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Effects of simulated acid rain on soil respiration and its components in a subtropical mixed conifer and broadleaf forest in southern China

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Soil respiration is a major pathway in the global carbon cycle and its response to environmental changes is an increasing concern. Here we explored how total soil respiration (R_T) and its components respond to elevated acid rain in a mixed conifer and broadleaf forest, one of the major forest types in southern China. R_T was measured twice a month in the first year under four treatment levels of simulated acid rain (SAR: CK, the local lake water, pH 4.7; T1, water pH 4.0; T2, water pH 3.25; and T3, water pH 2.5), and in the second year, R_T, litter-free soil respiration (R_S), and litter respiration (R_L) were measured simultaneously. The results indicated that the mean rate of R_T was 2.84 ± 0.20 μmol CO_2 m^{-2} s^{-1} in the CK plots, and R_S and R_L contributed 60.7% and 39.3% to R_T, respectively. SAR marginally reduced (P = 0.08) R_T in the first year, but significantly reduced R_T and its two components in the second year (P < 0.05). The negative effects were correlated with the decrease in soil microbial biomass and fine root biomass due to soil acidification under the SAR. The temperature coefficients (Q_{10}) of R_T and its two components generally decreased with increasing levels of the SAR, but only the decrease of R_T and R_L was significant (P < 0.05). In addition, the contribution of R_L to R_T decreased significantly under the SAR, indicating that R_L was more sensitive to the SAR than R_S. In the context of elevated acid rain, the decline trend of R_T in the forests in southern China appears to be attributable to the decline of soil respiration in the litter layer.

Environmental impact

Soil respiration plays a crucial role in the global carbon cycle. Its responses to acid rain in the forests of southern China, however, are poorly understood despite the fact that acid rain has become a serious environmental threat in this region. Here, we investigated this issue in a subtropical mixed conifer and broadleaf forest, with a special intention of quantifying the contribution of respiration derived from the litter-free soil and litter layer to the total soil respiration under acid rain. Our results support an important role of acid rain in regulating forest soil respiration in this region with soil respiration and its components significantly decreased under acid rain, and importantly, the litter respiration tended to respond more sensitively than the litter-free soil respiration.

Introduction

Acid rain is one of the most serious environmental problems in ecosystems and has received worldwide attention.[1] The main cause of acid rain is attributed to the release of sulfur dioxide (SO_2) and nitrogen oxides (NO_x)[2] that originate mostly from human activities such as the combustion of fossil fuels.[3,4] Accompanying rapid economic development and population growth, southern China has become the third largest area affected by acid rain, following Europe and North America.[1] In southern China, the annual mean pH value of precipitation is generally below 4.5 in recent years, and pH values as low as 3.5 have been observed.[5] Most ecosystems in this region have received large quantities of acidic inputs.[6] One of the consequences of the elevated acid rain is an alteration of the biogeochemical cycles and the stability of ecosystems.[7]

Ecosystem carbon (C) balance is determined by biological processes including C fixation (*e.g.*, plant photosynthesis) and C emission (*e.g.*, soil respiration).[8] Studies have shown that over two thirds of the gross primary productivity (GPP) in terrestrial ecosystems will return to the atmosphere through ecosystem respiration,[9] of which approximately 70% is from the soils.[10] In fact, second only to gross photosynthesis (100–120 PgC per year), soil respiration (68–98 PgC per year) exceeds all other terrestrial–atmospheric carbon exchanges,[11,12] and becomes

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a key component influencing terrestrial C cycles.[13] Effects of acid rain on soil respiration, therefore, can greatly affect the direction and extent of ecosystem C response.

Acid rain may have various impacts on soil chemistry and nutrients in forest ecosystems.[14] Acid rain is known to increase soil acidity and release base cations and toxic metals that are bound in the soil.[15] In one way, H^+ ions in the acid rain can displace cations from their binding sites in soil, reducing their exchange capacity, and increasing their concentrations in soil water.[16] Therefore, cations such as K^+, Na^+, Ca^{2+}, and Mg^{2+} are leached out and become unavailable to plants as nutrients.[4] In the other way, toxic ions such as Al^{3+} that are usually bound to the negatively charged surface of soil particles can be displaced by the H^+ ions in acid rain. This results in high concentrations of toxic ions in the soil solution, which may in turn be toxic to the fine roots and the microbial community.[17]

Since acid rain changes the conditions of soil and plant roots in forests, it may have remarkable effects on soil respiration.[18] However, mixed results in the responses of soil respiration to acid rain have been found in different ecosystems and regions. Positive,[19] neutral,[20] and negative[21] effects of acid rain on soil respiration have been reported. In addition, soil respiration can be divided into many different sources. Kuzyakov,[22] for example, distinguished five main biogenic sources of soil respiration. The potential effects and mechanisms of acid rain on soil respiration and its components may be different due to different controlling factors and variable biological processes in different terrestrial ecosystems. Until now, unfortunately, few studies of acid rain effects on soil respiration components have been conducted in forests.

In China, most of the land originally covered with primary forests has been degraded due to human activities over the past several centuries.[23] The area of a remnant mature native forest is less than 9% of the total forest area.[24] A mixed conifer and broadleaf forest (MF) usually occurs where evergreen monsoon rainforest is destroyed, and the MF occupies a significant area in southern China.[25,26] However, information regarding soil respiration, particular soil respiration components in these forests, is poorly known,[27,28] and its response to increased acid rain has seldom been studied.

We carried out a field experiment to investigate acid rain effects on soil respiration in a MF at the Dinghushan Forest Ecosystem Research Station in southern China. Both total soil respiration and its components (*i.e.* litter respiration and litter-free soil respiration) were measured, with an intention of better understanding the mechanisms of soil respiration responses to acid rain. The overall aims of this study were (1) to determine if acid rain affects soil respiration in subtropical forests with highly acidic soil, and, if so, to identify possible mechanisms of the observed effects; (2) to quantify the relative contributions of soil respiration from the litter-free soil and from the litter layer to total soil respiration under acid rain conditions; and (3) to evaluate the effects of acid rain on temperature sensitivities of total soil respiration and of its two components.

Materials and methods

Site description

The Dinghushan Forest Ecosystem Research Station is located in the central region of Guangdong Province in southern China (112°30′–112°33′E, 23°09′–23°11′N). The climate in the region is a typical south subtropical monsoon climate with a distinct seasonal pattern. The annual mean temperature is 21.0 °C with the maximum and minimum monthly mean temperatures being 28.0 °C in July and 12.6 °C in January, respectively. The annual precipitation is 1927 mm, of which nearly 75% falling from March to August and only 6% from December to February.[29] Acid rain is an environmental threat in this area with the frequency of acid rain having increased from 63% in 2003 to 97% in 2009,[30] and the annual average pH value of precipitation having varied from 4.90 in 2003 to 4.10 in 2007.[31]

At the station, there are three types of forests: the pine forest, the MF, and the broadleaf forest.[32] Our study site is located in the MF, with a stand age of about 100 years, occupying an area of 50% of the station. The major species in the canopy layer of the forest are *Pinus massoniana*, *Schima superba* Chardn. & Champ., and *Castanopsis chinensis* Hance.[33] The annual litter fall and surface accumulated litter of the forest are 701 ± 84 g per m^2 per year and 686 ± 96 g m^{-2}, respectively, and *Pinus massoniana* and *Schima superba* contributed 31% and 30% of the total leaf litter, respectively.[34] The soil is classified as lateritic red earth, with depth ranging from 30 to 60 cm.[35] The soil properties at the study site were measured using the samples collected in the control plots (0–10 cm depth) in March 2011, and showed that soil pH, total organic C, total N, C/N ratio, and available N were 3.8 ± 0.1, 37.8 ± 6.4 mg g^{-1}, 2.6 ± 0.9 mg g^{-1}, 14.5, and 13.5 ± 5.7 mg kg^{-1}, respectively. A previous study by Lu *et al.*[36] showed that soils at this study site are susceptible to acid rain with low cation exchange capacity (CEC, <125 $mmol_c$ kg^{-1} soil), low base saturation (BS, <8%), and negative water-extracted acid neutralizing capacity (ANC, <−0.3 $mmol_c$ kg^{-1} soil).

Experimental treatments

Sixteen plots were established within the study site in January 2011, with 1.7 m × 1.7 m for each plot at about 2 m intervals between adjacent plots. Around each SAR plot, the thick PVC panels were inserted at the top 15 cm soil layer to prevent surface runoff and lateral movement of water from the outside surrounding soil. A completely randomized design was used with four simulated acid rain (SAR) treatments. Four replicates were used for each treatment, and plots were randomly selected to receive the treatments. The SAR treatments were established by irrigating the plots with water of different pH values: CK (pH 4.7, the local lake water as the control), T1 (pH 4.0), T2 (pH 3.25), and T3 (pH 2.5). The SAR treatments were set based on the pH value of precipitation recorded in the region. The T1 treatment was set as the annual average value (pH 4.10 in 2007), and the T2 treatment as the lowest value (pH 3.17 in 2007) observed in the natural rain.[31] Considering

that the pH value of precipitation could decrease in the future, we set the T3 treatment 0.75 units lower than the T2 treatment. To reflect the tendency of the molar ratio of S : N of precipitation,[31] acidic solutions were prepared by adding a mixture of H_2SO_4 and HNO_3 (2 : 1 mole ratio) to the local lake water. The SAR treatments were initiated in March 2011 and the SAR events were manipulated twice a month. The SAR solution was applied to each plot below the canopy using a gasoline engine sprayer. The amount applied to each plot was 10 L per manipulation. The annual total H^+ load each plot received was 0.02, 0.1, 0.6, and 2.6 kmol per ha per year in the CK, T1, T2, and T3 treatments, respectively, which was equal to about 0.1, 0.3, 2.0, and 10.0 times of that in the natural rainfall of the study site.

For the measurement of total soil respiration (R_T), a PVC collar (20 cm in diameter) was installed with minimum disturbance to the intact soil surface and anchored 5 cm into the soil in each SAR treatment plot in March 2011. In the same plot, another collar for the measurement of litter-free soil respiration (R_S) was installed at about 0.5 m distance but with the entire litter layer carefully removed in March 2012. Throughout the experiment, a mesh enclosure covering an area of about 0.25 m^2 was established to allow precipitation to pass through, but prevented litter from falling or leaching onto the R_S collar. The difference between R_T and R_S was calculated as the respiration from the litter layer (*i.e.* R_L).

One month after the initial SAR application, R_T was measured twice a month from April 2011 to March 2013, using a Li-8100 Infrared Gas Analyzer (Li-Cor Inc., Lincoln, NE, USA) with a survey chamber attached. R_S was measured twice a month from April 2012 to March 2013. All measurements were made between 9:00 am and 11:00 am. Previous work at this study site has demonstrated that soil respiration in forests measured during this period was close to the daily mean.[36] The soil temperature (°C) at 5 cm depth and soil moisture (volumetric water content, %) of the top 5 cm soil layer were monitored adjacent to each PVC collar at the time of soil respiration measurements, using a digital thermometer and a PMKit, respectively.[37]

The 0–10 cm soil samples in all plots were collected using an auger (2.5 cm interior diameter) for measuring soil pH in March and December 2011, June 2012, and March 2013. Soil samples were also collected in June 2011 and June 2012 to determine soil microbial biomass carbon. Dead roots, litter, and plant residues in the soil samples were removed and the samples were passed through a 2 mm mesh sieve. The soil pH values were measured using a pH meter (1 : 2.5 soil–water ratio).[38] The soil microbial biomass C was estimated according to the method of Vance *et al.*[39] The fine root biomass (diameter ≤ 2 mm) in the 0–10 cm soil profile was sampled using a stainless-steel corer (6.8 cm interior diameter) in June 2011 and June 2012. It was dried at 60 °C for 48 h and weighed.[40]

Statistical analysis

We used repeated measures ANOVA to examine the effects of SAR treatments on soil respiration, soil temperature, soil moisture, and soil pH values among the treatments for the study period. Seasonal difference of soil respiration rate, soil temperature, and soil moisture was tested using one-way ANOVA. We also tested the SAR effects on the means of annual soil respiration, the contribution of R_S and R_L to R_T, soil microbial biomass C, and fine root biomass using one-way ANOVA. Previous work at this study site demonstrated that soil respiration increases exponentially with soil temperature and linearly with soil moisture.[33,37] Therefore, we first developed the relationship between soil respiration and soil temperature with an exponential equation:[41]

$$R = a\mathrm{e}^{(bT)} \tag{1}$$

and the relationship between soil respiration and soil moisture with a linear regression equation:[42]

$$R = aM + b \tag{2}$$

where R is the soil respiration rate (μmol CO_2 m^{-2} s^{-1}), T is the soil temperature (°C), M is the volumetric soil moisture (%), and a and b are constants fitted to the equation. Considering that the soil temperature and moisture may interactively regulate soil respiration, we also fit soil respiration with the soil temperature and soil moisture together using a two-factor soil respiration model:[43]

$$R = (a + cM)\mathrm{e}^{(bT)} \tag{3}$$

where a is the parameter related to basal soil respiration when both $T = 0$ and $M = 0$; and b and c are parameters related to the soil temperature and moisture sensitivities of soil respiration, respectively. The Q_{10} value, defined as the difference in respiration rates over a 10 °C interval, was calculated using the exponential relationship between soil respiration and soil temperature based on eqn (3):[44]

$$Q_{10} = \mathrm{e}^{(10b)} \tag{4}$$

One-way ANOVA was used to test the difference in the regression slope (b value) among the SAR treatments. We used measurements of soil respiration, soil temperature, and soil moisture of the whole year from April 2012 to March 2013. A non-linear least squares method was used to derive the model parameters using the SAS NLIN procedure.[45] The relationship between R_L and R_T was modeled with a linear function (*i.e.* proportional function);[44] the significance of the slopes among the SAR treatments was tested using one-way ANOVA. All data analyses were carried out using the SAS software version 9.1 (ref. 45) (SAS Institute Inc., Cary, NC, USA). Statistically significant differences are indicated by $P < 0.05$ unless otherwise stated.

Results

Soil temperature and soil moisture

Both soil temperature and soil moisture showed strong seasonal variations for all treatments (Fig. 1a and b). Soil was

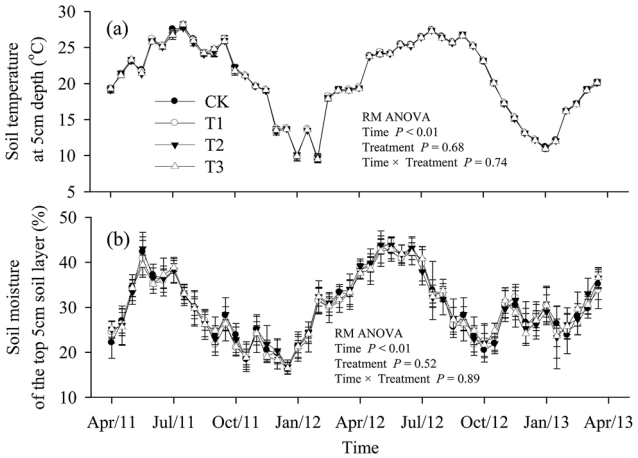

Fig. 1 Seasonal dynamics of the soil temperature and soil moisture under different simulated acid rain (SAR) treatments. (a) Soil temperature at 5 cm depth; (b) volumetric soil moisture of the top 5 cm soil layer. Error bars are standard errors of the mean (n = 4 for all the treatments). The inserted figures indicate P values of repeated measures ANOVAs. The SAR treatments are: CK = control, T1 = pH 4.0, T2 = pH 3.25, and T3 = pH 2.5.

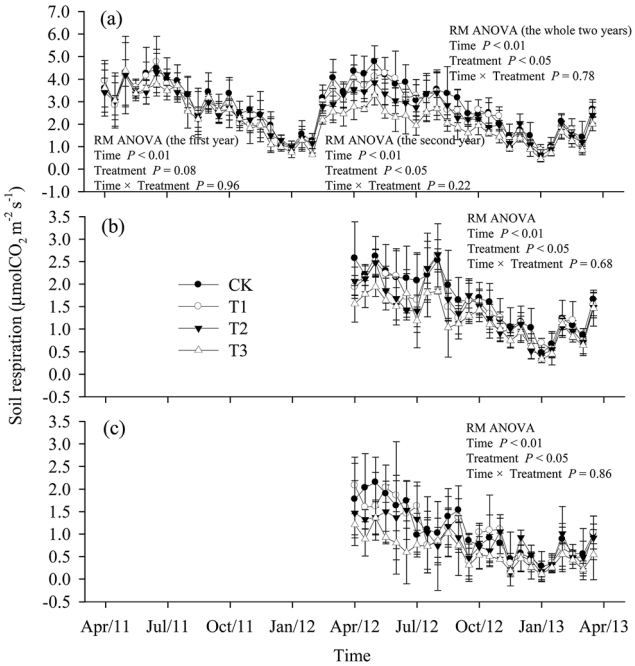

Fig. 2 Seasonal dynamics of total soil respiration, litter-free soil respiration, and litter respiration under different simulated acid rain (SAR) treatments. (a) Total soil respiration; (b) litter-free soil respiration; (c) litter respiration. Error bars are standard errors of the mean (n = 4 for all treatments). The inserted figures indicate P values of repeated measures ANOVAs. The SAR treatments are: CK = control, T1 = pH 4.0, T2 = pH 3.25, and T3 = pH 2.5.

warmer and wetter in the warm-wet seasons (from April to September) than in the cool-dry seasons (from October to March of the next year) (P < 0.05 for both). The annual mean soil temperature and moisture in the control plots were 20.60 ± 0.04 °C and 29.90 ± 0.21%, respectively. There were no differences in either soil temperature or soil moisture among different treatments throughout the two experimental years (P > 0.05 for both).

Total soil respiration and its two components

R_T and its two components in different treatments also showed clear seasonal patterns during the study period, with significantly higher rates in the warm-wet seasons and lower rates in the cool-dry seasons (P < 0.05 for all) (Fig. 2a–c). The mean rate of R_T in the CK plots was 2.84 ± 0.20 μmol CO_2 m^{-2} s^{-1}, and R_S and R_L contributed 60.7% and 39.3% to R_T, respectively (Table 1). Repeated measures ANOVA showed that the SAR treatments marginally reduced R_T in the first year (P = 0.08), but significantly reduced R_T, R_S, and R_L in the second year (P < 0.05) (Fig. 2a–c). R_T, R_S, and R_L generally decreased with increasing levels of the SAR. Compared with the CK treatment, the mean rate of R_T was 5.7–15.4% lower in the acid treatment plots in the first year. In the second year, the mean rates of R_T, R_S, and R_L were 6.9–38.0%, 8.0–26.6%, and 5.2–42.8% lower in the acid treatment plots, respectively, with significantly lower values in the T2 and T3 treatments than those in the CK treatment for all (Table 1).

In addition, the SAR treatments significantly affected the contribution of R_S and R_L to R_T (Fig. 3). The contribution of R_L to R_T decreased with increasing levels of SAR (with a significantly lower value in the T3 treatment than that in the CK treatment, P < 0.05) and the portion of R_S increased accordingly. Moreover, as shown in Fig. 4, the relationship between R_L and R_T could be strongly explained by a linear regression model for each of the SAR treatment, implying that R_L was significantly and positively correlated with R_T. One-way ANOVA showed that there was a significant (P < 0.05) difference in the slope of the linear regression between different SAR treatments, with the slope of the T3 treatment significantly lower than that of the CK and T1 treatments. All these analyses provided evidence that strong acid rain decreased the proportion of R_L in R_T.

Table 1 Annual mean values of soil respiration under different SAR treatments[a]

	Year	CK	T1	T2	T3
R_T	1st year	2.97 ± 0.21[a]	2.80 ± 0.24[ab]	2.71 ± 0.09[ab]	2.51 ± 0.31[b]
	2nd year	2.72 ± 0.30[a]	2.53 ± 0.24[ab]	2.33 ± 0.06[b]	1.83 ± 0.17[c]
	All	2.84 ± 0.20[a]	2.67 ± 0.22[ab]	2.52 ± 0.07[b]	2.17 ± 0.21[c]
R_S	2nd year	1.65 ± 0.24[a]	1.52 ± 0.20[a]	1.43 ± 0.07[ab]	1.21 ± 0.14[b]
R_L	2nd year	1.07 ± 0.11[a]	1.01 ± 0.10[ab]	0.90 ± 0.05[b]	0.61 ± 0.05[c]

[a] Table shows means and standard deviations of total soil respiration (R_T), litter-free soil respiration (R_S), and litter respiration (R_L) under different SAR treatments. Mean values within a row with different lowercase letters have significant treatment differences (P < 0.05). n = 4 for all the treatments. The treatments are: CK = control, T1 = pH 4.0, T2 = pH 3.25, and T3 = pH 2.5. The 1st year: April 2011 to March 2012; the 2nd year: April 2012 to March 2013; all: April 2011 to March 2013. Unit: μmol CO_2 m^{-2} s^{-1}.

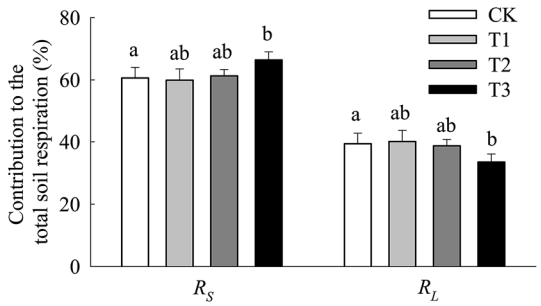

Fig. 3 The contribution of litter-free soil respiration (R_S), and litter respiration (R_L) to total soil respiration under different simulated acid rain (SAR) treatments from April 2012 to April 2013. Error bars are standard errors of the mean ($n = 4$ for all the treatments). The SAR treatments are: CK = control, T1 = pH 4.0, T2 = pH 3.25, and T3 = pH 2.5.

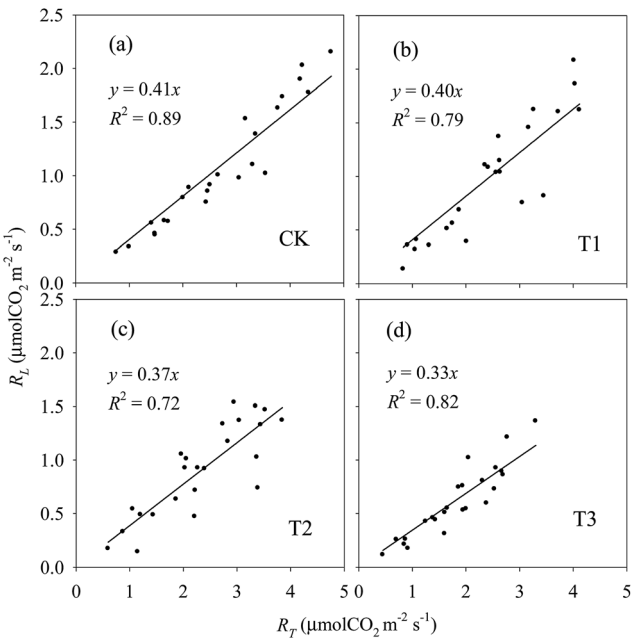

Fig. 4 Relationship between litter respiration (R_L) and total soil respiration (R_T) under different simulated acid rain (SAR) treatments. (a), (b), (c), and (d) represent CK, T1, T2, and T3, respectively. All P values for the regression lines in (a)–(d) are less than 0.001. $n = 24$ for all the treatments. The SAR treatments are: CK = control, T1 = pH 4.0, T2 = pH 3.25, and T3 = pH 2.5.

Relationships of soil respiration with soil temperature and moisture

R_T, R_S, and R_L showed significantly positive exponential relationships with the soil temperature ($P < 0.001$ for all, with R^2 between 0.49 and 0.67, 0.51 and 0.75, and 0.32 and 0.49, respectively) as well as significantly positive linear relationships with soil moisture ($P < 0.01$ for all, with R^2 between 0.41 and 0.50, 0.30 and 0.39, and 0.44 and 0.52, respectively) (Fig. 5). Since soil temperature and moisture interactively controlled soil respiration at the site, we further fit soil respiration with soil temperature and soil moisture together. Including the soil temperature and moisture in the soil respiration model significantly improved model fittings with higher coefficient of determination ($P < 0.001$ for all, with R^2 between 0.74 and 0.91, 0.71–0.88, and 0.69–0.90 in R_T, R_S, and R_L, respectively) (Table 2). The fitted Q_{10} values of R_T, R_S, and R_L were 1.78, 1.72, and 1.83, respectively, in the CK plots (Table 2). The Q_{10} of R_T and its two components generally decreased with increasing levels of the SAR, but only the decline of b values for the exponential curves in R_T and R_L was significant ($P < 0.05$).

Soil pH value, soil microbial biomass C, and fine root biomass

Repeated measures ANOVA showed that the SAR treatments significantly reduced the soil pH value of the 0–10 cm layer ($P < 0.05$) (Fig. 6). During the study period, the mean soil pH value was 3.76 ± 0.07 in the CK plots. The values in the T1, T2, and T3 treatments were decreased by 0.01, 0.07, and 0.15, respectively, and the reduction in the T3 treatment was significant. In addition, we observed that these declining trends had been strengthened over time. The significant differences of soil pH values among the SAR treatments were only found in June 2012 and March 2013 (16 and 24 months after the initial SAR application, respectively). Similar to the dynamics of the soil pH value, microbial biomass C and fine root biomass were not different among the treatments in June 2011 (4 months after initial SAR application). However, in June 2012 (16 months after the initial SAR application), microbial biomass C in the T2 and T3 treatments were 24.7% and 30.9% lower, respectively, than that in the CK treatment; fine root biomass in the T3 treatment was 33.9% and 32.7% lower, respectively, than those in the CK and T1 treatments (significantly different with $P < 0.05$ for both) (Fig. 7a and b).

Discussion

Soil respiration in the MF was significantly depressed after exposure to the SAR, indicating that the response of soil respiration in subtropical forests was susceptible to acid rain. This result was similar to the results of previous studies in a northern subtropical secondary forest.[46,47] We also found that both R_L (soil respiration derived from the litter layer) and R_S (soil respiration derived from litter-free soil) were reduced by the SAR treatment. One possible mechanism might be due to reduced soil microbial biomass related to R_L under the SAR. Efflux of CO_2 derived from the litter layer was an important component of soil respiration in the MF. This component accounted for 39.3% of R_T in the CK plots (Fig. 3), close to the value that was reported in a tropical montane cloud forest (37%),[48] and slightly higher than the worldwide average percentage of 33% for forest ecosystems.[49] Soil litter layer respiration depended on the litter decomposition rate. The SAR significantly reduced the soil pH value and elevated soil acidification (Fig. 6). Falappi *et al.*[50] pointed out that due to the toxicity of high H^+, soil acidification can change the community structures and the biological activity of soil decomposers. Indeed, we found that soil microbial biomass C was reduced by the aggravation of soil acidification (Fig. 7a), which was similar to that reported in previous

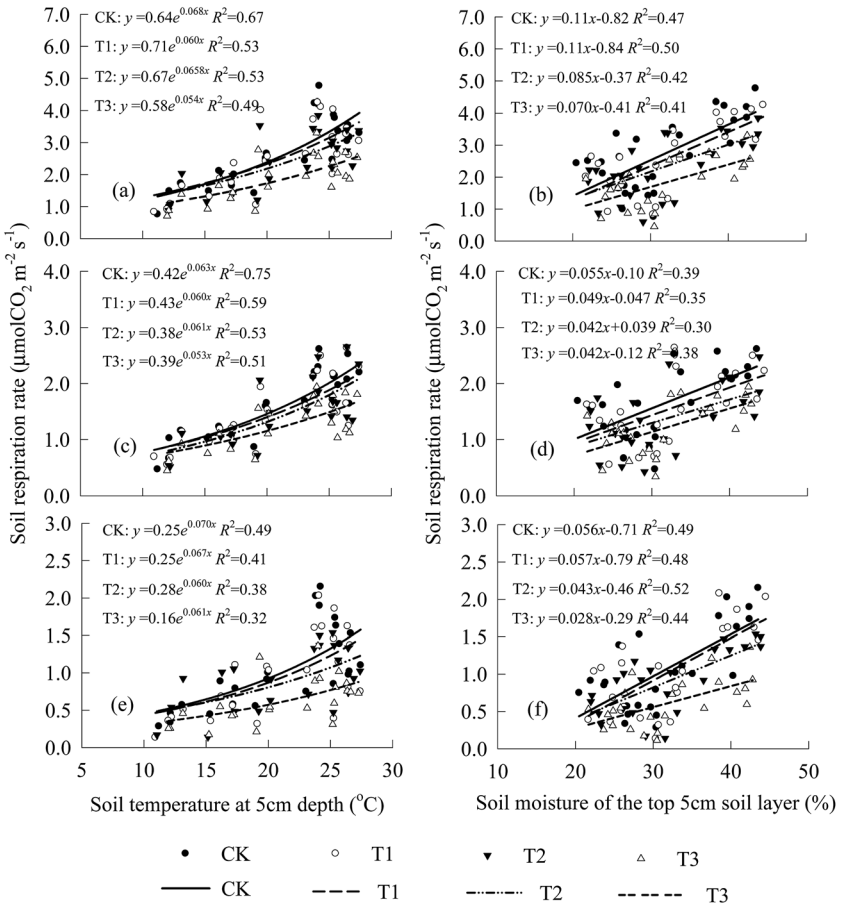

Fig. 5 Relationship of total soil respiration (a and b), litter-free soil respiration (c and d), and litter respiration (e and f) with soil temperature or soil moisture under different simulated acid rain (SAR) treatments. All P values for the regressions in (a)–(f) are less than 0.001. $n = 24$ for all the treatments. The SAR treatments are: CK = control, T1 = pH 4.0, T2 = pH 3.25, and T3 = pH 2.5.

Table 2 Relationship of soil respiration to the soil temperature and soil moisture, and significance tests of model parameters[a]

	Treatment	a	c	b	Q_{10}	R^2
R_T	CK	0.2407 ± 0.0642	0.0183 ± 0.0058	0.0578 ± 0.0054[a]	1.78	0.78
	T1	0.2864 ± 0.0591	0.0220 ± 0.0057	0.0547 ± 0.0093[a]	1.73	0.89
	T2	0.2926 ± 0.0325	0.0194 ± 0.0029	0.0495 ± 0.0046[ab]	1.64	0.91
	T3	0.2486 ± 0.0755	0.0197 ± 0.0085	0.0419 ± 0.0033[b]	1.52	0.74
R_S	CK	0.2712 ± 0.0645	0.0074 ± 0.0017	0.0542 ± 0.0082[a]	1.72	0.88
	T1	0.2256 ± 0.0720	0.0086 ± 0.0032	0.0523 ± 0.0122[a]	1.69	0.75
	T2	0.2915 ± 0.0576	0.0069 ± 0.0027	0.0473 ± 0.0034[a]	1.60	0.83
	T3	0.2781 ± 0.0709	0.0085 ± 0.0036	0.0488 ± 0.0046[a]	1.63	0.71
R_L	CK	0.1445 ± 0.0647	0.0114 ± 0.0026	0.0602 ± 0.0098[a]	1.83	0.80
	T1	0.1686 ± 0.0370	0.0119 ± 0.0025	0.0584 ± 0.0068[ab]	1.79	0.90
	T2	0.1391 ± 0.0303	0.0131 ± 0.0059	0.0469 ± 0.0041[b]	1.60	0.75
	T3	0.1498 ± 0.0384	0.0131 ± 0.0093	0.0465 ± 0.0083[b]	1.59	0.69

[a] Relationship of total soil respiration (R_T), litter-free soil respiration (R_S), and litter respiration (R_L) (μmol CO_2 m^{-2} s^{-1}) with soil temperature at 5 cm below the soil surface (T, °C) and soil moisture of the top 5 cm soil layer (M, % vol) was developed using $R = (a + cM)e^{(bT)}$ (parameter estimate ± standard deviation). R^2 in the table is the determination coefficient. $Q_{10} = e^{(10b)}$ is temperature sensitivity coefficient. All P values for the regressions are less than 0.001. $n = 24$ for all the treatments. Different letters within the column of b denote significant difference ($P < 0.05$) among the treatments. The treatments are: CK = control, T1 = pH 4.0, T2 = pH 3.25, and T3 = pH 2.5.

studies.[51] The reduction of soil microbial biomass would inhibit litter decomposition,[52] and therefore, lead to the reduction of microbial CO_2 production from litter, and a lower R_L.

A similar mechanism might be used to explain the lower R_S under the SAR. The contributors to R_S are microbial decomposition of soil organic matter (SOM) and root respiration. In this

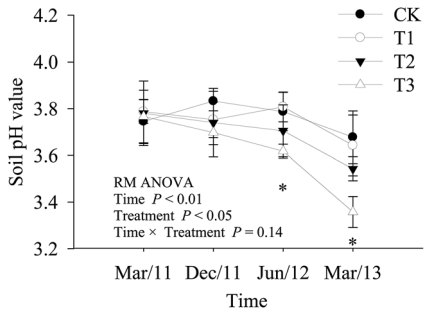

Fig. 6 Dynamics of the soil pH value under different simulated acid rain (SAR) treatments. Error bars are standard errors of the mean ($n = 4$ for all the treatments). The inserted figures indicate P values of repeated measures ANOVAs. The asterisk (*) indicates significant difference ($P < 0.05$) among the treatments. The SAR treatments are: CK = control, T1 = pH 4.0, T2 = pH 3.25, and T3 = pH 2.5.

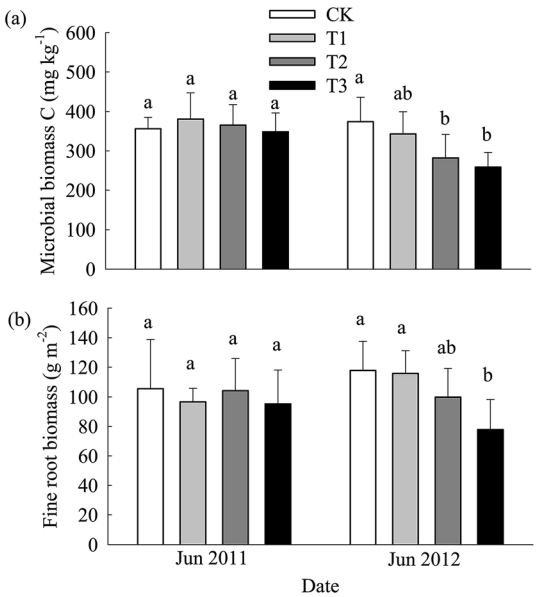

Fig. 7 Soil microbial biomass carbon (a) and fine root biomass (b) under different simulated acid rain (SAR) treatments. Error bars are standard errors of the mean ($n = 4$ for all the treatments). Different lowercase letters denote significant difference ($P < 0.05$) between treatments. The SAR treatments are: CK = control, T1 = pH 4.0, T2 = pH 3.25, and T3 = pH 2.5.

study, the reduction of soil microbial biomass under soil acidification might slow the mineralization and decomposition rates of SOM, thus inhibiting soil CO_2 emission.[53] In addition, respiration from plant roots might decrease after soil acidification. Although we did not separate root respiration in R_T in this study, root respiration often contributes a large proportion to R_T, with a worldwide average percentage of 46% for forest ecosystems.[54] Cumulative effects of soil acidification lead to the depletion of soil nutrients[55] and the excitation of free moving toxic ions (*e.g.*, Al^{3+}, Mn^{2+}),[56,57] and thus decrease fine root biomass and inhibit seedling growth.[58] In this study, fine root biomass showed a negative response to the increasing levels of the SAR treatments (Fig. 7b), which would be expected to reduce CO_2 emission from roots.

Although SAR reduced R_L and R_S simultaneously in this study, the contributions of R_L to R_T decreased significantly and the portion of R_S increased accordingly under the SAR (Fig. 3). These relative changes were supported by not only the decreased percentages of R_S and R_L under the SAR (26.6% and 42.8% lower, respectively, in the T3 treatment compared with the CK treatment), but also the significant decrease in the slope of the linear regression model between R_L and R_T (Fig. 4). All these indicated that R_L tended to respond more sensitively to the SAR than R_S. As far as we know, previously few studies have quantified the contribution changes of soil respiration components to R_T in ecosystems exposed to acid rain.[47] Therefore, such data will be necessary to improve the current models used to evaluate the effects of elevated acid rain on forest soil carbon cycles in the future.

Soil temperature and moisture are generally considered two important factors in controlling soil respiration. Our results showed a strong positive exponential relationship between soil respiration and the soil temperature, as well as a strong positive linear relationship between soil respiration and soil moisture for each SAR treatment (Fig. 5), which were in agreement with that reported in previous studies.[40,59] The dual temperature and moisture controls on soil respiration in this forest may be related to the monsoon tropical climate in the region in which high temperature occurs simultaneously with high moisture.[32] Therefore, high plant growth rates and soil microbial activity in the warm-wet season stimulated greater soil respiration in this region.[60]

The Q_{10} value reflects the sensitivity of soil respiration to the soil temperature. The mean Q_{10} value was 1.78 in the CK plots (Table 2), which was below the median of 2.4 reported in a review of global soil respiration studies,[49] but was within the range of reported values for a subtropical forest in China (1.75–2.55).[61] In this study, the Q_{10} of R_S (1.72) was lower than that of R_T, while the Q_{10} of R_L (1.83) was higher. Boone *et al.*[62] suggested that Q_{10} varies among ecosystems and across temperature ranges, partly due to the various Q_{10} values of different components of soil respiration. Furthermore, our results confirmed that acid rain can influence soil temperature sensitivity with Q_{10} of R_T and R_L decreasing significantly under the SAR treatment (Table 2). Similar results have been reported by Zhang *et al.*[46] It has been suggested that the decrease in Q_{10} under environmental changes could result from concurrent changes in the soil temperature[45] and soil drying which may reduce root and microbial activities,[63] and/or from shifts in the structure of the soil microbial community.[64] In our experiment, there were no treatment effects on soil temperature or moisture ($P > 0.05$) (Fig. 1a and b). Therefore, the experimental changes in the Q_{10} likely reflected shifts in the metabolic pathways and status of plant and soil microbes under soil acidification.[60] If Q_{10} indeed declines under the SAR in forest ecosystems, it may have important implications for modeling future carbon/climate interactions, particularly in tropical and subtropical regions. However, caution should be taken when interpreting this result. Since the decline of Q_{10} was only significant in R_T and R_L but not in R_S, the component differences should also be taken into account when the Q_{10} is used in ecosystem carbon

projecting models. On the other side, this result implied that the change of Q_{10} of R_T may mainly be due to the change of Q_{10} of R_L, which also supported the conclusion that R_L responded more sensitively to the SAR than R_S.

It should be noted that soils at this study site were highly acidic with a soil pH value of around 3.8 at 0–10 cm depth, and around 4.2 at 10–20 cm depth (data not shown). The low soil pH at this site was due to the long term accumulation of the acid rain effect in the last three decades that caused a linear decline of the soil pH value from 4.60–4.75 in 1980s to 3.84–4.02 in 2005 of the top 0–20 cm.[65] It is interesting to see that a very acidic soil like the lateritic soil we studied can be acidified even more when exposed to additional input of H^+ (Fig. 6). This result is consistent with that reported in previous studies that soils at this study site were highly sensitive to acid rain due to their low cation exchange capacity (CEC), low base saturation (BS), negative water-extracted acid neutralizing capacity (ANC), and high leaching potential.[36] Meanwhile, the length of time in which the SAR is applied can influence the accumulation effects of H^+ loads. The declining trends of soil pH had been strengthened over time during the study period (Fig. 6), which indicated that soil acidification under the SAR was a gradual process in the forest. Therefore, the negative effects of the SAR on soil microbial biomass, fine root biomass (Fig. 7a and b), and on soil respiration had been strengthened over time (Fig. 2a and Table 1).

This study, due to some technical difficulties, had some limitations in the separation of different sources of soil respiration and the estimation of their responses to acid rain. First, we set a plot size of 1.7 m × 1.7 m based on the common practices in manipulation experiments, availability of adequate plots, and heterogeneity of plots. Herbs in these plots remained undisturbed and were assigned to the SAR application, but there were no trees in the plots. Hence, it is difficult to detect the whole ecosystem responses. Second, we did not calculate the effects of SAR on aboveground plant growth and fresh fallen litter input, although this part would influence the accumulation of the litter layer and the amount of litter respiration. Third, R_L in this study was more of a measurement of the presence of litter layer influences on R_T rather than actual respiration from the litter layer, because we did not account for the potential synergistic effect between soil and the litter layer.[8] Despite these limitations, partitioning R_T into R_L and R_S in response to acid rain can significantly enhance our capability of predicting the magnitudes and variation in R_T to environmental changes and improves our understanding of controls on belowground carbon cycling processes.

Conclusion

Our results support an important role of acid rain in regulating soil respiration. R_T and its two components were significantly decreased under the SAR. These responses were primarily due to the negative responses of both soil microbial biomass and fine root biomass in the subtropical forest ecosystem. The finding that litter respiration tended to respond more sensitively to acid rain indicates that, with the elevated acid rain projected in the future, the declining trend of soil respiration in the forest ecosystems in southern China will be gradually driven by the decline of respiration in the litter layer. However, the determination of soil respiration cannot completely reflect the ecosystem C storage change in terms of sustainably increasing acid rain. Further studies on multiple aspects of soil C cycles including C inputs from root exudates and fresh litter are needed in long-term simulated acid rain experiments.

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