

## Response of Litter CO<sub>2</sub> Release to Simulated Acid Rain in a Coniferous and Broad-leaved Mixed Forest at Dinghushan: Postprint

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### Abstract

Investigating the effects of increased acid rain on CO<sub>2</sub> release from forest litter is of great significance for understanding how forest ecosystem carbon cycles respond to environmental changes. A simulated acid rain experiment was initiated in January 2011 in a coniferous-broadleaf mixed forest (mixed forest) at Dinghushan, with four different treatment levels established: control CK (natural lake water with pH 4.5), T1 (pH=4.0), T2 (pH=3.25), and T3 (pH=2.5). From January 2013 to December 2013, a one-year continuous observation of forest litter CO<sub>2</sub> release rates under different acid rain intensity treatments was conducted to explore the effects of acid rain on litter C emissions in the mixed forest. The results showed: the annual CO<sub>2</sub> release flux from litter in the control plot was  $(1507.41 \pm 155.19) \text{ gCO}_2 \cdot \text{m}^{-2} \cdot \text{a}^{-1}$ , with the wet season and dry season accounting for 68.7% and 31.3% of the annual flux, respectively. Simulated acid rain inhibited forest litter CO<sub>2</sub> release, manifested as: compared with CK, the annual CO<sub>2</sub> release flux under T2 and T3 treatments was significantly reduced by 15.4% and 42.7%, respectively ( $P < 0.05$ ); and this inhibitory effect exhibited seasonal differences, with significant differences among treatments appearing only in the wet season. Litter CO<sub>2</sub> release rate showed a significant exponential correlation with soil temperature and a significant linear correlation with soil moisture, while acid rain treatment reduced the temperature sensitivity of litter CO<sub>2</sub> release. The inhibitory effect of simulated acid rain on CO<sub>2</sub> release from mixed forest litter was related to changes in soil microbial activity caused by cumulative soil acidification, manifested as: soil pH value and microbial biomass carbon significantly decreased under simulated acid rain. The results of this study quantify the impact of acid rain on CO<sub>2</sub> release flux from mixed forest litter and demonstrate that acid rain is one of the important factors affecting soil carbon cycling in mixed forests.

## Full Text

### Responses of Litter CO Emission to Simulated Acid Rain in a Mixed Conifer and Broadleaf Forest at Dinghushan Nature Reserve

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## Abstract

Understanding how increased acid rain affects forest litter CO release is crucial for elucidating carbon cycling responses to environmental change in forest ecosystems. A simulated acid rain experiment was initiated in January 2011 in a mixed conifer and broadleaf forest (mixed forest) at Dinghushan Nature Reserve, with four treatment levels: control CK (natural lake water, pH 4.5), T1 (pH 4.0), T2 (pH 3.25), and T3 (pH 2.5). From January to December 2013, we conducted year-long continuous observations of forest litter CO release rates under different acid rain intensities to explore the effects of acid rain on litter carbon emissions in the mixed forest. The results showed that the annual litter CO flux in control plots was  $(1507.41 \pm 155.19) \text{ gCO} \cdot \text{m}^{-2} \cdot \text{a}^{-1}$ , with the wet season and dry season accounting for 68.7% and 31.3% of the annual flux, respectively. Simulated acid rain suppressed forest litter CO release: compared with CK, the annual CO flux under T2 and T3 treatments decreased significantly by 15.4% and 42.7% ( $P < 0.05$ ), respectively. This inhibitory effect exhibited seasonal variation, with significant differences among treatments occurring only during the wet season. Litter CO release rates showed significant exponential relationships with soil temperature and significant linear relationships with soil moisture. Additionally, acid rain treatments reduced the temperature sensitivity of litter CO release. The suppressive effect of simulated acid rain on litter CO emission in the mixed forest was associated with changes in soil microbial activity caused by cumulative soil acidification, as evidenced by significant declines in soil pH and microbial biomass carbon under acid rain treatments. These results quantify the impact of acid rain on litter CO efflux in mixed forests and demonstrate that acid rain is an important factor affecting soil carbon cycling in these ecosystems.

**Keywords:** simulated acid rain, litter CO emission, mixed conifer and broadleaf forest, Dinghushan Nature Reserve

## Introduction

Acid rain, also known as acid precipitation, is defined as atmospheric precipitation with pH values below 5.6. Southern China represents the world's third major acid rain region after Europe and North America, and it is the only region where the affected area continues to expand. Monitoring data indicate that acid rain frequency in many cities of this region has reached 80%, and precipitation with  $\text{pH} < 4.5$  occurs over large areas. Moreover, China's acid rain distribution overlaps with acidic soil distribution, leading to increasingly severe soil acidification over the past several decades. Forests are the main component of terrestrial ecosystems, and the damage of acid rain and soil acidification to forest structure and function, including forest decline, has long been a concern.

Litter is a critical component of material cycling in forest ecosystems and an important carbon source for soil respiration. Previous estimates suggest that surface litter and soil organic matter decomposition contribute 74% of global soil CO<sub>2</sub> emissions. Therefore, in-depth study of litter is essential for understanding forest soil carbon pool cycling and the source-sink dynamics of soil respiration. Current research primarily employs litter removal and addition approaches to investigate how litter affects soil respiration and consequently influences organic matter and nutrient accumulation and dynamics. While the response of forest litter decomposition rates to environmental changes has received widespread attention, studies on how litter decomposition processes respond to increased acid rain are relatively rare compared to other environmental factors such as nitrogen deposition and temperature increase. Most scholars have evaluated acid rain effects on litter decomposition using only the single indicator of decomposition rate. To date, few studies have quantified CO<sub>2</sub> flux released from litter under acid rain, limiting accurate estimation of acid rain impacts on forest ecosystem carbon cycling.

Furthermore, due to human activities, zonal forests in China's tropical and subtropical regions have been extensively destroyed. Naturally successional mixed conifer and broadleaf forests formed by natural invasion of broadleaf species into artificially planted *Pinus massoniana* forests represent the main forest type in this region. The mixed forest is currently the most widely distributed vegetation type in Dinghushan Nature Reserve, with litter production and standing crop reaching  $(8.50 \pm 0.62) \text{ t} \cdot \text{hm}^{-2}$  and  $(4.97 \pm 1.03) \text{ t} \cdot \text{hm}^{-2}$ , respectively, making it an extremely important source of soil respiration. In recent years, acid rain increase and soil acidification have become prominent issues in Dinghushan Nature Reserve, yet no studies have quantified the impact of acid rain on litter CO<sub>2</sub> release flux in mixed forests. Therefore, this study took the Dinghushan mixed forest as the research object, dynamically monitored litter CO<sub>2</sub> release rates and related environmental factors under simulated acid rain treatments, and quantified the degree of acid rain influence on ground litter carbon emissions to provide data support for accurate assessment and modeling of forest ecosystem carbon balance.

### 1.1 Study Area and Plot Description

The study site was located in Dinghushan Nature Reserve, Zhaoqing City, Guangdong Province (112°30'39" - 112°33'41" E, 23°09'21" - 23°11'30" N). The region has distinct seasonal climate patterns, belonging to the subtropical monsoon climate type. The mean annual temperature is 20.9 °C, with the coldest month being January (average 12.6 °C) and the hottest month being July (average 28.0 °C). Mean annual precipitation is 1929 mm, with rainfall from April to September accounting for approximately 80% of the annual total (wet season), and October to March representing the dry season. Dinghushan Nature Reserve features three typical subtropical vegetation types: *Pinus massoniana* coniferous forest, mixed conifer and broadleaf forest, and monsoon evergreen broadleaf forest. Our experimental plots were established in the mixed forest, which is mainly distributed between 80-400 m elevation, with a stand age of approximately 100 years. Dominant tree species include *Castanopsis chinensis*, *Schima superba*, *Cryptocarya concinna*, and *Pinus massoniana*. The mixed forest soil is latosol with a thickness of 40-70 cm. Pre-study soil background surveys of the 0-10 cm layer showed organic carbon, total nitrogen, C/N ratio, and available nitrogen of  $(3.8 \pm 0.1) \text{ mg} \cdot \text{g}^{-1}$ ,  $(37.8 \pm 6.4) \text{ mg} \cdot \text{g}^{-1}$ ,  $(2.6 \pm 0.9)$ ,  $14.5$ , and  $(13.5 \pm 5.7) \text{ mg} \cdot \text{kg}^{-1}$ , respectively.

In January 2011, sixteen 1.7 m × 1.7 m plots were established in the mixed forest for the simulated acid rain experiment. To avoid interference of soil and plant roots between plots, a 2 m wide buffer zone was set between each plot, and plastic boards were installed around each plot perimeter, inserted 15 cm below the ground surface and extending 10 cm above ground. The simulated acid rain treatment design followed Liang et al. (2013), with four gradient treatments: CK (local natural lake water, pH 4.5), T1 (pH 4.0), T2 (pH 3.25), and T3 (pH 2.5), with H<sub>2</sub>SO<sub>4</sub>:HNO<sub>3</sub> ratio of 1:1. Each treatment had four replicates in a completely randomized design. Simulated acid rain treatments began in March 2011, applied twice monthly using a gasoline-powered sprayer. Each plot received 10 liters of simulated acid rain per application. Two soil respiration collars (20 cm diameter) were installed in each plot at random positions, inserted 5 cm into the soil. In one collar, litter was allowed to enter normally; in the other, litter was excluded using a 0.5 m × 0.5 m litter collection net with 1 mm × 1 mm mesh size placed 0.5 m above ground to prevent litter from falling into the collar.

### 1.3 Measurement of Litter CO<sub>2</sub> Release Rate

CO<sub>2</sub> release rate was measured using a Li-8100 soil respiration system (Li-Cor Inc., NE, USA). From January 2013 to December 2013 (22 to 33 months after initiating simulated acid rain treatments), measurements were taken twice monthly, before each acid rain application, between 9:00-12:00 AM, as values during this period best reflect daily average rates. Synchronized with CO<sub>2</sub> release measurements, soil temperature at 5 cm depth and soil moisture at 0-5 cm were measured using the temperature and humidity probes equipped with the

Li-8100. The litter CO<sub>2</sub> release rate for each plot was calculated as the difference between CO<sub>2</sub> release rates measured in the litter-present and litter-excluded PVC collars.

#### 1.4 Determination of Soil pH, Microbial Biomass Carbon, and Soil Organic Carbon

During the experimental period, soil samples were collected twice: in June 2011 and June 2013 (3 and 27 months after simulated acid rain initiation). In each plot, three random points were selected to collect 0–10 cm soil, which was mixed and placed in cloth bags. In the laboratory, soil was sieved through a 2 mm mesh. Fresh sieved soil was used for microbial biomass carbon determination by chloroform fumigation-extraction method. Another portion of fresh soil was air-dried for soil pH and organic carbon determination. Soil pH was measured using KCl extraction method, and soil organic carbon was determined by potassium dichromate external heating method.

#### 1.5 Data Analysis

All data were analyzed using SPSS 11.0 and plotted using SigmaPlot 10.0. Repeated measures ANOVA was used to test for significant differences in litter CO<sub>2</sub> release rates among treatments during the experimental period. One-way ANOVA was used to test differences in annual litter CO<sub>2</sub> flux, soil pH, soil microbial biomass carbon, and soil organic carbon among treatments. The relationship between litter CO<sub>2</sub> release rate and temperature was fitted using an exponential model:  $y = ae^{bT}$ , where  $y$  is litter CO<sub>2</sub> release rate,  $T$  is soil temperature,  $a$  is the litter CO<sub>2</sub> release rate at 0 °C, and  $b$  is the temperature response coefficient. The  $Q_{10}$  value (the factor by which litter CO<sub>2</sub> release rate increases for every 10 °C increase in soil temperature) was calculated as:  $Q_{10} = e^{10b}$ .

#### 2.1 Dynamics of Litter CO<sub>2</sub> Release Under Simulated Acid Rain and Its Relationship with Soil Temperature and Moisture

Litter CO<sub>2</sub> release rates and soil temperature and moisture all showed clear seasonal variation across treatments, with higher values in the wet season (April–September) and lower values in the dry season (October–March) [FIGURE:1:a,b,c]. Repeated measures ANOVA indicated significant differences in litter CO<sub>2</sub> release rates among different simulated acid rain treatments ( $P < 0.05$ ), demonstrating that simulated acid rain inhibited litter CO<sub>2</sub> release rates. Variance analysis of each measurement showed that significant differences among treatments occurred only in the wet season [FIGURE:1:c], indicating that the inhibitory effect of simulated acid rain on litter CO<sub>2</sub> release rates had seasonal variation.

Annual litter CO<sub>2</sub> flux was calculated from each measurement [Figure 2: see original paper]. In CK plots, annual flux was  $(1507.41 \pm 155.19) \text{ gCO}_2 \cdot \text{m}^{-2} \cdot \text{a}^{-1}$ , with the wet season accounting for 68.74% and the dry season 31.26%.

For annual flux, CK and T1 treatments showed no significant difference, while T2 and T3 treatments were significantly lower than CK and T1. Compared with CK, annual CO<sub>2</sub> flux under T2 and T3 treatments decreased by 2.32% and 42.72%, respectively. Similarly, the reduction in litter CO<sub>2</sub> flux caused by simulated acid rain was greater in the wet season than in the dry season, with significant differences among treatments occurring only in the wet season.

Litter CO<sub>2</sub> release rates in all treatments showed significant exponential relationships with soil temperature ( $P < 0.01$ ,  $R^2$  ranging from 0.35 to 0.59) and significant linear relationships with soil moisture ( $P < 0.01$ ,  $R^2$  ranging from 0.25 to 0.45) [FIGURE:3, TABLE:1]. Based on the exponential relationships between litter CO<sub>2</sub> release rates and soil temperature under each treatment, the temperature sensitivity coefficient  $Q_{10}$  values for CK, T1, T2, and T3 treatments were calculated as 2.14, 2.18, 1.86, and 1.77, respectively [FIGURE:3, TABLE:1], showing a declining trend with decreasing pH of acid treatments.

## 2.2 Dynamics of Soil pH, Microbial Biomass Carbon, and Organic Carbon Under Simulated Acid Rain and Their Relationship with Litter CO<sub>2</sub> Release

Average values of soil pH, microbial biomass carbon, and organic carbon measured twice in CK plots were ( $3.77 \pm 0.10$ ), ( $360.26 \pm 40.68$ )  $\text{mg} \cdot \text{kg}^{-1}$ , and ( $36.73 \pm 5.63$ )  $\text{g} \cdot \text{kg}^{-1}$ , respectively [FIGURE:4:a,b,c]. Variance analysis showed that soil pH and microbial biomass carbon did not change significantly in June 2011 (3 months after simulated acid rain initiation) ( $P > 0.05$ ). However, in June 2013 (27 months after initiation), these two indicators differed significantly among treatments ( $P < 0.05$ ): compared with CK, soil pH decreased significantly by 0.21 and 0.32 units under T2 and T3 treatments, respectively, while soil microbial biomass carbon decreased significantly by 22.62% and 31.70%. The differences between the two measurement periods also showed that the declining trends of soil pH and microbial biomass carbon under simulated acid rain became more significant with prolonged treatment time. Simulated acid rain had no significant effect on soil organic carbon content in either measurement ( $P > 0.05$ ).

Correlation analysis among soil pH, microbial biomass carbon, organic carbon, and litter CO<sub>2</sub> flux data indicated significant positive correlations between soil pH, microbial biomass carbon, and litter CO<sub>2</sub> flux, but no significant correlations between soil organic carbon and the other three indicators.

## Discussion and Conclusion

Annual litter CO<sub>2</sub> flux in forests is related to forest type, site conditions, and climate. In this study, the annual litter CO<sub>2</sub> flux in control plots of the mixed forest was ( $1507.41 \pm 155.19$ )  $\text{gCO}_2 \cdot \text{m}^{-2} \cdot \text{a}^{-1}$  [Figure 2: see original paper], which is comparable to results from Dinghushan mixed forest by Deng et al. (2007) ( $1471.2 \pm 50.0$ )  $\text{gCO}_2 \cdot \text{m}^{-2} \cdot \text{a}^{-1}$ , with an average annual contribution of 33.29%

to soil respiration). This value is close to the global average contribution rate of litter to soil respiration in forest ecosystems (33%) but higher than the average contribution rate (20.2%) analyzed by Chen et al. (2008) for 62 forest sites in China, while lower than values measured in tropical montane cloud forests (37%). The relatively high annual litter CO<sub>2</sub> flux in Dinghushan mixed forest is due to high litter production and standing stock, as well as the hot and humid climate of southern subtropical regions that favors litter decomposition. Many studies in subtropical and temperate forests have shown that acid rain significantly delays decomposition rates of fallen leaves, which is also an important reason for suppressed forest soil respiration under acid rain. The innovation of this study lies in its in-situ quantification of the inhibition degree of acid rain on annual litter CO<sub>2</sub> flux in mixed forests (significant decrease of 15.35%–42.72%).

The inhibitory effect of simulated acid rain on annual litter CO<sub>2</sub> flux in mixed forests is related to soil acidification-induced declines in soil microbial activity, manifested as significant decreasing trends in soil pH and microbial biomass carbon under acid rain. The significant positive correlations among annual litter CO<sub>2</sub> flux, soil pH, and microbial biomass carbon further support this mechanism. Similar to results from many tropical and subtropical forests, soil pH decreased significantly after 27 months of simulated acid rain treatment in this study. Notably, the control plot soil was strongly acidic (pH < 4), indicating that already acidified soils experienced further acidification under simulated acid rain. On one hand, litter decomposition and CO<sub>2</sub> release depend on specialized microbial activities, while accumulated H<sup>+</sup> during soil acidification is toxic to soil microorganisms, altering decomposer community composition, structure, and activity. On the other hand, acid rain treatment leads to decreased enzyme activity and consequently slower litter decomposition. For example, Ji et al. (2013) found that urease and cellulase, which contribute significantly to litter decomposition, were inhibited under acid rain stress. Although this study did not measure soil enzymes, the reduction in soil microbial biomass carbon indicates that soil microbial activity was suppressed by acid rain, thereby inhibiting litter decomposition and CO<sub>2</sub> release.

In tropical and subtropical forests, relationships between soil respiration and soil temperature and moisture are generally described by exponential and linear regression equations, respectively. As an important component of soil respiration, litter CO<sub>2</sub> release rates in this study showed significant exponential relationships with soil temperature and linear relationships with soil moisture across all treatments. The inhibitory effect of simulated acid rain on litter CO<sub>2</sub> release occurred mainly in the wet season because the Dinghushan region has abundant water and heat during this period, resulting in higher microbial activity and thus more pronounced inhibition by acid rain. Additionally, the  $Q_{10}$  value is an important index measuring the sensitivity of soil respiration to temperature changes. Consistent with previous soil respiration studies,  $Q_{10}$  showed a declining trend under simulated acid rain, indicating reduced temperature sensitivity of litter CO<sub>2</sub> release, possibly related to changes in soil microorganisms and enzyme activity under acid rain.

The litter layer and soil have certain buffering capacities against acid rain in the short term, but when large H inputs exceed the ecosystem's load, soil acidification becomes inevitable. Therefore, in the early stage of this study, simulated acid rain did not significantly reduce soil pH, but this declining effect became significant with prolonged treatment time, indicating that soil acidification under acid rain is a gradual cumulative process. Meanwhile, the responses of soil microbial activity and litter CO<sub>2</sub> release rate to simulated acid rain also became more significant with extended treatment duration. Additionally, Yi et al. (2006) reported that the 0–15 cm soil carbon storage in Dinghushan mixed forest was 26.9 t · hm<sup>-2</sup>. Based on our results, the reduced CO<sub>2</sub> annual flux under T2 and T3 treatments corresponds to 0.63 and 1.76 tC · hm<sup>-2</sup>, respectively, equivalent to 2.35% and 6.53% of the mixed forest's 0–15 cm soil carbon storage, demonstrating that acid rain is an important factor affecting carbon cycling in Dinghushan mixed forest.

In summary, simulated acid rain inhibited litter CO<sub>2</sub> release in Dinghushan mixed forest through soil acidification-induced declines in soil pH and microbial biomass carbon, which reduced soil microbial activity. Nevertheless, soil organic carbon content did not change significantly under simulated acid rain, suggesting that forest carbon cycling is a complex process controlled by many factors, and that acid rain effects on soil ecosystems are long-term cumulative processes. Future research should continue exploring long-term effects of acid rain on litter CO<sub>2</sub> release while strengthening long-term and continuous monitoring of changes in soil organic carbon and related indicators to better understand the comprehensive impacts of acid rain on forest soil carbon cycling.

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