Ecosystems (2016) 19: 450–460
DOI: 10.1007/s10021-015-9941-2

ECOSYSTEMS

Soil Moisture Alters the Response of Soil Organic Carbon Mineralization to Litter Addition

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Abstract

Increasing rainfall and longer drought conditions lead to frequent changes in soil moisture that affect soil organic carbon (SOC) mineralization. However, how soil moisture affects response of SOC mineralization to litter addition in forest ecosystems remains unexplored. We added ^{13}C-labeled litter to subtropical forest soils with three mass water contents (L, 21%; M, 33%; H, 45%). Carbon dioxide production was monitored, and the composition of soil microbial communities was determined by phospholipid fatty acid (PLFA). When no litter was added, SOC mineralization was greater in the M-treated soil. Litter addition promoted SOC mineralization, but this promotion was altered by soil moisture and litter type. Priming effects induced by *P. massoniana* leaf litter in the M-moistened soil were significantly ($P < 0.05$) higher than those in other treatments. Litter-derived C was approximately 55% incorporated into 18:1ω9c and 16:0 PLFAs, and this proportion was not significantly affected by soil moisture. Soil moisture affected the distribution of litter-^{13}C in i15:0, i17:0, and cy19:0 individual PLFAs. The primed C evolution was significantly related to the ratio of Gram-positive to Gram-negative bacteria. These results suggest that changes in soil moisture could affect SOC mineralization in forest ecosystems.

Key words: soil moisture; litter addition; priming effect; soil microbial community; soil organic carbon; forest eocsystem.

Introduction

Although future changes in precipitation strongly depend on climatic zone and region, considering recent climate changes, increasing rainfall and longer drought conditions may be expected (IPCC 2007). These conditions cause frequent changes in soil moisture and consequently influence the availability of carbon (C) and nutrients (Schimel and others 2007; Butterly and others 2009) and the mineralization of soil organic C (SOC; Navarro-García and others 2012; Wang and others 2013a). Therefore, as highlighted by Kuzyakov (2010), studying the influence of soil moisture on the priming effect is of particular importance for understanding the potential influence of climate change on the C cycle.

The priming effect is defined as the promotion or retardation of SOC mineralization by the addition

Received 10 March 2015; accepted 23 October 2015;
published online 4 March 2016

Author contributions Qingkui Wang designed this experiment and wrote this paper. Zhangquan Zeng measured the soil microbial community by PLFA and gave some advice when writing the paper, and Micai Zhong measured soil respiration and analyzed these data

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of an external organic substrate to the soil (Kuzyakov and others 2000). This effect has been extensively studied in response to the addition of organic C to soil, ranging from easily degraded C sources (Hamer and Marschner 2005; Qiao and others 2014) to plant residues (Potthast and others 2010; Wang and others 2013a). Most studies on the priming effect have focused on the quantity and quality of external substrates (Hamer and Marschner 2005; Potthast and others 2010) and on nutrient availability (Nottingham and others 2009; Zhang and Wang 2012; Wang and others 2014). Soil moisture is a key factor influencing SOC mineralization in terrestrial ecosystems (Liu and others 2009; Moyano and others 2013). Although some studies on SOC mineralization in relation to soil moisture have been conducted (Schimel and others 2007; Misson and others 2010; Manzoni and others 2012), little is known about how soil moisture affects the priming effect in forest ecosystems.

The composition and activity of the soil microbial community affect the magnitude and direction of the priming effect (Blagodatskaya and Kuzyakov 2008; Garcia-Pausas and Paterson 2011; Yao and others 2012; Wang and others 2014). The response of the soil microbial community to litter addition may be affected by changes in soil moisture because soil moisture plays a vital role in regulating microbial activity and community composition (Hackl and others 2005; Chen and others 2007; Brockett and others 2012). For example, short-term increases in soil microbial activity can occur after rewetting of dry soils, as shown by a flush of C mineralization (Navarro-García and others 2012; Göransson and others 2013). Although many studies have investigated the influence of soil moisture on microbial community composition (Chen and others 2007; Brockett and others 2012; Zumsteg and others 2013), none of these studies have monitored changes in microbial community composition with priming effect changes. Moreover, changes in soil microbial community composition may alter litter-C flow within the soil microbial community (Rubino and others 2010; Garcia-Pausas and Paterson 2011; Wang and others 2014). Some recent studies have successfully traced ^{13}C-labeled substrates through soil microbial communities using ^{13}C-stable isotopic techniques, and important information on microbial utilization of a given substrate has been obtained through GC-C-IRMS analyses of individual phospholipid fatty acids (PLFAs; Moore-Kucera and Dick 2008; Dungait and others 2011; Wang and others 2014). However, data on the effect of soil moisture on C flow from ^{13}C-labeled substrates into soil microbial community are unavailable.

In the subtropical forest ecosystem of China, summer droughts have become more severe, and the frequency of heavy rains has increased, thereby resulting in frequent changes in soil moisture. In the present paper, we report the responses of SOC mineralization in an incubation experiment as affected by soil moisture and the addition of *Cunninghamia lanceolata* and *Pinus massoniana* litters. This study aims to illustrate how soil moisture affects the priming effect and litter-C flow into the soil microbial community in forest ecosystems. Our initial hypotheses are that the priming effect would increase by increasing soil moisture and that the relative contribution of SOC- and litter-derived C in CO_2 fluxes depended on soil moisture. To separate litter from SOC mineralization, we used ^{13}C-labeled litter and monitored ^{13}C flow through the main microbial groups. We believe that this study is the first to assess the influence of soil moisture on the priming effect in forest ecosystems.

Materials and Methods

^{13}C-Labeled Leaf Litter and Soil Sampling

In South China, *C. lanceolata* and *P. massoniana* are the main tree species used for timber production; these trees are cultivated over total areas of approximately 11.3 and 12.0 million ha, respectively. Therefore, litters from the two tree species were chosen for the incubation experiment. The site has a humid mid-subtropical monsoon climate with a mean annual temperature of 16.5°C and precipitation of 1200 mm. The seedlings of *C. lanceolata* and *P. massoniana* were labeled with $^{13}CO_2$ gas in a growth chamber. After 3-month labeling, the isotopic δ^{13}C values of *C. lanceolata* and *P. massoniana* leaf litters were 996 and 1318‰, respectively. Other chemical properties of the *C. lanceolata* and *P. massoniana* leaf litters are shown in Table 1. The soil used in the present experiment was collected from the 0–10 cm layer of a *C. lanceolata* forest located at the Huitong National Research Station of Forest Ecosystem (26°50′N, 109°36′E) in Huitong County, Hunan Province, South China. Fresh soil samples were transported to the laboratory and immediately sieved (<2 mm). Visible organic residues were removed by hand picking. The soil was classified as ultisol according to the second edition of USDA soil Taxonomy. The sand, silt, and clay contents of the soil

Table 1. Chemical Property of the Labeled *Cunninghamia lanceolata* and *Pinus massoniana* Leaf Litter Used in the Incubation Experiment

	C (g kg^{-1})	N (g kg^{-1})	P (g kg^{-1})	C/N	C/P	K (g kg^{-1})	Ca (g kg^{-1})
C. lanceolata	471.4	17.7	1.11	26.6	424.7	11.6	6.6
P. massoniana	477.1	19.2	1.51	24.9	316.0	6.2	1.6

samples were 11.7, 44.7, and 43.6%, respectively. The total C and N contents of the soil were 26.7 and 2.05 g kg^{-1}, respectively. Soil pH was measured at a soil-to-H_2O ratio of 1:2.5 (w/v) using a pH meter.

Incubation Experiment

Approximately, 8.5 kg of fresh soils collected was preincubated for 5 days in a bucket containing a beaker with distilled H_2O to prevent desiccation and a beaker with 1 M NaOH solution to trap the evolved CO_2. The experimental design included 27 samples divided into 9 treatments with 3 true replicates per treatment (Table 2). Nine soil samples were amended with *C. lanceolata* (CL) leaf litter, another nine soil samples were amended with *P. massoniana* (PM) leaf litter, and the remaining nine samples were used as control soils. The leaf litter was ground and then added as 5% of the SOC. The nine treatments included three soil mass water contents, that is, 21% (L), 33% (M), and 45% (H) representing 44, 69, and 95% of the water-holding capacity of the soil.

All soil samples were air-dried in the laboratory, and then wetted with distilled water to achieve L, M, and H soil moisture levels, respectively. Ground leaf litter was homogeneously incorporated with the soil to produce a mixture. This mixture and a vial containing 20 ml of 0.2 M NaOH solution were placed into 500-ml flasks to create a microcosm. Microcosms were incubated in the dark for 45 days at 25°C. The CO_2 evolved from the soil was measured on days 1, 3, 6, 10, 15, 21, 29, and 45 by alkali-trapping in the vials. After each sampling, the flasks were flushed with reconstituted humid and C-free air.

Table 2. Description of Experimental Treatments

Treatment	Soil moisture (%)	Leaf litter (g C kg^{-1} dry soil)	Leaf litter type
L	21	0	
M	33	0	
H	45	0	
L + CL	21	1.43	*C. lanceolata*
M + CL	33	1.43	*C. lanceolata*
H + CL	45	1.43	*C. lanceolata*
L + PM	21	1.43	*P. massoniana*
M + PM	33	1.43	*P. massoniana*
H + PM	45	1.43	*P. massoniana*

At the end of each sampling interval above, 10 ml of NaOH solution was used to determine the amount of CO_2-C evolved from soil via titration with 0.1 M HCl. The CO_2 evolved from the soil sample was calculated from the difference in the values of CO_2 evolved in the flasks with soil and without soil. The remaining 10 ml of NaOH solution was used to analyze the isotopic composition of the trapped CO_2 by a stable isotope-ratio mass spectrometer (Picarro G2131-i Analyzer, USA) with 0.2‰ analytical precision.

Microbial Community Composition

Soil microbial community composition was determined using PLFAs as biomarkers for different microbial groups. Lipid extraction and PLFA analyses were performed as described by Wang and others (2013a). After incubation, part of the soil was sampled and freeze-dried for PLFA analysis. Briefly, 5 g of freeze-dried soil was extracted using chloroform:methanol:phosphate buffer (1:2:0.8). The PLFAs extracted were purified on silica columns with chloroform, acetone, and methanol, amended with methyl-nonadecanoate as an internal standard for quantification, and converted to fatty acid methyl esters (FAMEs) by alkaline methanolysis. The concentration and isotopic composition of individual FAME were analyzed by tandem gas chromatography-mass spectrometry (Thermo Fisher, USA). Qualitative standard mixes (37 Comp. FAME Mix and Bacterial Acid Methyl Esters CP Mix, Sigma-Aldrich) were used to identify the peaks. The total bacterial biomass was calculated by summing i15:0, a15:0, 15:0, i16:0, 16:1ω7c, 16:1ω9c, 16:0, a17:0, i17:0, cy17:0, 17:0, 18:0, cy19:0, and 20:0 PLFAs (Hill and others 2000). PLFAs i15:0, a15:0, i16:0, i17:0, and a17:0 were used as markers for Gram-positive bacteria, whereas PLFAs 16:1ω7c, 16:1ω9c, cy17:0, and cy19:0 were used as markers for Gram-negative bacteria (Moore-Kucera and Dick 2008). PLFAs 18:1ω9c, 18:1ω9t, and 18:2ω9,12c were used as markers for fungi, and PLFAs 10Me16:0,

10Me17:0, and 10Me18:0 were used as markers of actinomycetes (Hill and others 2000).

Calculation and Statistical Analysis

A mass balance equation was used to calculate the amount of CO_2-C derived from litter and SOC under incubation (Blagodatskaya and others 2011):

$$C_L = C_t(\delta_t - \delta_S)/(\delta_L - \delta_S) \quad (1)$$

$$C_S = C_t(\delta_L - \delta_t)/(\delta_L - \delta_S), \quad (2)$$

where C_t ($C_t = C_L + C_S$) is the total amount of CO_2-C during the considered time interval, δ_t is the corresponding isotopic composition, C_L is the amount of C derived from the added litter, δ_L is the isotopic composition of the litter, C_S is the amount of C derived from SOC, and δ_S is the isotopic composition of CO_2-C in the control (non-amended soil) during incubation.

The priming effect (PE, %) induced by the added litter was calculated by comparing the amount of CO_2-C in litter-containing soil samples with the amount of CO_2-C in the control soil sample:

$$PE = 100 \times (CO_2\text{-}C_{treatment} - CO_2\text{-}C_{control}) / CO_2\text{-}C_{control}, \quad (3)$$

where $C_{treatment}$ is the accumulated amount of CO_2 derived from SOC in treatments with litter addition and $C_{control}$ is the amount of CO_2 derived from the SOC without litter addition under the corresponding soil moisture level.

The percentage of plant-derived labeled C in each PLFA was determined using a mass balance approach (Rubino and others 2010):

$$Pi = (\delta^{13}C_t - \delta^{13}C_c)/(\delta^{13}C_l - \delta^{13}C_c), \quad (4)$$

where $\delta^{13}C_t$ is the $\delta^{13}C$ enrichment (‰) of individual PLFA in the soils treated with litter at the end of incubation, and $\delta^{13}C_c$ is the $\delta^{13}C$ enrichment (‰) of individual PLFA in the control soil, and $\delta^{13}C_l$ is the $\delta^{13}C$ of labeled litter (‰). The total labeled litter-derived C in each PLFA was calculated by multiplying each Pi by the individual PLFA abundances.

All statistical analyses were conducted using SPSS version 17.0 for Windows (SPSS Inc., Chicago, USA). Two-way analysis of variance followed by Tukey's test was used to analyze the effects of soil moisture and litter addition on SOC mineralization and litter decomposition, primed C evolution, soil microbial community composition, and percentage distribution of ^{13}C among the main individual PLFAs. Pearson's correlation coefficients were calculated to quantify the relationship between the cumulative primed C evolution and the SOC mineralization and microbial community composition. Significant differences were determined at $P < 0.05$.

Results

SOC Mineralization and Priming Effect

SOC mineralization in no-leaf litter addition treatments differed among soil moisture levels (Figure 1). SOC mineralization increased over 45 days period according to the order: M > H > L, and ranged from 192 to 241 mg C kg^{-1} soil. Moreover, differences in the rate of SOC mineralization among treatments gradually diminished with increasing incubation time.

The temporal evolution of cumulative primed C evolution after leaf litter addition is shown in Figure 2. A high rate of primed C evolution was recorded during the first 21 days. At the late stage (from 29 to 45 days) of incubation, decrease in the cumulative primed C evolution was observed in some treatments but not in the M + PM treatment, suggesting the negative priming effect occurred. After the addition of *C. lanceolata* leaf litter, the cumulative primed C evolution in the M-treated soil was 77.8 and 17.9% higher than those in the L- and H-treated soils, respectively. After the addition of *P. massoniana* leaf litter, the cumulative primed C evolution in the M-treated soil was 139.6 and 98.5% higher than those in the L- and H-treated soils, respectively.

Soil moisture affected the priming effect of SOC mineralization (Figure 3). The highest priming effect occurred in the M treatments, showing priming effect induced by *P. massoniana* leaf litter addition, was higher than *C. lanceolata* leaf litter addition. The priming effect induced by *C. lanceolata* leaf litter addition was 23.2, 32.9, and 30.2% in the L, M, and H soil treatments, respectively. *P. massoniana* leaf litter addition induced priming effect of 26.1, 50.0, and 27.3% in the L, M, and H soil treatments, respectively.

Leaf Litter Decomposition

Leaf litter decomposition under different soil moisture levels showed a similar pattern (Figure 4). The decomposition of leaf litters began soon after addition during the first 21 days and then gradually slowed down thereafter. Litter showed the highest decomposition in the M soil moisture treatment

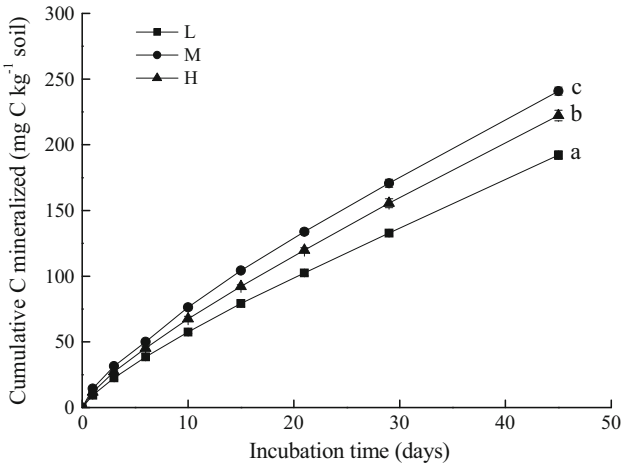

Figure 1. Evolution of cumulative SOC-C mineralized in control samples (without leaf litter addition) under different soil moisture levels (*L* low, *M* medial, *H* high). The *vertical bars* are standard deviations.

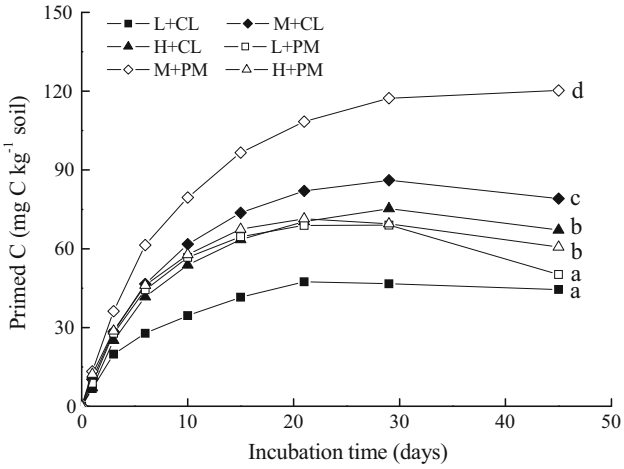

Figure 2. The cumulative primed C evolution from SOC after *C. lanceolata* (CL) and *P. massoniana* (PM) litter addition under different soil moisture levels (*L* low, *M* medial, *H* high) after the 45-day incubation.

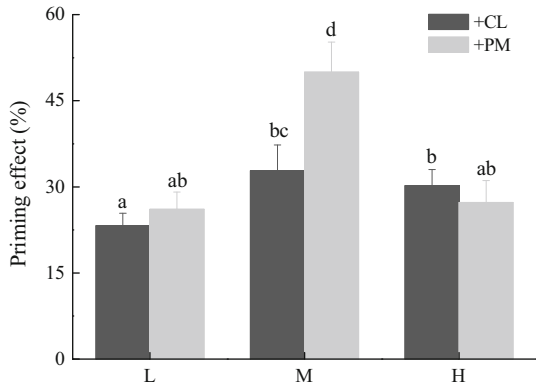

Figure 3. Priming effect of SOC mineralization under different moisture levels after incubation with *C. lanceolata* (CL) and *P. massoniana* (PM) leaf litter. *Bars* represent means ± standard deviations of three replicates. *Different letters above the bars* indicate significant differences at the 0.05 level.

and the lowest decomposition in the L soil moisture treatment. *C. lanceolata* leaf litter decomposition did not differ in the L and H soil moisture treatments, and *P. massoniana* leaf litter decomposition did not differ in the M and H soil moisture treatments. Considering the total litter decomposition observed during the 45 days incubation period, the proportion of the decomposed litter to the added leaf litter ranged from 19.7 to 32.8%.

Relative contribution of SOC-derived CO_2 to total CO_2 fluxes ranged from 54.8 to 64.0%, and contribution of litter-derived CO_2 varied from 36.0 to 45.2% (Figure 5). SOC-derived C contributed more to CO_2 fluxes than litter-derived C at the same moisture level. CO_2 derived from *C. lanceolata* leaf litter had lower contribution to CO_2 fluxes at the H moisture level, but CO_2 derived from *P. massoniana* leaf litter had lower contribution at the

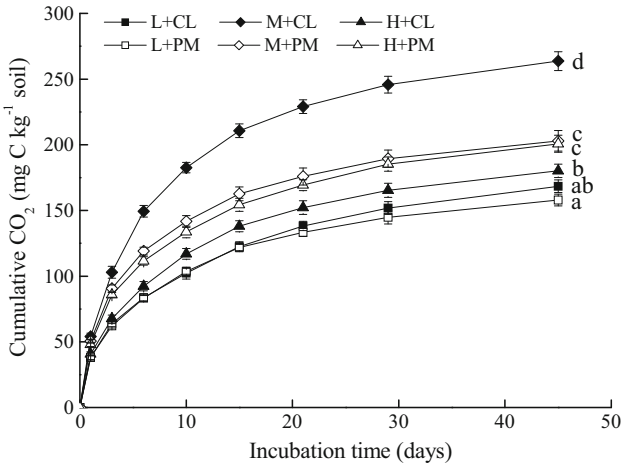

Figure 4. The cumulative amount of CO_2-C derived from *C. lanceolata* (CL) and *P. massoniana* (PM) leaf litters under different soil moisture levels (*L* low, *M* medial, *H* high). The *vertical bars* are standard deviations.

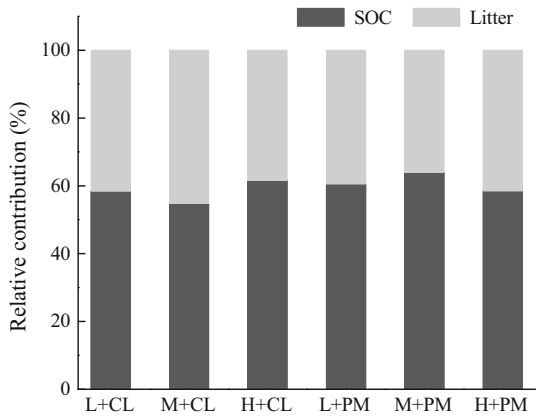

Figure 5. Relative contribution of SOC- and litter-derived C to CO_2 fluxes under different moisture.

M moisture level. On average, *C. lanceolata* litter-derived CO_2 showed higher contribution to CO_2 fluxes than *P. massoniana* litter.

Soil Microbial Community Composition and PLFA $\delta^{13}C$

Without litter addition, the bacterial PLFA concentration increased in the M treatment compared with that in the L treatment, thereby resulting in a higher ratio of bacteria to fungi (Table 3). The concentrations of actinomycetes, Gram-negative bacteria, and Gram-positive bacteria were also higher in the M treatment than in other treatments. Microbial communities in the L treatment were distinguished from microbial communities in the M and H treatments by higher abundances of Gram-positive bacteria (i15:0) and lower abundances of fungi 18:1ω9c and 18:2ω9,12c (Figure 6). The addition of leaf litters increased the microbial biomass, but decreased the ratio of bacteria to fungi compared with the treatments without litter addition at the corresponding soil moisture levels (Table 3). The addition of *C. lanceolata* leaf litter did not alter the effect of soil moisture on soil microbial concentration and community composition. By contrast, the addition of *P. massoniana* leaf litter altered the effect of soil moisture on the concentration and community composition of some microbial groups. The concentration of total PLFAs, fungi, and Gram-negative bacteria increased in the L + PM treatment, and the ratio of Gram-positive to Gram-negative bacteria decreased in the M + PM treatment.

Approximately, 55% incorporation of litter-derived ^{13}C into 18:1ω9c and 16:0 PLFAs was observed. These percentages decreased according to the order 18:2ω9,12c, i17:0, and cy19:0 PLFAs (Table 4). Incorporation of litter-derived ^{13}C into the i15:0 and cy19:0 PLFAs was lower under the L soil moisture. Most of the new litter-derived C was incorporated into non-specific bacteria and fungal PLFAs, accounting for over 72.3% of the total litter-derived C incorporated into PLFAs. Under the M moisture treatment, incorporation of *C. lanceolata* litter C into the total bacteria and Gram-negative bacteria was slightly higher than that of *P. massoniana* litter C; incorporation into fungi showed the opposite trend. *P. massoniana* litter C incorporated into fungi was higher under the L moisture treatment than that under the M moisture treatment.

The relationships between cumulative primed C evolution and mineralized SOC and the ratio of Gram-positive to Gram-negative bacteria are illustrated in Figure 7. Significant correlations were found between primed C evolution and mineralized SOC and between primed C evolution and ratio of Gram-positive to Gram-negative bacteria; by contrast, no relationship between primed C evolution and concentrations of total PLFAs, bac-

Table 3. Changes in the Concentration (nmol g^{-1} soil) of PLFAs and the Two PLFA Ratios in Soils Treated with *C. lanceolata* (CL) and *P. massoniana* (PM) Litters Under Different Soil Moisture Levels at the End of the 45-Day Incubation

	Total PLFA	Bacteria	Fungi	Actinomycete	Bacteria:fungi	GP	GN	GP:GN
L	70.2 ± 2.5a	49.6 ± 1.9a	14.9 ± 0.7a	5.71 ± 0.12a	3.33 ± 0.09b	19.42 ± 0.66a	12.84 ± 0.41a	1.51 ± 0.01ab
M	79.0 ± 3.3ab	57.0 ± 2.1b	15.1 ± 0.8a	6.83 ± 0.36b	3.78 ± 0.07c	22.47 ± 0.84b	14.46 ± 0.58b	1.55 ± 0.03b
H	75.6 ± 6.0a	53.2 ± 4.9ab	15.9 ± 0.7a	6.45 ± 0.44ab	3.35 ± 0.20b	19.42 ± 2.05ab	12.84 ± 1.31ab	1.50 ± 0.06ab
L + CL	91.7 ± 5.9bc	62.9 ± 3.9bc	21.8 ± 1.6b	7.10 ± 0.52bc	2.89 ± 0.06a	22.62 ± 1.41b	14.90 ± 0.84b	1.52 ± 0.02ab
M + CL	95.0 ± 3.9c	65.5 ± 2.7bc	22.4 ± 1.1b	6.99 ± 0.66bc	2.92 ± 0.04a	24.54 ± 0.69b	16.12 ± 0.96b	1.52 ± 0.08ab
H + CL	91.2 ± 2.7bc	63.0 ± 1.4bc	21.1 ± 2.2b	7.05 ± 0.38bc	3.01 ± 0.35ab	23.27 ± 1.46b	16.00 ± 0.72b	1.45 ± 0.07ab
L + PM	86.6 ± 2.7b	59.4 ± 2.5b	20.0 ± 0.7b	7.17 ± 0.70bc	2.97 ± 0.04a	22.25 ± 0.64b	14.38 ± 0.68b	1.55 ± 0.05ab
M + PM	102.1 ± 7.9c	69.7 ± 6.0c	25.8 ± 2.2c	6.69 ± 0.34bc	2.70 ± 0.05a	24.89 ± 2.11b	17.36 ± 1.92c	1.44 ± 0.04a
H + PM	98.2 ± 0.9c	65.0 ± 5.0bc	22.9 ± 1.3bc	7.23 ± 0.15c	2.84 ± 0.16a	24.57 ± 1.02b	16.61 ± 0.33c	1.77 ± 0.47b

Data expressed as mean ± SD (n = 3) are reported for different taxa and two PLFA ratios under different treatments at the end of 45-day incubation. GN and GP indicate Gram-negative and Gram-positive bacteria, respectively. L, M, and H denote low, medial, and high soil moisture, respectively. Different letters following the data in the same column denote significance.

teria and fungi, and Gram-positive and Gram-negative bacteria was observed.

Discussion

Priming effect caused by increased organic materials (for example, litter, root, and root exudates) under rising atmospheric CO_2 concentrations and temperatures will affect SOC mineralization (Kuzyakov 2010; Zhang and Wang 2012), but this priming effect is influenced by frequent changes in soil moisture caused by increases in rainfall and longer drought conditions. Our study on the effects of soil moisture on the response of SOC mineralization to litter addition yielded some important findings in subtropical forest soils. First, the priming effect of SOC mineralization was highest under the medial soil moisture level, but the response of priming effect to soil moisture is strongly related to litter species. Moreover, relative contribution of SOC- and litter-derived C to CO_2 fluxes depends on soil moisture conditions. Second, the response of the soil microbial community to soil moisture is affected by litter addition. Finally, bacterial community shifts are partly responsible for the differences in soil moisture influence on the priming effect. Although some important findings were yielded in our experiment, caution should be exercised when our results are applied to what will happen to priming effects, losses of SOC, and differential incorporation of litter and SOC into different microbial groups in the field in response to global climate changes. In our experiment, ground leaf litter was used to add into the soil rather than intact litter. The time course of decomposition of ground litter incorporated into soil will also differ from intact litter on the soil surface. The basic principles derived from our results, however, can be used to interpret patterns in the field, and it is also quite possible that these results would apply more directly to the effects of moisture interacting with leaf litter decomposition in the field.

Without addition of leaf litter, increased CO_2 production occurred in the M treatment, which suggests that native SOC mineralization is controlled by soil moisture. Several studies also demonstrate that soil respiration increases with soil moisture (Saiz and others 2007; Borken and Matzner 2009; Abera and others 2012). Findings in forest soils are inconsistent with the observations of Dijkstra and Cheng (2007) and Geisseler and others (2011) in arable soils. The different responses of SOC mineralization to changes in soil moisture may be attributed to differences in soil texture and moisture levels between experiments.

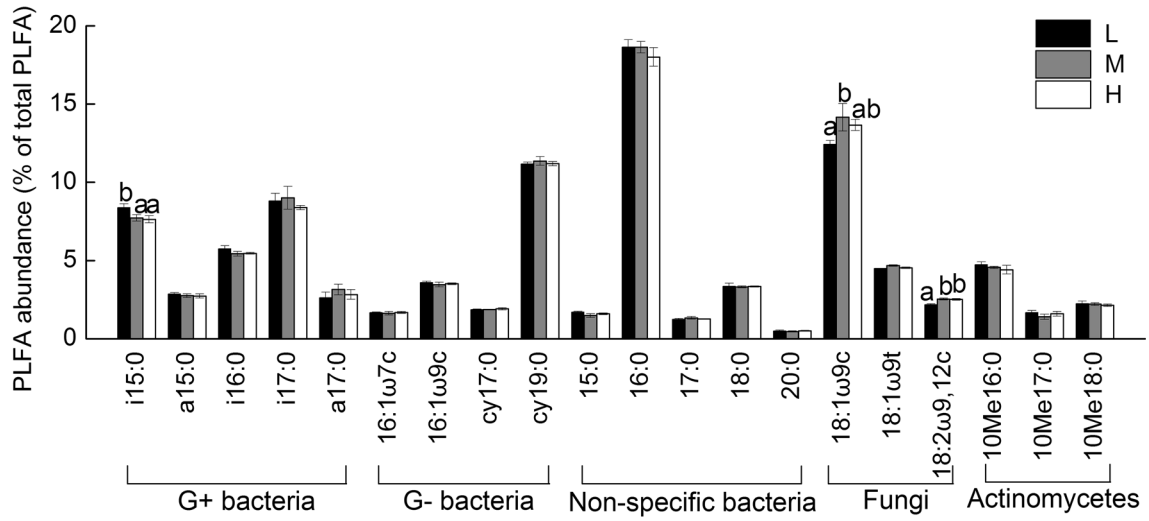

Figure 6. Relative abundances of individual phospholipid fatty acids (PLFAs) in control soils (without leaf litter addition) under different soil moisture levels (*L* low, *M* medial, *H* high). The *vertical bars* are standard deviations.

Table 4. Percentage of Distribution of Litter-^{13}C Among the Main Individual PLFAs Under Different Soil Moisture Levels at the End 45-Day Incubation

	L + CL	M + CL	H + CL	L + PM	M + PM	H + PM
G + bacteria						
i15:0	3.54 ± 0.31a	4.60 ± 0.29b	4.51 ± 0.34b	3.22 ± 0.18a	4.40 ± 0.33b	4.70 ± 0.40b
i16:0	4.08 ± 0.44a	3.24 ± 0.36a	3.63 ± 0.39a	4.42 ± 0.63a	3.42 ± 0.38a	3.29 ± 0.41a
i17:0	4.63 ± 0.49a	8.60 ± 1.03c	6.34 ± 0.57b	4.00 ± 0.35a	6.46 ± 0.44b	7.70 ± 0.97bc
a17:0	3.05 ± 0.32c	2.40 ± 0.26bc	2.24 ± 0.29bc	2.42 ± 0.30bc	1.21 ± 0.17a	1.86 ± 0.22b
G-bacteria						
cy17:0	4.55 ± 0.83b	4.42 ± 0.62b	2.57 ± 0.47a	2.76 ± 0.33a	2.64 ± 0.30a	3.44 ± 0.32ab
cy19:0	3.52 ± 0.34a	7.04 ± 0.65b	5.93 ± 0.74b	4.53 ± 0.38ab	6.09 ± 0.53b	6.11 ± 0.71b
Actinomycetes						
10Me17:0	1.17 ± 0.09b	1.70 ± 0.13c	1.00 ± 0.10b	2.44 ± 0.18d	0.50 ± 0.04a	1.65 ± 0.13c
10Me18:0	1.17 ± 0.11a	0.93 ± 0.10a	0.95 ± 0.08a	1.14 ± 0.10a	0.90 ± 0.07a	1.00 ± 0.08a
Non-specific bacteria						
15:0	1.03 ± 0.11b	1.03 ± 0.08b	0.85 ± 0.09b	0.24 ± 0.03a	0.67 ± 0.07b	1.14 ± 0.10b
16:0	30.33 ± 3.74a	25.64 ± 2.13a	27.20 ± 3.01a	28.80 ± 2.78a	27.00 ± 2.90a	26.00 ± 2.45a
17:0	0.49 ± 0.06a	0.79 ± 0.10a	0.61 ± 0.08a	0.49 ± 0.04a	0.46 ± 0.05a	0.69 ± 0.06a
18:0	1.18 ± 0.10a	1.72 ± 0.21b	2.13 ± 0.17bc	1.53 ± 0.14ab	2.86 ± 0.32c	2.17 ± 0.28c
Fungi						
18:2ω9,12c	8.71 ± 1.02a	7.39 ± 0.84a	8.09 ± 0.97a	8.92 ± 0.75a	7.23 ± 0.68a	8.18 ± 1.01a
18:1ω9c	28.96 ± 3.10a	26.22 ± 2.46a	29.68 ± 3.07a	30.28 ± 3.09a	31.87 ± 3.44a	27.56 ± 2.73a
18:1ω9t	3.59 ± 0.27a	4.27 ± 0.32a	4.27 ± 0.38a	4.80 ± 0.36a	4.31 ± 0.41a	4.52 ± 0.39a

Data are mean ± SD (n = 3) of three replicates at the end of the 45-day incubation. L, M, and H denote low, medial, and high soil moisture, respectively. CL and PM denote C. lanceolata and P. massoniana litters. Different letters in the same row denote significance.

Soil moisture is a driver of soil microbial activity, and microbes are generally believed to be the key factors affecting SOC mineralization in many ecosystems (Liu and others 2009; Moyano and others 2013). Higher primed C evolution in the M treatment compared with that in the L treatment was attributed to increases in labile C and nutrient flux, which could further stimulate microbial growth and activities (Schimel and others 2007; Iovieno and Baath 2008; Butterly and others 2009). This finding is supported by our data of soil microbial biomass (Table 3). Fierer and Schimel

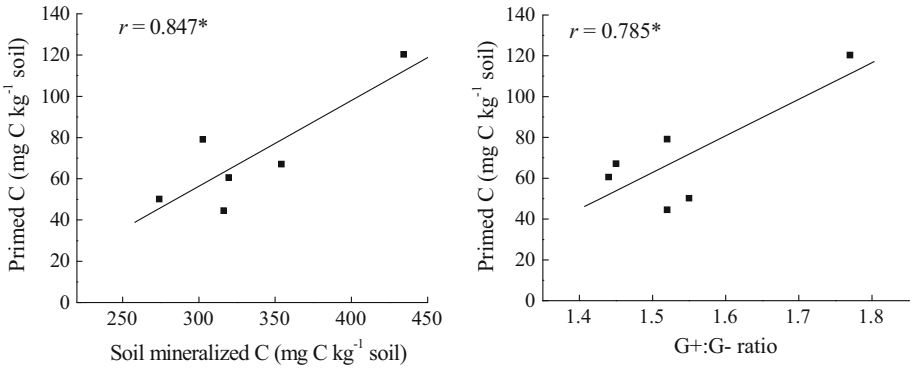

Figure 7. Relationship between cumulative primed C evolution at the end of 45-day incubation and both mineralized SOC and ratio of Gram-positive to Gram-negative bacteria.

(2002) and Iovieno and Baath (2008) determined that increases in C mineralization associated with changes in soil moisture occurred over relatively short periods of time (5–7 days). We also determined that differences in the rate of SOC mineralization between treatments gradually diminished with time, which suggests that the initial flush of labile C and nutrients had been consumed. Although we did not measure levels of the oxygen diffusion, we postulate that lower SOC mineralization in the H treatment compared with that in the M treatment is due to oxygen deficiency in the soil, which inhibits microbial activity and decomposition (Liu and others 2009; Geisseler and others 2011). In future research, we will determine soil pore size distributions, water potentials, and oxygen diffusion to explain further how soil moisture affects SOC mineralization.

Litter species affected the priming effect of SOC mineralization. At the same soil moisture level, differences in the priming effect induced by *C. lanceolata* and *P. massoniana* leaf litter addition were in accordance with observations from previous works (Blagodatskaya and Kuzyakov 2008; Potthast and others 2010; Wang and others 2013b), indicating that the quality of the substrate added to soils affects the magnitude of the priming effect. In a previous study, Wang and others (2014) found that the leaf litter with higher C:P ratios promoted greater SOC mineralization. In the present study, *C. lanceolata* leaf litter which features a higher C:P ratio (425) tended to cause higher priming effects than *P. massoniana* leaf litter with a lower C:P ratio (316) under the H soil moisture levels, but *C. lanceolata* litter caused lower priming effects than *P. massoniana* leaf litter under the L and M soil moisture levels. We postulate that in this experiment, the functions of other elements in controlling priming effect may be more important than that of the C:P ratio.

Contrary to our hypothesis, the priming effect was relatively more extensive in the M treatment than in other treatments. This finding does not agree with previous observation in agricultural soils (Dijkstra and Cheng 2007). Dijkstra and Cheng (2007) found that priming effects in the soils with 85% of water-holding capacity were higher than those in soils with 45% of water-holding capacity. The authors thus believed that the effect of soil moisture on the priming effect depends on the soil types. In the present study, the soil was clay loam with 43.6% clay, by contrast, the soil used by Dijkstra and Cheng (2007) was sandy loam. Moreover, the highest soil moisture (95% water-holding capacity) in our study was greater than that in the study of Dijkstra and Cheng (2007). Thus, we assume that differences in soil texture and moisture levels are responsible for distinct responses of priming effect to soil moisture. SOC mineralization with increasing soil moisture followed a uniform pattern after the addition of *C. lanceolata* and *P. massoniana* but the magnitude of the priming effect differed. This result suggests that the soil moisture dependency of the priming effect is affected by the litter species, as noted in other studies on agricultural soils (Geisseler and others 2011; Abera and others 2012). Differences in substance quality may be a possible mechanism for effect of litter species on the response of priming effect to soil moisture. In the present study, the two types of leaf litter had different initial P concentration and C:P. Li and others (2002) reported that soils from *C. lanceolata* forests had greater phenolic and lignin contents than soils from *P. massoniana* forests, but we did not determine these contents in the present study. Thus, our further work could include investigation of the interactive effect of soil moisture and litter quality on the priming effect.

Soil microbes can utilize leaf litter added to soil as energy and C sources to decompose native SOC. Soil moisture showed minimal effects on the distribution of ^{13}C in soil microbial groups for the same litter species, although ^{13}C incorporation showed significant differences in some individual

PLFAs (for example, i15:0, i17:0, cy19:0). The increase in soil microbial activity only lasted for relatively short time periods because of depletion of initial flush of labile C and nutrients associated with changes in soil moisture (Iovieno and Baath 2008). This resulted in no detectable effect of soil moisture on the distribution of ^{13}C in soil microbial groups. Therefore, the number of sampling times should be increased during the early incubation to qualify the dynamic activity and composition of soil microbial communities in future research. The ^{13}C incorporated into Gram-positive bacteria was twice as much as that incorporated into Gram-negative bacteria likely because of the larger concentration of Gram-positive bacteria than Gram-negative bacteria in the samples. Some studies also determined that incorporation of ^{13}C derived from exudates and glucose into Gram-positive bacteria was higher than that into Gram-negative bacteria (Rubino and others 2010; Dungait and others 2011). This finding suggests that the function of Gram-positive bacteria in decomposing litter is greater than that of Gram-negative bacteria.

In conclusion, greater SOC mineralization was observed in the M treatment (69% water-holding capacity) when no litter was added, which suggests that soil water availability is vital to SOC mineralization in acid soils from subtropical forests. However, we also note that increases in soil moisture may result in oxygen deficiency, which can inhibit microbial activity and SOC mineralization because the soil is clay loam with high clay content (Saiz and others 2007; Liptzin and others 2011). Therefore, SOC mineralization may decrease when soil moisture reaches full water-holding capacity. Priming effects were affected by changes in soil moisture, and higher priming effects were observed in the M-treated soils. Litter species affected the response of priming effects to changes in soil moisture, which indicates that soil moisture presents different effects on CO_2 emissions. Distinct contribution of SOC-derived C to total CO_2 fluxes under different moisture levels suggested that relative contribution of SOC- and litter-derived C to CO_2 fluxes was dependent on soil moisture conditions. Higher amounts of fresh litter C were incorporated into the 16:0 and 18:1ω9c PLFAs, which suggests that these two microorganisms perform significant functions in degrading added litter. Future work could focus on investigating bacterial and fungal communities by next generation pyrosequencing and DNA-based stable isotope probing to elucidate the importance of the soil microbial community to the soil C cycle further.

ACKNOWLEDGMENTS

This study was supported by the Strategic Priority Research Program of the Chinese Academy of Sciences (Grant No. XDB15010301), the State Key Laboratory of Forest and Soil Ecology (Grant Nos. LFSE2015-17 and LFSE2013-13), and the National Natural Science Foundation of China (Grant No. 31570466). We appreciate the assistance of Peng Wang during incubation experiment. We greatly thank the two anonymous reviewers for helpful comments to improve this manuscript.

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