

Species-dependent responses of soil microbial properties to fresh leaf inputs in a subtropical forest soil in South China

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Abstract

Aims

Forest disturbance from extreme weather events due to climate change could increase the contribution of fresh green leaves to the litter layer of soil and subsequently alter the composition and activity of the soil microbial properties and soil carbon cycling. The objective of this study was to compare the effect of naturally fallen litter and fresh leaves on the soil microbial community composition and their activities.

Methods

Fresh leaves and normal fallen litter were collected from four tree species (*Pinus elliottii*, *Schima superba*, *Acacia mangium*, *A. auriculataeformis*) in subtropical China and mixed with soil. Soil microbial community composition was determined using PLFAs, and its activity was quantified by soil respiration. During a 12-month period, the decomposition rate of litter was measured bimonthly using a litterbag method. Soil microbial samples were collected after 6 and 12 months. Soil respiration was measured monthly.

Important Findings

We found that fresh leaves decomposed faster than their conspecific fallen litter. Although total microbial biomass and bacterial

biomass were similar among treatments, soil fungal biomass was higher in fresh leaf than fallen litter treatments, resulting in greater values of the Fungal phospholipid fatty acids (PLFAs)/Bacterial PLFAs ratio. Fungal PLFA values were greater for *Schima superba* than the other species. The effect of litter type on soil respiration was species-dependent. Specifically, fallen litter released 35% more CO₂ than fresh leaves of the conifer *P. elliottii*. The opposite pattern was observed in the broadleaf species whose fresh leaf treatments emitted 17–32% more CO₂ than fallen litter. Given future predictions that global climate change will cause more disturbances to forests, these results indicate that conifer and broadleaf forests in subtropical China may respond differently to increased fresh litter inputs, with net soil microbial respiration decreasing in conifer forests and increasing in broadleaf forests.

Keywords: fresh leaf input, forest disturbance, soil microbial community, soil respiration, southern China

Received: 9 July 2012 Revised: 31 January 2013 Accepted: 18 February 2013

INTRODUCTION

Soil respiration plays a significant role in the global carbon and nutrient cycles and also function as a driver for changes in climate. Globally, soil respiration accounts for the release of ~80 Pg of carbon as CO₂ annually (Raich *et al.* 2002),

and tropical and subtropical forests contribute more than any other vegetation type (30% of the total). During the decomposition of litter that was added to soil from above-ground and below-ground sources, large amounts of C are released into the atmosphere. Previous studies estimated that above-ground litter inputs in many forest ecosystems have

contributed more than one-third of the annual C loss through soil respiration (Raich and Nadelhoffer 1989; Bowden *et al.* 1993). For example, Bowden *et al.* (1993) found that above-ground litter contributed 37% of the annual soil respiration, suggesting that the above-ground litter input exerts an important influence on soil C dynamics.

Global climate change does not only include elevated CO₂ or changes in temperature or precipitation but also include increasing frequency of extreme weather events (Cotrufo *et al.* 2005; Liu *et al.* 2009; de Dato *et al.* 2010). Several studies based on modelling and empirical data imply that hurricane or typhoon intensity and frequency should increase with increasing global mean temperature (Emanuel 1987; Camargo and Sobel, 2005; Emanuel 2005; Wu *et al.* 2006; Solomon *et al.* 2007). Strong winds can defoliate forests, and consequently, a large quantity of green leaves and wood may fall to the forest floor and affect nutrient cycling (Dale *et al.* 2001; Xu 2006). The abundance and type of above-ground litter inputs thus could be impacted by climate change that alters the frequency, intensity, duration and timing of catastrophic wind (e.g. typhoon, cyclone and hurricane) or other extreme weather events (Dale *et al.* 2001; Xu 2006; Lin *et al.* 2011).

In the forests of South China, litterfall is very sensitive to typhoon frequency and intensity (Lin *et al.* 2011). In this and nearby regions, typhoon-generated litter can contribute 17–80% of annual litter production in various tropical forests, depending on the frequency and intensity of typhoons (Xu and Hirata 2002; Lin *et al.* 2003; Xu *et al.* 2004a; Xu 2006; Wang *et al.* 2007; Wu *et al.* 2008; Lin *et al.* 2011). However, typhoon disturbance did change not only the quantity but also the characteristics of litter input. Litter generated from typhoons usually contains a greater proportion of fresh green leaves having more nutrients and decomposes more rapidly than typical litter comprised of senescent leaves, resulting in a rapid loss of N and P (Xu *et al.* 2004a). Based on a 3-year litterbag study, both broadleaf and conifer litter decomposition were increased by typhoon-generated litter and decomposed faster than typical litter comprised primarily of senescent fallen leaves (Xu *et al.* 2004a, Xu 2006). In an Okinawan subtropical forest, typhoon disturbance contributed an average of 30% of the annual litterfall mass, and from 30 to 39% (for different nutrient elements) of total annual nutrient inputs (Xu *et al.* 2004b). As a major pathway for carbon and nutrients between vegetation and soils, it seems likely that such massive inputs of litter usually having higher concentrations of many nutrients than normal litterfall will, thus, have consequences for belowground processes, such as soil respiration and microbial community composition.

In soil ecosystems, microbes are responsible for a major part of total soil respiration and nutrient cycling. Many recent studies indicate that aboveground litter inputs can significantly impact soil microbial biomass, composition and activity (Fontaine *et al.* 2004; Jin *et al.* 2010; Hossain and Sugiyama 2011). Cleveland *et al.* (2007) found that addition

of litter-derived labile C led to rapid shifts in microbial composition and greatly increased soil respiration. Zhao *et al.* (2011) found that removal of all plants in subtropical plantations has greatly decreased fungal PLFAs and Fungal PLFA/Bacterial PLFA (F/B) ratio in South China, probably caused by the reduction of litter inputs and rhizodeposition. Although vegetation or litter manipulation experiments in various ecosystems have observed the changes of soil microbial community composition, little work focused on the changes of litter quality or chemistry on the soil microbial community. A better understanding of the microbial community dynamics and their interactions with different types of plant litters would provide more accurate estimates of soil respiration rates and, thus, improve theoretical models for forest management in the context of climate change (Wu *et al.* 2011).

The objective of this study was to compare the effect of naturally fallen litters and fresh green leaves on the composition of the soil microbial community and soil CO₂ efflux in southern China. We hypothesized that, compared with normal fallen litter, (i) fresh leaf addition would increase soil microbial biomass and alter soil microbial community composition; (ii) fresh leaves would decompose faster and, thus, increase soil respiration. To test these hypotheses, soil microbial activity and the rates of decomposition were measured for fresh leaves and fallen litters of four tree species.

MATERIALS AND METHODS

Study site

A common garden experiment was conducted at the Heshan National Field Research Station of Forest Ecosystem (112° 50' E, 22° 34' N), which is located in a subtropical hilly land region of South China. The climate in this region is subtropical monsoon, including a rainy and warm season (April–October) and a dry and cool season (November–March). The vegetation of this region is dominated by evergreen broadleaf forests. Typhoon season is usually from June to late October. In 2009, the annual precipitation was 1532.6 mm, and the mean annual temperature was 22.5°C (Fig. 1). The soil was classified a sandy loam Acrisol (Wu *et al.* 2011).

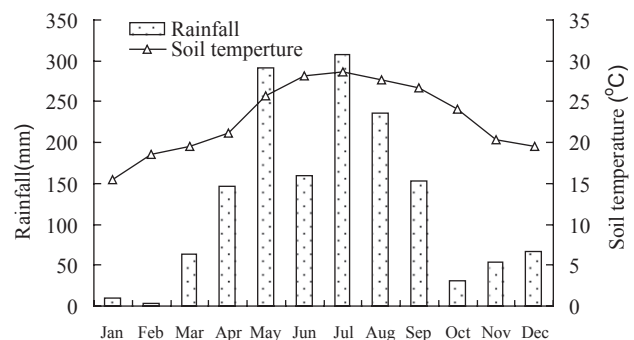


Figure 1: annual soil temperature (0–10 cm) and rainfall in 2009 in the experiment site.

Experimental design

Four species, which are distributed widely as plantations in South China (Li et al. 2001), were chosen in this experiment, namely *Pinus elliottii* (Conifer), *Schima superba* (Broadleaf), *Acacia mangium* (Broadleaf and N-fixing) and *A. auriculaeformis* (Broadleaf and N-fixing). In this region, all four tree species are evergreen year round. Litter and fresh leaves of the four selected species were collected from conspecific plantations in a nearby site established in 1984. Our previous study has found that the highest annual litterfall and litter stock of the four species plantations in 20 yrs old were ~10.5 t/ha/yr and 13.5 t/ha, respectively (Zou et al. 2006). However, more mature forests of this region could accumulate as much as 25.8t/ha on the forest floor (Zhang et al. 1998).

The fallen leaf litter was collected in September–December 2008 in litter traps (1 × 1 m nylon net) and fresh leaves were collected from the mature green leaves of living trees in November 2008. All fallen litter and fresh leaves were air-dried for 1 month to obtain constant water content without destroying their original physical and chemical structure.

A Randomized Complete Block Design was employed with four tree species and two sources of litter plus a control without any litter. Four blocks (2 × 2 m field plots) were selected. The experiment site was located on an unplanted old field, which was covered by natural regenerated vegetation from 1984. The dominant tree species in this site are *Litsea cubeba* and *Evodia lepta* (Wang et al. 2010). In each block, the original top soil was collected, air-dried and sieved (<5 mm) to remove gravel and debris, then it was mixed thoroughly and filled back into each block. Soil was processed in such a way to create a common garden setting allowing testing effect of litter differences (species and senescence) on soil respiration and microbial community. In each block, 18 PVC chambers (20 cm in diameter; 20 cm in height) were insert to a depth of 10 cm of the newly filled soils. The bottom of each chamber was covered with a nylon screen (0.05 mm mesh). In each chamber, 3.5 kg air-dried and well mixed soils were added to a depth of 10 cm. The eight-litter treatments plus a control without any litter were assigned among the 18 chambers, with each treatment assigned to a pair of abutting chambers to allow for soil samplings in June–December. In litter treatments, fallen litter and fresh leaves, with a weight equalling 105 g air-dried litters (leaves) (~3% of the soil weight in each chamber), were mixed with the soil in each chamber to maximize litter influence on soil microbial community and soil processes. In tropics and subtropics, abundant and diverse macro-fauna are active in migrating litter into subsoil. Surface soil is normally well mixed with litter due to the soil animal activity.

Soil microbial community composition and activity

Before the experiment started, four soil samples were collected from the chambers filled with the initial, well-mixed soil. The general soil properties of this soil were shown in

Table 1. In late June 2009, soils in one of the replicated chambers (for each litter treatment in each block) were sampled. In December 2009, the remaining chamber was also sampled. Soil samples collected were divided in half: one part was used for determination of soil physico-chemical characteristics and the other part was used for soil microbe analysis. Soil to be used for soil microbe analysis was stored at –20°C before processing. The soil microbial community was characterized using phospholipid fatty acids (PLFAs) analysis. The lipid extraction procedure followed the method described by Bossio and Scow (1998). For each sample, different PLFAs were considered to be representative of different groups of soil microorganisms. The abundance of individual fatty acids was determined as relative nmol per g of dry soil and nomenclature followed those in Tunlid et al. (1989). Bacteria were considered to be represented by the PLFAs of i15:0, a15:0, 15:0, i16:0, 16:1ω9, 16:1ω7, 16:1ω5c, 16:1 2OH, i17:0, a17:0, 17:0, cy17:0, cy19:0 (Bossio and Scow 1998; Smithwick et al. 2005; Hamman et al. 2007), and fungi biomass was estimated from the concentrations of 18:2ω6c (Hamman et al. 2007) and 18:1ω9c (Smithwick et al. 2005). The ratios of fungi/bacteria were calculated from the above PLFAs. Other PLFAs were also used to analyse the composition of microbial community. Total microbial biomass was estimated from the sum of all the extracted PLFAs.

Soil CO₂ efflux represents CO₂ release resulting from soil heterotrophic (primarily by soil microbes) and root activities. Because roots were excluded in the field plots in this experiment, soil respiration only originated from heterotrophic activity and was considered as the primary indicator of soil microbial activities. The static chamber and gas chromatography technique was used. During flux measurements, a chamber top was attached to the chamber bottom (the PVC chambers described above) and sealed with adhesive tape. Air was sampled from each chamber from 09:00 to 11:00 at each sampling date. Diurnal studies in this region demonstrated that soil CO₂ fluxes measured during this time were close to daily means (Tang et al. 2006). Soil respiration was measured once per month during the 1-year experiment. Gas samples were collected with 60-ml plastic syringes at 0, 10, 20 and 30 min after the chamber closure and analysed for CO₂ within 36 hours using gas

Table 1: means (±1SE, *n* = 4) of general soil chemical and microbial properties of the soil tested in the experiment

| | |
|---|--------------|
| pH | 4.17 ± 0.03 |
| SOC (g/kg) | 17.2 ± 0.51 |
| Total N (g/kg) | 1.36 ± 0.04 |
| C/N | 12.71 ± 0.11 |
| Total PLFAs (nmol g ⁻¹ dry soil) | 55.03 ± 8.39 |
| Fungal PLFAs (nmol g ⁻¹ dry soil) | 2.0 ± 0.13 |
| Bacterial PLFAs (nmol g ⁻¹ dry soil) | 13.08 ± 0.77 |
| F/B | 0.15 ± 0.01 |

chromatography (Agilent 4890D, Agilent Co. USA). Gas flux was calculated from the linear regression of concentration versus time using the data points from each chamber to minimize the negative effect of close chamber on CO₂ production (Zhang *et al.* 2008). Coefficients of determination (r^2) for all linear regression analyses performed exceeded 0.98. Accumulative respiration flux was calculated by the following formula:

$$R_{\text{accumulative}} = ((R_{i+1} + R_i) / 2) * (T_{i+1} - T_i) * 24$$

where $R_{\text{accumulative}}$ stands for the accumulative respiration data, R for the soil respiration data in each sampling time ($\text{mg C m}^{-2}\text{h}^{-1}$), i for the sampling time and T for the data of sampling.

From January to June 2009, paired chambers from each experimental unit, adjacent to one another, were available for gas efflux measurement. The efflux of each litter treatment was calculated as the mean from the two chambers. After June, to avoid the disturbance effect of soil sampling in June 2009, only the remaining un-sampled chamber in each experimental unit was used for further gas efflux measurement. Both soil temperature and soil moisture at 5-cm depth were also recorded at the same time when soil respiration was measured.

Litter decomposition

A separate litter-decomposition study was conducted concurrently using the litter-bag method. Fallen litters or fresh leaves were added to nylon litter bags (15×15 cm, 10-g dried leaf litter per bag). The mesh size was 1 mm^2 . On the 30th of December 2008, litterbags were placed randomly on the surface of the four 2×2 m plots described above. Four replicate litter bags for each litter and leaf treatments were collected bimonthly between January 2009 and December 2009. In the laboratory, adhering soil particles were removed from the litter by rinsing with deionized water. The litter was then dried to constant mass at 75°C and weighed to calculate mass loss. The remaining litter samples were pulverized and analysed for the contents of C and N. Litter C content was measured with the wet-combustion method (Liu *et al.* 1996). The N concentrations were determined by micro-Kjeldahl digestion method and then measured colorimetrically by FIA (Lachat Instruments, USA).

General soil properties

Soil samples collected for soil physico-chemical analyses were ground to pass through sieve of 60 mesh. Total nitrogen (TN) concentration of the soil was measured after micro-Kjeldahl digestion using a flow injection autoanalyser (FIA, Lachat Instruments, USA). Soil organic carbon (SOC) was measured with the Walkley–Black method (Liu *et al.* 1996). Soil C/N values were calculated as the ratio of SOC to TN. Soil pH was determined using a 1:2.5(w/v) ratio of soil to deionized water.

Statistical analyses

Repeated measures two-way analysis of variance (RM-ANOVA) was performed to determine the effect of species and litter type and their interaction on soil respiration and litter decomposition. An LSD *post hoc* test was run after RM-ANOVA to explore the difference among litter type treatments. One-way ANOVA was used to test the litter quality difference among litter-type treatments and followed by an LSD *post hoc* test. We performed a three-way ANOVA to test for the effects of sample time, species, litter type and the two-way interaction of species and litter type on soil physico-chemical and microbial characteristics. The relationship between soil respiration rates, soil temperature and soil moisture were further examined by nonlinear regression models (Mo *et al.* 2008). 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 ($Q_{10} = e^{10\beta}$). These statistical analyses were performed using SPSS version 15 software (SPSS, Inc, Chicago, IL). Difference is significant at the 0.05 level.

Canonical correspondence analysis (CCA) was performed to explore the soil microbial community composition in relation to litter treatments. PLFA signatures were treated as ‘species’ variables, and litter treatments (four species with two sources of litter) were treated as nominal (0, 1) environmental variables. Soil physico-chemical properties (i.e. pH, SOC, TN and C/N ratio), which were usually regarded to affect microbial composition, were treated as covariates. Scaling was focused on inter-species distances with biplot scaling. A Monte Carlo permutation was employed to determine the significance of first and second axes. This procedure was performed with Canoco 4.5 software (Ithaca, NY).

RESULTS

Litter decomposition

Values of C/N ratio and N concentration in litters differed among the four species (Table 2). The N concentration measured in fresh leaves of PE (*P. elliptica*) and SS (*S. superba*) were higher than in their conspecific normal fallen litter (although the difference was not statistically significant). In contrast, the two N-fixing tree species, AM (*A. mangium*) and AA (*A. auriculiformis*), had a lower N concentration in fresh leaves than in conspecific normal fallen litter (Table 2). In the fallen litter, the highest litter C/N ratio value was found in PE (59.45 ± 3.64), followed by SS (39.43 ± 3.98), being 50 and 29% higher than those of their conspecific fresh leaves, respectively (Table 2). In the two N-fixing species, ratios of C/N were similar between the fallen litter and fresh leaves (Table 2). The residual mass during the 12-month incubation varied significantly between litter types (Fig. 2). Normal fallen litter had significantly slower decomposition rates than fresh leaves for all species ($P < 0.001$, Fig. 2).

Soil microbial community structure

Species significantly affected both fungal PLFAs and the F/B ratio ($P = 0.02$ and $P < 0.01$, respectively, Table 3). In both samplings, SS soils had greater quantities of fungal PLFAs than other species soils in either fresh leaf or fallen litter groups (Fig. 3b), and the soils of SS fresh leaf treatment always had the greatest fungal PLFAs and F/B values, which were greater than all other treatment combinations (Fig. 3b and d). Soil fungal PLFAs and F/B ratio were also affected by

litter type ($P = 0.01$ and $P < 0.01$, respectively, Table 3). The soils of fresh green leaves always had greater fungal PLFAs and F/B ratio than the soils of conspecific fallen litter (Fig. 3b and d). Total PLFAs and the F/B ratio differed in the two sampling times ($P < 0.001$ for all, Table 2). The quantity of total PLFAs was greater in June 2009 than in December 2009 ($P < 0.001$, Fig. 3a), while the F/B ratio had the opposite trend ($P < 0.001$, Fig. 3d).

Soil microbial community composition varied among different species and litter types (Fig. 4a and b). In both samplings (June and December in 2009), soils treated with fresh leaf of SS was characterized mainly by high concentrations of the fungal PLFAs (18:1 ω 9c and 18:2 ω 6c). In the June data, axes 1 explained 34% variation of PLFAs, and the fresh leaf PE treatment soils had the similar soil microbial community composition as the fallen litter treatment soils of AA and AM, while the conspecific litter treatments of PE soils were similar to the fresh leaf treatments of AA and AM soils (Fig. 4a). The sampling time also greatly affected the effect of litter treatments on soil microbial community compositions. In the June data, the treatment variables explained 41.7% of PLFAs' variation, while in the December data, the value was only 20%.

Soil respiration

Soil respiration showed a seasonal pattern with slower rates (20–60 mg C m⁻² h⁻¹) in the dry season and faster rates (70–150 mg C m⁻² h⁻¹) in the rainy season (Fig. 5). Species and litter type did not affect soil respiration as main effects

Table 2: means (\pm 1SE) of initial C and N concentration of leaf litter in the four study tree species in subtropical China ($n = 4$)

| Tree species | C Conc. (%) | N Conc. (mg g ⁻¹) | C/N |
|---------------|---------------------------------|---------------------------------|---------------------------------|
| Fallen litter | | | |
| PE | 45.13 \pm 0.75 ^{cd} | 7.61 \pm 0.36 ^d | 59.45 \pm 3.64 ^a |
| SS | 47.20 \pm 1.93 ^{bc} | 12.02 \pm 0.75 ^{cd} | 39.43 \pm 3.98 ^{bc} |
| AM | 52.39 \pm 4.78 ^a | 17.18 \pm 0.79 ^a | 30.53 \pm 3.13 ^d |
| AA | 48.71 \pm 1.63 ^{abc} | 15.82 \pm 0.83 ^{ab} | 30.85 \pm 2.02 ^d |
| Fresh leaves | | | |
| PE | 51.23 \pm 5.11 ^{ab} | 12.92 \pm 0.97 ^{bc} | 39.84 \pm 5.53 ^b |
| SS | 43.44 \pm 1.38 ^{de} | 14.95 \pm 3.89 ^{abc} | 30.51 \pm 8.43 ^d |
| AM | 46.26 \pm 2.38 ^{cd} | 13.76 \pm 2.46 ^{abc} | 34.22 \pm 5.07 ^{bcd} |
| AA | 45.45 \pm 1.85 ^{cd} | 14.88 \pm 0.96 ^{abc} | 30.34 \pm 0.30 ^{cd} |

Note: Different lowercase letters indicate significant (LSD, $P < 0.05$) differences in each litter trait among species.

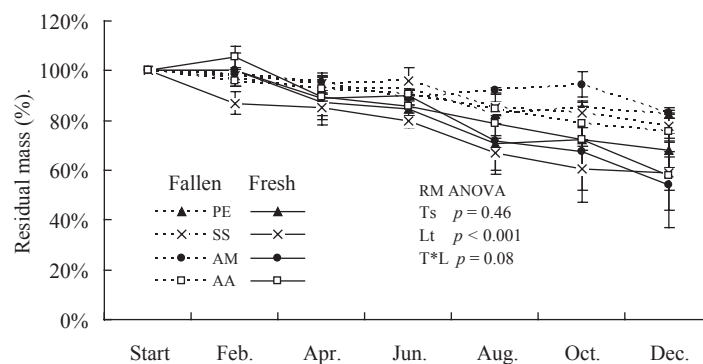


Figure 2: changes of residual mass of leaf litter in the litter decomposition experiment in 2009. Ts: tree species effect, Lt: litter type effect. The error bars represent one standard error (SE) ($n = 4$).

Table 3: three-way ANOVA table of the effect of independent variables of time, species, litter type and the two-way interaction of species and litter type on soil microbial characteristics ($n = 4$).

| Factors | Time (df = 1) | | Species (df = 3) | | Litter (df = 1) | | S*L (df = 4) | |
|-----------------|---------------|------|------------------|------|-----------------|------|--------------|------|
| | F | P | F | P | F | P | F | P |
| Total PLFAs | 36.66 | 0.00 | 1.32 | 0.28 | 0.95 | 0.34 | 0.12 | 0.95 |
| Fungal PLFAs | 1.93 | 0.17 | 3.87 | 0.02 | 7.33 | 0.01 | 1.14 | 0.34 |
| Bacterial PLFAs | 2.02 | 0.16 | 0.15 | 0.93 | 1.25 | 0.27 | 0.05 | 0.98 |
| F/B | 26.13 | 0.00 | 14.70 | 0.00 | 15.26 | 0.00 | 5.14 | 0.00 |

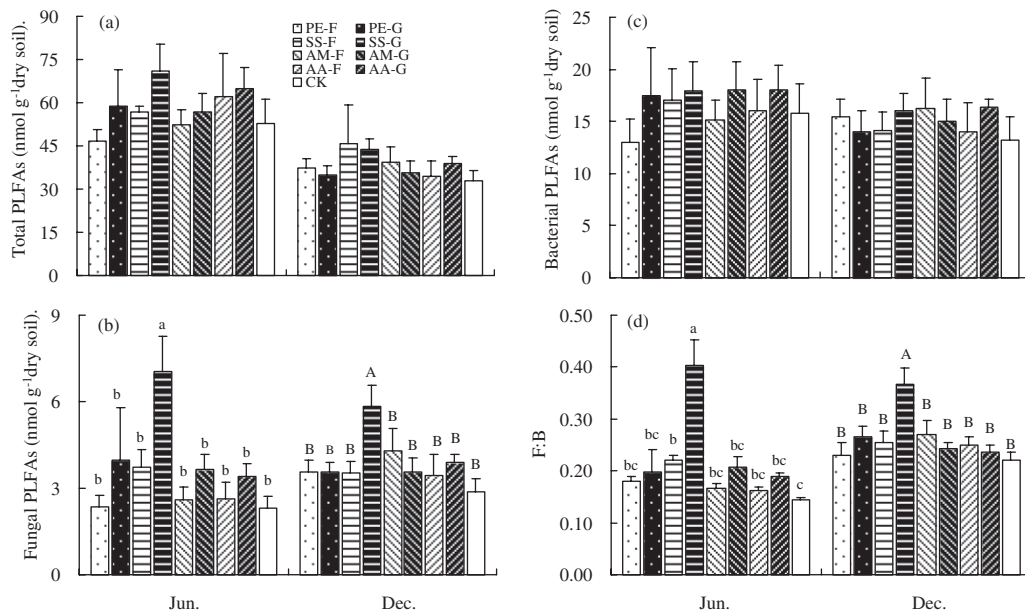


Figure 3: soil microbial PLFAs in the common garden experiment in 2009. F: Fallen litters, G: Fresh green leaves. The error bars represent one SE ($n = 4$). Superscript letter indicated a significantly difference ($P = 0.05$) was detected by one-way ANOVA, and bars sharing the same superscript letter were not significantly different at $P = 0.05$ (LSD).

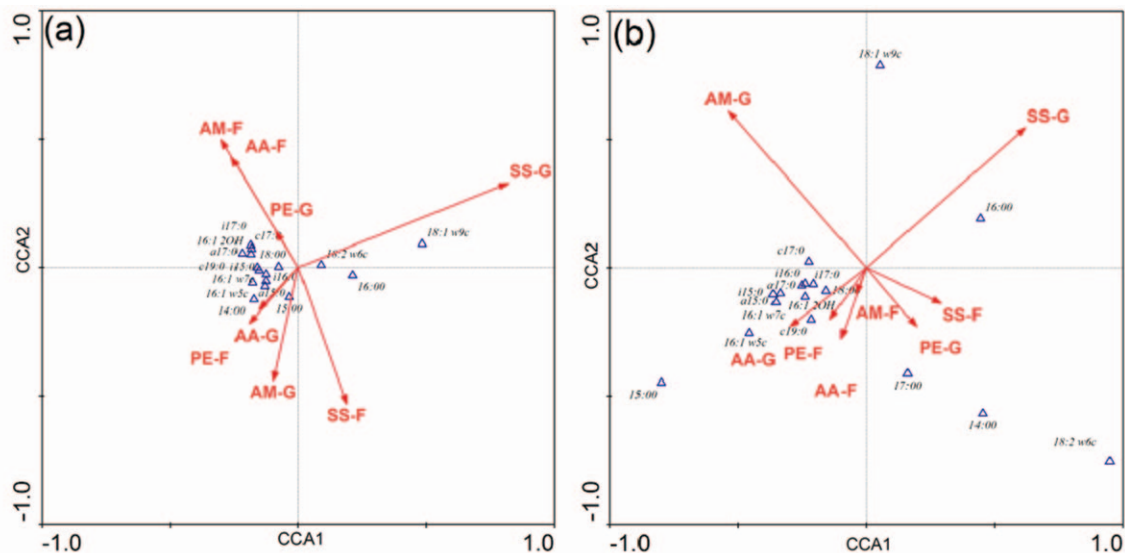


Figure 4: CCA biplots of soil PLFAs and different litter treatments variables (PE, SS, AM, AA, F and G) in June (a) and December (b), 2009. Soil properties (organic carbon, pH, total nitrogen and C/N ratio) were treated as covariables. Eigenvalues of June data (a) are 0.015 ($F = 10.32$, $P = 0.002$) and 0.001 for first (horizontal) and second (vertical) axes, respectively. The first two axes of June data (a) explain 37.4% of the variation. Eigenvalues of December data (b) are 0.004 ($F = 2.12$, $P = 0.91$) and 0.003 for first (horizontal) and second (vertical) axes, respectively. The first two axes of December data (b) explain 17.4% of the variation.

(RM-ANOVA: S: $P > 0.05$; L: $P > 0.05$) but rather in a two-way interaction of species and litter type (RM-ANOVA: S \times L: $P = 0.02$). To define this interaction effects, an LSD *post hoc* test was run. In PE, the conifer species, fallen dry litter treatment had greater soil respiration than fresh green leaf treatment (Fig. 5a, $P = 0.01$). The opposite pattern was observed for the broadleaf species where fresh leaf treatments respired

more CO₂ from soil than natural senescent litter ($P = 0.076$, $P = 0.023$ and $P = 0.14$ for SS, AM and AA, respectively).

The pattern was more demarcated for cumulative soil respiration data (Fig. 5). Among the four species, addition of litter induced 20–68% higher CO₂ emission than control. The annual soil respiration in the control was 4.13 t C ha⁻¹ yr⁻¹, which was significantly less than other treatments, except SS

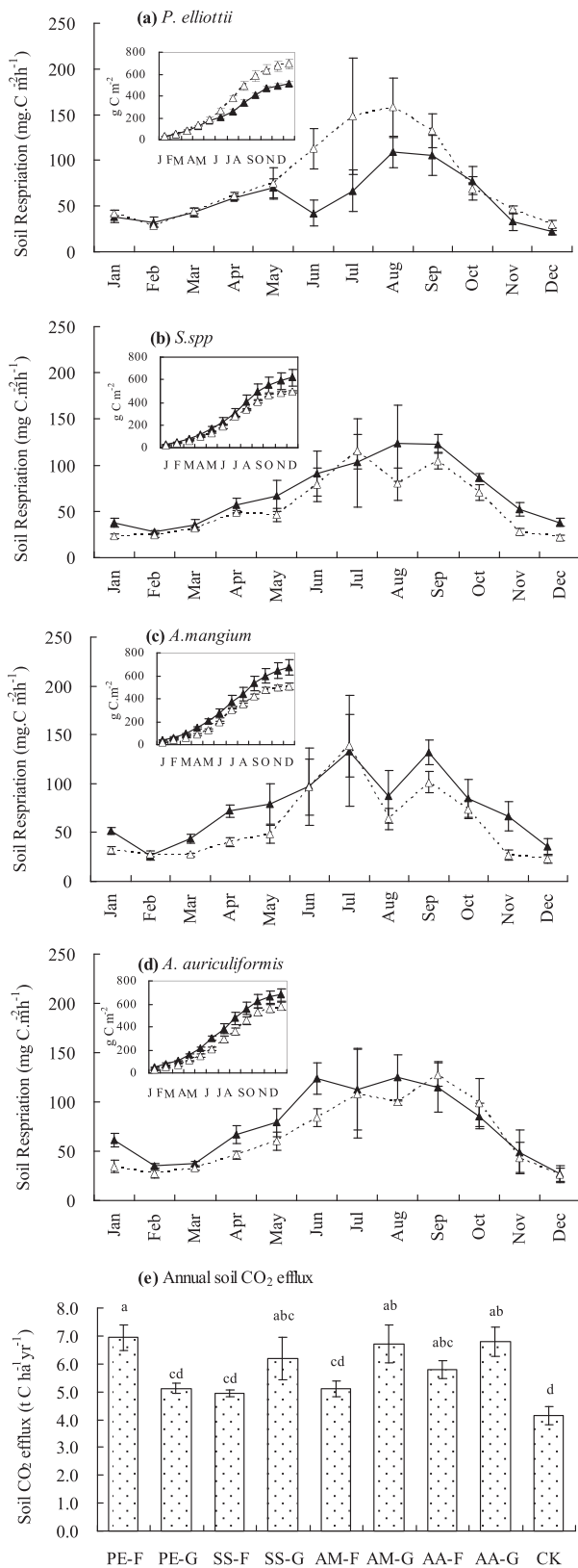


Figure 5: monthly variation of soil respiration in different litters of PE (a), SS (b), AM (c), AA (d) treatments in 2009 (RM-ANOVA: Ts: $P > 0.05$ Lt: $P > 0.05$ T \times L: $P = 0.02$) and annual soil CO₂ efflux (e), the inset figure indicating the cumulative soil respiration for each species. PE, SS, AM, AA, F, G. The error bars represent one SE ($n = 4$).

and AM fallen litter plots ($4.96 \text{ t C ha}^{-1} \text{ yr}^{-1}$ and $5.10 \text{ t C ha}^{-1} \text{ yr}^{-1}$, respectively) and PE fresh leaf plots ($5.12 \text{ t C ha}^{-1} \text{ yr}^{-1}$). Until May 2009, the addition of different litter types did not show any difference in CO₂ release for the conifer PE. However, in the following 4 months (June–September), the fallen litter treatments ($100\text{--}150 \text{ mg C m}^{-2} \text{ h}^{-1}$) released much more CO₂ than the fresh leaf treatments (Fig. 5a), and finally 35% more CO₂ was released from the fallen litter ($6.94 \text{ t C ha}^{-1} \text{ yr}^{-1}$) than from the fresh leaf treatments (Fig. 5e, $P = 0.009$). For the three broadleaf species, fresh leaves always had higher soil respiration than the corresponding fallen litter for the entire duration of the experiment (Fig. 5b–d). The annual soil respiration in the fresh-leaf treatments of SS ($6.19 \text{ t C ha}^{-1} \text{ yr}^{-1}$), AM ($6.71 \text{ t C ha}^{-1} \text{ yr}^{-1}$) and AA ($6.79 \text{ t C ha}^{-1} \text{ yr}^{-1}$) was 25, 32 and 17% higher than the conspecific fallen litters, respectively (Fig. 5e).

The relationship between soil respiration and soil temperature was well fit by the exponential growth regression model ($P < 0.01$ for all treatments, Fig. 6). Soil temperature explained 71.75–84.08% of the variations in the fallen litter treatments, and 51.14–72.71% in the fresh leaf treatments. Q₁₀-value ranged from 1.895 to 2.759, with higher values in fallen litter treatments than their conspecific fresh leaf treatments (Fig. 6). A regression of residuals from a quadratic equation model did not reveal a significant influence of soil moisture on soil respiration rates (data not shown).

Relationship between soil respiration and litter characteristics

In both months, June and December, there was no correlation between the initial litter quality and soil respiration. However, if the conifer PE was removed from all treatments, the cumulative soil respiration was correlated positively with their litter N concentration and negatively correlated with litter C/N ratio in the June sampling (Fig. 7a and b). Litter N concentration and C/N explained about 94.6% ($r^2 = 0.946$) and 91% ($r^2 = 0.91$) of the variance of broadleaf species soil accumulated respiration in June, respectively. If conifer PE was included, they only explained 46% ($r^2 = 0.46$) and 20% ($r^2 = 0.20$), respectively (Fig. 7). However, no such relationships were observed in the December data.

DISCUSSION

Litter decomposition

As what we hypothesized, the rate of decomposition was faster in fresh leaves than their conspecific senescent fallen litters. This result is in accordance with previous studies in subtropical and tropical forests (Xu et al. 2004a; Xu 2006), where researchers have found that typhoon-generated fresh leaves, regardless of broadleaf or conifer species, decomposed much faster than normal leaf litter. The same result was observed in a tropical forest in Indonesia (Yoneda 1997). Rates of decomposition may be related to the nutrient content of litter. In the present study, the fresh leaves had higher concentrations of N than fallen litter, but only in the non-N-fixing species. These data

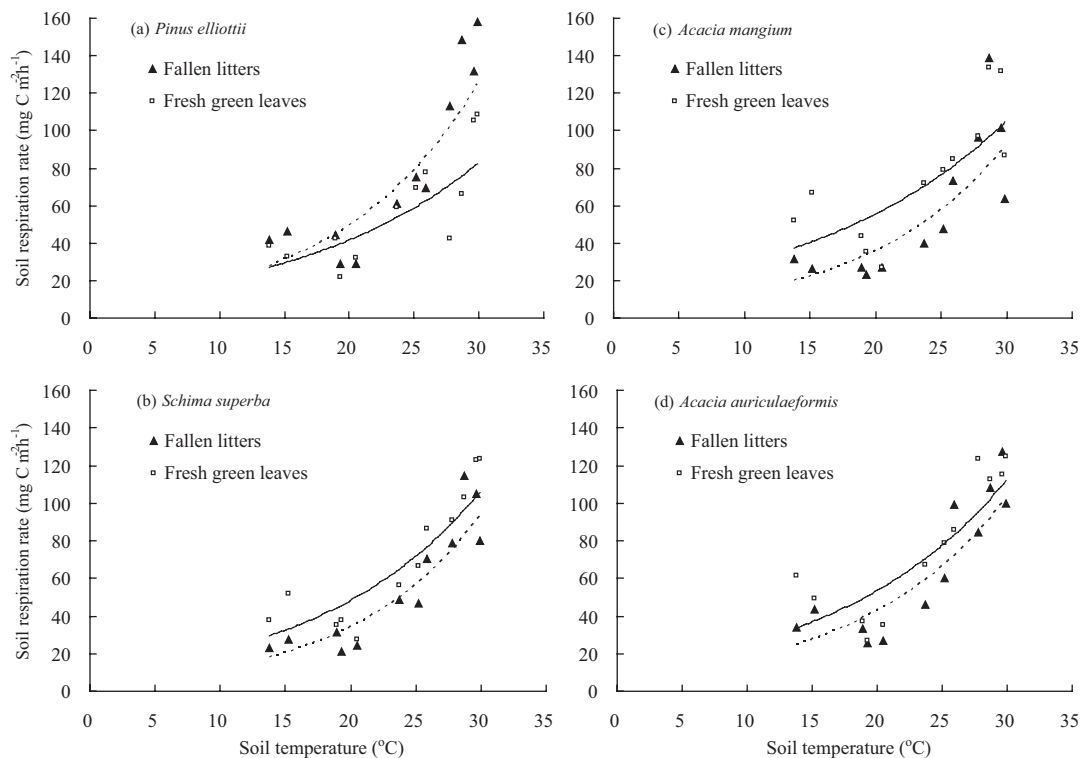


Figure 6: relationship between soil respiration rate and soil temperature under all tree species treatments. Fitted exponential lines represent the treatments of normal fallen litters (dashed black) and fresh green leaves (solid black). The equation and statistics are (a) *P. elliotii*: Fallen litters $y = 7.7151e^{0.093x}$, $r^2=0.7271$, $P < 0.001$, $n = 12$, $Q_{10} = 2.535$; Fresh leaves $y = 10.466e^{0.069x}$, $r^2 = 0.5996$, $P = 0.003$, $n = 12$, $Q_{10} = 1.994$; (b) *S. superba*: Fallen litters $y = 4.48e^{0.1015x}$, $r^2=0.8408$, $P < 0.001$, $n = 12$, $Q_{10} = 2.759$; Fresh leaves $y = 9.9254e^{0.0791x}$, $r^2 = 0.7266$, $P < 0.001$, $n = 12$, $Q_{10} = 2.206$; (c) *A. mangium*: Fallen litters $y = 5.4079e^{0.0946x}$, $r^2 = 0.7299$, $P < 0.001$, $n = 12$, $Q_{10} = 2.575$; Fresh leaves $y = 15.526e^{0.0639x}$, $r^2 = 0.5114$, $P = 0.009$, $n = 12$, $Q_{10} = 1.895$; (d) *A. auriculaeformis*: Fallen litters: $y = 7.3477e^{0.088x}$, $r^2 = 0.7175$, $P = 0.001$, $n = 12$, $Q_{10} = 2.411$; Fresh leaves: $y = 12.102e^{0.0743x}$, $r^2 = 0.6086$, $P = 0.003$, $n = 12$, $Q_{10} = 2.102$.

support those of Xu (2006) who made a similar conclusion about fresh leaves of always being premature and usually containing higher concentrations of nutrients (i.e. N and P) and decomposing more rapidly than normal fallen leaves. A different mechanism may operate for N-fixing species. Nitrogen concentration was not the sole dominant factor to control litter decomposition. Rather, other features, such as P and lignin concentration, have influenced decomposition rate (Li *et al.* 2001). In subtropical China, lignin concentration correlated negatively with decomposition rate and explained 54% variance of decomposition rate in eight tree species (Chen *et al.* 2011). Hence, these factors are likely to vary considerably between the species and litter types we investigated, and their influence on decomposition warrants further investigation.

Soil microbial community composition and respiration

Generally, soil microbial biomass and activity is limited by the availability of labile C (Wardle *et al.* 1998). The aboveground litter inputs thus could induce a 'bottom-up control' on soil microbial biomass and activity due to the increased input of labile C (Wardle *et al.* 1998; Allen *et al.* 2010). In the current study, as we hypothesized, the addition of fresh leaves into soils increased

fungal PLFAs and the F/B ratio compared with normal fallen litter in the June sampling. As fresh leaves had greater labile C and nutrients availability than normal litters (Xu *et al.* 2004a), and litter-derived labile C could lead to rapid shifts in microbial composition (Cleveland *et al.* 2007), the result in the present study indicated that increased inputs of fresh leaves resulting from forest disturbance and enhanced growth under climate change could change soil microbial community composition, which may further affect soil respiration (see below).

The results from soil respiration measurements indicated that litter addition, independent of species or litter types, lead to 20–68% higher annual CO₂ emission than the control, although such increases were not always statistically significant. This result was consistent with the findings of recent litter manipulation experiments that litter addition increased soil respiration rate (Sulzman *et al.* 2005; Crow *et al.* 2009; Berger *et al.* 2010), and could lead to a priming effect on soil respiration (Fontaine *et al.* 2004; Chemidlin Prévost-Bouré *et al.* 2010; Sayer *et al.* 2011).

Temperature was the dominant factor to control soil respiration in this study. This is consistent with many studies reported in South China (Mo *et al.* 2008; Wang *et al.* 2011; Wu *et al.* 2011). The sensitivity of soil respiration to soil temperature in this study was higher in fallen litter treatments (2.411–2.759)

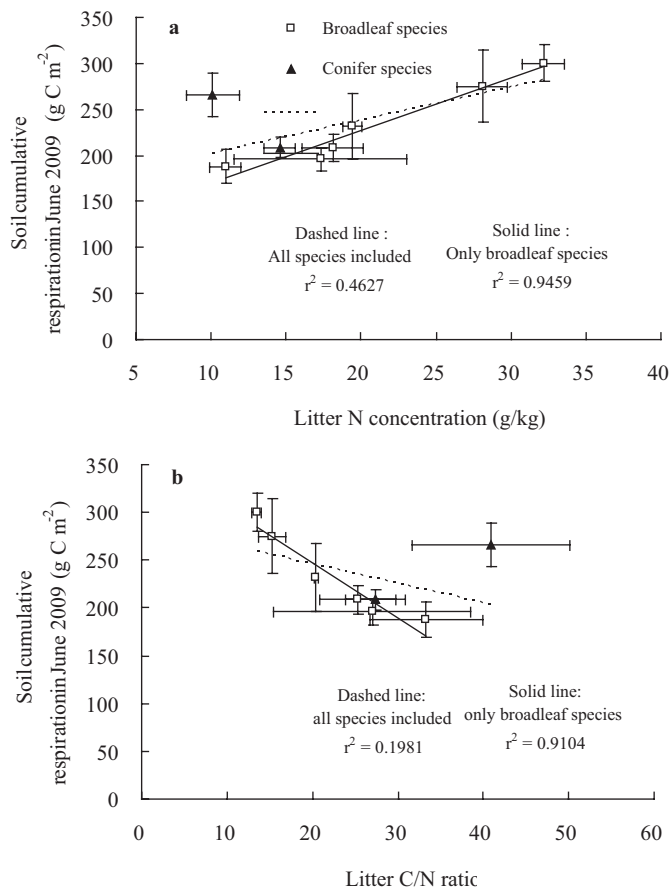


Figure 7: relationship between cumulative CO₂ efflux and litter N concentration and C/N ratio in June 2009. The error bars represent one SE ($n = 4$).

than those in fresh leaf treatments (1.895–2.206). Although these values were all within previous reported range (Wang et al. 2011; Chen et al. 2009), this result still indicated that litter quality affected the temperature sensitivity of soil respiration. Generally, fresh leaf usually contains more labile C than normal fallen litter (Xu et al. 2004a). According to fundamental principles of enzyme kinetics, the temperature sensitivity of microbial decomposition should be inversely related to litter carbon quality (Bosatta and Ågren 1999). The result in the current study thus was consistent with the enzyme kinetics principles. Furthermore, Fierer et al. (2005) through a litter incubation experiment has proved that the quality of organic C substrates consumed by microorganisms was inversely related to the observed Q_{10} of litter decomposition. With an expecting of increased temperature due to climate change, this result indicated that the decomposition of normal fallen litters was more sensitive to temperature change than that of fresh leaves.

The response of soil respiration to fresh leaf inputs depended on whether the source of litter was conifer or broadleaf. In broadleaf species, we found fresh leaf induced cumulative soil respiration was 17–32% higher than that of fallen litters, while

in conifer PE, fallen litters had 35% higher soil CO₂ emission than fresh leaf treatments. Thus, the effects of fresh leaf inputs on soil microbial activities were species dependent. The modifications of soil microbial community composition by litter addition may partly explain this result. In June 2009, CCA analysis indicated that PE-F, AA-G and AM-G treatments were similar in microbial community composition, while PE-G, AA-F and AM-F treatments were similar in a different direction (Fig. 4a).

The residual mass data shows that fresh leaves decomposed faster than corresponding fallen litters in broadleaf species. Hence, the litter-derived CO₂ emissions should be higher under fresh leaves treatments, which was in accordance with the observed soil respiration patterns under broadleaf species. However, this study also found that fallen PE litters (lower litter quality) decomposed slower than fresh PE leaves (higher litter quality) but induced more soil CO₂ flux. This result is similar with the finding by Giardina et al. (2001), who reported that soil C mineralization rates under *P. contorta*, a species producing low quality litters, was much faster than that under *Populus tremuloides*, which had higher litter quality and faster litter mass loss rate (Taylor and Parkinson 1988). Many studies have found that increased needle litter inputs could enhance soil-derived CO₂ emission-priming effect (Sulzman et al. 2005; Crow et al. 2009; Berger et al. 2010). Several studies has reported that low quality compounds, such as cellulose or wheat straw amended to soils induced more effect on SOM mineralization than glucose or fructose (Fontaine et al. 2003; Shen and Bartha 1997). We thus hypothesize that trends of faster soil respiration at PE fallen litter treatments were associated with greater soil-derived CO₂ emission.

CONCLUSIONS

In subtropical forests of South China and other typhoon affected regions, typhoon disturbance is an important factor to control forest litter fall. Some climate models predict an increase in the intensity and frequency of tropical cyclones in a future warmer climate (Knutson and Tuleya 2004; Solomon et al. 2007). Hence, the fresh leaf inputs would increase in forest litterfall in the context of climate change. This study found that soil microbial community composition and activity response to fresh leaf inputs were species dependent to soil heterotrophic respiration would be enhanced by fresh leaf inputs of broadleaf species but depressed by conifer fresh leaves, soil fungal biomass was higher in fresh leaf than fallen litter treatments soils, resulting in greater values of the F/B PLFA ratio, and fungal PLFA values were greater for *S. superba* than the other species. These results thus indicated that more accurate estimates of soil microbial community response to different litter inputs is critical to improve our knowledge of forest C cycling in the context of climate change.

ACKNOWLEDGEMENTS

National Natural Science Foundation of China (30870442), National Basic Research Program of China (2009CB421101 and

2011CB403200), NSFC-Guangdong Joint Project (U1131001), Guangdong Natural Science Foundation (S2011040005712), the Knowledge Innovation Program of the Chinese Academy of Sciences (KSCX2-EW-J-28) and the 'Strategic Priority Research Program — Climate Change: Carbon Budget and Related Issues' of the Chinese Academy of Sciences (XDA05070307). We also thank Prof. Yiqi Luo from University of Oklahoma for his comments on an earlier version of this paper.

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