Hawaii (82 mg CO_2-C m^{-2} h^{-1}, Townsend et al., 1995), is in the same range as found in tropical forests of South America (51–115 mg CO_2-C m^{-2} h^{-1}, Davidson et al., 2004; Sotta et al., 2004 and references therein), as well as in a previous study in adjacent forests (45–87 mg CO_2-C m^{-2} h^{-1}, Tang et al., 2006). Our results also showed that N addition significantly reduced soil respiration (Fig. 1d and Fig. 2a). These results were similar to those found in several temperate forests (Bowden et al., 2000, 2004; Maier & Kress, 2000; Burton et al., 2004; Micks et al., 2004), but were contradictory to those found in a tropical rain forest in Costa Rica, in which soil respiration rates were significantly stimulated by two years of N addition (Cleveland & Townsend, 2006). Furthermore, we found an effect of N additions on the temperature response of soil respiration, with a significant decline in Q_{10} from 2.6 (Control, Low-N and Medium-N treatments) to 2.2 in the High-N treatment (Table 2). This result suggests that N addition changes the temperature control on soil respiration. However, because the change only occurred in the High-N treatment it is more likely due to other mechanisms.

The reductions in soil respiration after N additions in our forest may be related to the following mechanisms. First, autotrophic respiration from plant roots may be decreased after N additions. Root biomass in organic horizons and mineral horizon to 20 cm was highest in the control plots and lowest in the High-N plots in a temperate forest (Bowden et al., 2004). This is consistent with our results where measurements of fine root biomass revealed a negative response to increasing level of N addition and a significant difference between Control and High-N plots ($P = 0.033$, Fig. 2b). It was reported that fine root biomass was significantly correlated with soil respiration rate in a moist tropical forest ($R^2 = 0.74$, Davidson et al., 2004) and that chronic N

additions could reduce belowground root input to the soil (Haynes & Gower, 1995; Boxman *et al.*, 1998).

Second, heterotrophic respiration from the microbial community may be reduced in N addition plots. During the period of April 2003–2004, measurements taken in a nearby forest showed that removal of the litter layer significantly reduced soil CO_2 efflux, and that the contribution of forest floor litter could account for 17% of total soil respiration (Tang *et al.*, 2006). Our results that soil respiration in Control plots was marginally correlated with litterfall ($P = 0.068$) and the correlation became significant when lagging litterfall data were used ($P = 0.035$) supported the importance of C input from litterfall on soil respiration. Although N addition had no significant effect on the mass of total litterfall, microbial respiration could be decreased in response to N additions if the rates of decomposition were decreased (Carreiro *et al.*, 2000; Burton *et al.*, 2004). In previous papers, we have reported that N addition significantly reduced litter decomposition rates in our forests (Mo *et al.*, 2006) and this negative effect became stronger with continued N addition (Fang *et al.*, 2007), implying a decrease in microbial activity in response to N addition (Mo *et al.*, 2006; Fang *et al.*, 2007). In this study, we found that high N addition significantly decreased soil microbial biomass C (Fig. 2c). Several studies in temperate forests also showed that N addition significant decreased soil microbial biomass (Arnebrandt *et al.*, 1990; Wallenstein, 2003; Bowden *et al.*, 2004; Compton *et al.*, 2004; Frey *et al.*, 2004). Lastly, the increase of soil extractable DOC under experimental N additions is in line with the recent literature reports that N deposition could increase regional and global DOC fluxes from terrestrial ecosystems to aquatic ecosystems (Deforest *et al.*, 2004; Findlay, 2005; Waldrop & Zak, 2006). But our data suggested such change may be minor compared with other C fluxes, such as the reduction of root biomass and microbial biomass, affecting root-affiliated autotrophic respiration and heterotrophic respiration.

A study of soil organic C content over two decades in the Dinghushan forest indicated an accumulation of soil C (0–20 cm depth) at about $54\,g\,C\,m^{-2}\,yr^{-1}$ (Zhou *et al.*, 2006). The reason for this accumulation is unclear, but one suggestion is that the elevated N deposition ($>30\,kgN\,ha^{-1}\,yr^{-1}$) over recent decades has increased litter C input, decreased soil respiration or both. Based on our data, a 9% reduction in soil respiration would be enough to explain the observed accumulation of soil C, which is less than the 14% decrease we observed at High-N from a couple of years N addition. The net result of N addition on the soil C balance in our experiment, however, remains unclear. Although the CO_2 efflux decreased at High-N, the C loss by DOC leaching may have increased and the C input from root exudates and litter may have decreased (due to lower root biomass in the High N). To resolve the effect of N deposition on soil C, studies on multiple aspects of soil C cycling are needed in long-term N addition experiments or across N deposition gradients.

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