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Labile carbon and nitrogen additions affect soil organic matter decomposition more strongly than temperature

Qianru Li[a,b], Yuqiang Tian[c], Xinyu Zhang[a,*], Xingliang Xu[a,*], Huimin Wang[a,b,f], Yakov Kuzyakov[a,d,e]

[a] Key Laboratory of Ecosystem Network Observation and Modeling, Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, 11A Datun Road, Chaoyang District, Beijing 100101, China
[b] College of Resources and Environment, University of Chinese Academy of Sciences, Beijing 100190, China
[c] State Key Laboratory of Earth Surface Processes and Resource Ecology, Beijing Normal University,Haidian District, Beijing 100875, China
[d] Department of Soil Science of Temperate Ecosystems, and Department of Agricultural Soil Science, University of Göttingen, Göttingen 37077, Germany
[e] Institute of Environmental Sciences, Kazan Federal University, Kazan 420008, Russia
[f] Jiangxi Key Laboratory of Ecosystem Processes and Information, Ji'an 343725, China

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ABSTRACT

Inputs of labile carbon (C) and nitrogen (N) affect the intensity and direction of priming effects (i.e., increase or decrease of soil organic matter (SOM) decomposition caused by labile inputs). Increased temperature is also an important factor affecting SOM decomposition. However, the effects of temperature on priming of SOM decomposition remain unclear. To investigate how temperature affects priming of SOM decomposition through changing microbial composition, we added ^{13}C-labeled glucose with or without NO_3^- or NH_4^+ to a subtropical plantation soil in southern China and incubated the soil at 15 °C and 25 °C for 10 days. Soil microbial composition was assessed by analysis of phospholipid fatty acids (PLFAs). Glucose led to positive priming (release of additional CO_2) at both temperatures. In contrast, glucose addition with NO_3^- or NH_4^+ resulted in negative priming. Temperature did not show a significant effect on SOM decomposition, while the effects of temperature on priming of SOM decomposition were dependent on labile C and N. Labile C addition induced stronger priming at 25 °C than at 15 °C, while combined C and N addition more strongly reduced priming at the high than the low temperature. Although PLFA composition was affected by temperature and labile C and N inputs, changes in PLFA composition were not correlated with priming. We conclude that temperature changes may have limited effects on SOM decomposition in this subtropical soil, while the availability of labile organics has a much stronger effect on priming under warming.

1. Introduction

Soil organic matter (SOM) contains the largest amount of carbon (C) in terrestrial ecosystems (German et al., 2011), almost four times higher than that in the atmosphere (Tarnocai et al., 2009). Even small changes in this soil C pool could significantly affect atmospheric CO_2 concentration, leading to positive feedbacks on climate change (Raich and Potter, 1995; Schlesinger and Lichter, 2001). Therefore, investigating the factors affecting SOM dynamics is a prerequisite for better understanding climate-carbon cycle feedbacks (Sun et al., 2014).

Temperature is an important factor affecting SOM dynamics. Increased temperature substantially accelerates SOM decomposition (Kirschbaum, 2006; Conant et al., 2011; Razavi et al., 2015), thus potentially contributing to global warming. Therefore, many studies have examined temperature sensitivity of SOM decomposition in the alpine, boreal, and temperate ecosystems (Lu et al., 2013), where the most dramatic increases in temperature have been predicted. Although greater understanding has been achieved, the results from various studies regarding the temperature sensitivity of SOM decomposition remain controversial (Giardina and Ryan, 2000; Fang et al., 2005; Bradford, 2013). Although a temperature increase is also expected in subtropical forests (Liski et al., 2003; Tan et al., 2012; Dai et al., 2016), few studies have explored the effects of warming on SOM decomposition (Wu et al., 2016).

* Corresponding authors.
E-mail addresses: zhangxy@igsnrr.ac.cn (X. Zhang), xuxingl@hotmail.com (X. Xu).

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Priming effect, defined as strong short-term changes in SOM decomposition rates caused by labile additions (Kuzyakov et al., 2000), has been shown to accelerate SOM decomposition by up to 380% or to decrease SOM decomposition by up to 50% (Kuzyakov, 2002; Cheng et al., 2014). Here, accelerated SOM decomposition is referred to as a positive priming effect while decreased SOM decomposition is regarded as a negative priming effect (Kuzyakov et al., 2000). Labile C inputs have a strong potential to affect SOM decomposition through inducing positive priming (Kuzyakov, 2010; Cheng et al., 2014) and, therefore, are another critical factor affecting SOM dynamics and the C cycle. Preferential substrate utilization (Cheng, 1999), microbial community shifts (Fontaine et al., 2003), mining of N (Craine et al., 2007), C starvation (Hobbie and Hobbie, 2013), and microbial activation (Blagodatskaya et al., 2007) have been invoked to explain the occurrence of priming effects; however, the mechanisms are still not fully understood. Microbial N mining could be an important mechanism responsible for priming of SOM decomposition, as N limitation occurs in most terrestrial ecosystems (LeBauer and Treseder, 2008). However, this possibility still needs extensive experimental investigation.

Both temperature and priming effects can accelerate SOM decomposition, but their effects may have distinct pathways. Generally, increased temperature enhances the rates of enzyme reactions through decreasing activation energy (Davidson et al., 2006; Blagodatskaya et al., 2016). At the same time, temperature can directly affect microbial metabolism or change microbial composition through influencing microbial interactions. Therefore, increased temperature can produce short-term and long-term effects on microbial decomposition of SOM. In contrast to temperature, other priming effects affect SOM decomposition mainly through labile inputs, which affect microbial growth and activities. In most soils, 10–40% of microbes are potentially active. Addition of labile organics to soils could stimulate potentially active microbes and contribute to microbial activities over short-term periods (Blagodatskaya and Kuzyakov, 2013). Additionally, labile inputs can cause microbial community shifts and/or lead to preferential substrate utilization by the microbes. Overall, both temperature and added substrates can cause changes in microbial composition (Zhou et al., 2015). Further, temperature can also affect microbial utilization of labile organics (Manzoni et al., 2012) and thus influence SOM priming. These interactions have not yet been evaluated.

Soil microbes need C and N in stoichiometric ratios for their growth and functioning (Lovell and Jarvis, 1995; Bardgett et al., 2008). Thus, inorganic N addition could modify the effects of added organics on microbial decomposition and microbe responses to increased temperature. Inorganic N forms have distinct properties, e.g., NH_4^+ is reduced and positively charged, while NO_3^- is oxidized and negatively charged. Microbes can directly use NH_4^+ for protein assimilation, but they need energy to reduce NO_3^- to NH_4^+ for further utilization. This indicates that NH_4^+ and NO_3^- could produce distinct effects on microbial decomposition of SOM (Britto and Kronzucker, 2013), but these effects remain unclear.

Forests play an important role in the global and regional C cycle; the C stock of forests, including soil and vegetation, has been estimated to be approximately 1,150 Pg C (Dixon et al., 1994). Approximately 40% of the total C is stored in subtropical and tropical forests (Dixon et al., 1994). In such forest types, most tree species are broad-leaved evergreens, and root exudation and litter decomposition occur nearly throughout the year, which can induce a positive priming effect and thus affect SOM dynamics (Qiao et al., 2014, 2016). Additionally, local temperatures are predicted to increase in these regions. Understanding how temperature affects priming of SOM decomposition will provide insights into climate C cycle feedbacks in subtropical forests. To address this question, we conducted an incubation experiment using subtropical plantation soil from southern China where such plantations are widely distributed and cover an area of approximately 25 million hectares (Wang et al., 2010). As microbial responses to labile inputs and temperature are very rapid, we incubated soil amended with glucose or glucose and inorganic N for a short period (10 days) at two temperatures (15 and 25 °C). Phospholipid fatty acid (PLFA) analysis was used to evaluate changes in microbial composition. We hypothesized that: (1) SOM decomposition is modified by labile C and N additions in subtropical soils, and that labile C addition induces positive priming, while N addition reduces SOM decomposition (NH_4^+ might have a stronger inhibitory effect than NO_3^- because energy is necessary for NO_3^- reduction); (2) priming of SOM decomposition increases with temperature; and (3) priming of SOM decomposition is related to changes in microbial composition.

2. Materials and methods

2.1. Site location and description

The study site is located at Qianyanzhou Forest Experimental Station in Jiangxi Province, southern China (115°04′13″E, 26°44′48″N), at an altitude of 100 m. The site is characterized by typical subtropical monsoon climate. Meteorological records for Qianyanzhou from 1989 to 2010 showed that average annual precipitation ranges from 1,300 mm to 1,600 mm, and the mean annual temperature is 18.0 °C, with the highest and lowest temperatures recorded as 39.5 °C and −5.8 °C, respectively. The native vegetation type is broad-leaf evergreens. However, the natural vegetation was destroyed by human activities and was changed to plantations in the 1980s. These plantations are dominated by Chinese fir (*Cunninghamia lanceolata*), slash pine (*Pinus elliottii*), masson pine (*Pinus massoniana*), and mixed with *Schima superba*, *Cinnamomum camphora*, and *Liriodendron chinense*. The main soil type in this region is red earth (Wang et al., 2004; Sun et al., 2006; Yuan et al., 2015) of the orthic acrisol category (FAO, 2014). The soil is a silty clay and contains 17% sand, 68% silt, and 15% clay by weight (Wen et al., 2010).

Because Chinese fir is the most important plantation tree species in this region, we collected the soil samples (top 10 cm), from a Chinese fir plantation that was established in 1998. The plantation was dominated by Chinese fir, with a few other species also present, such as masson pine and *Liquidambar formosana*. The dominant species of the understory layer were *Adinandra millettii*, *Callicarpa*, *Dicranopteris dichotoma* and *Woodwardia japonica*. The soil organic carbon (SOC) content in the sample was 18.2 g kg^{-1} with a field water-holding capacity (WHC) of 39.8% and soil pH of 4.3.

2.2. Soil collection and preparation

Before collecting soil samples, four plots (20 m × 20 m) were randomly selected in one Chinese fir plantation ecosystem and the plots were separated by buffer zones of more than 20 m. In each plot, five points were randomly set up to collect soil samples (the distance between points was more than 5 meters). After the aboveground vegetation and litter layer were removed, soil was collected from the top 10-cm soil layer using a polyvinylchloride cylinder (4.0-cm diameter). Four soil cores were collected from each point and 20 soil cores from one plot were pooled as one sample. In total, four soil samples were collected. After removing roots and stones, each soil sample was passed through a 2-mm sieve for homogenization. The sieved soil samples were stored at 4 °C until the incubation experiment started.

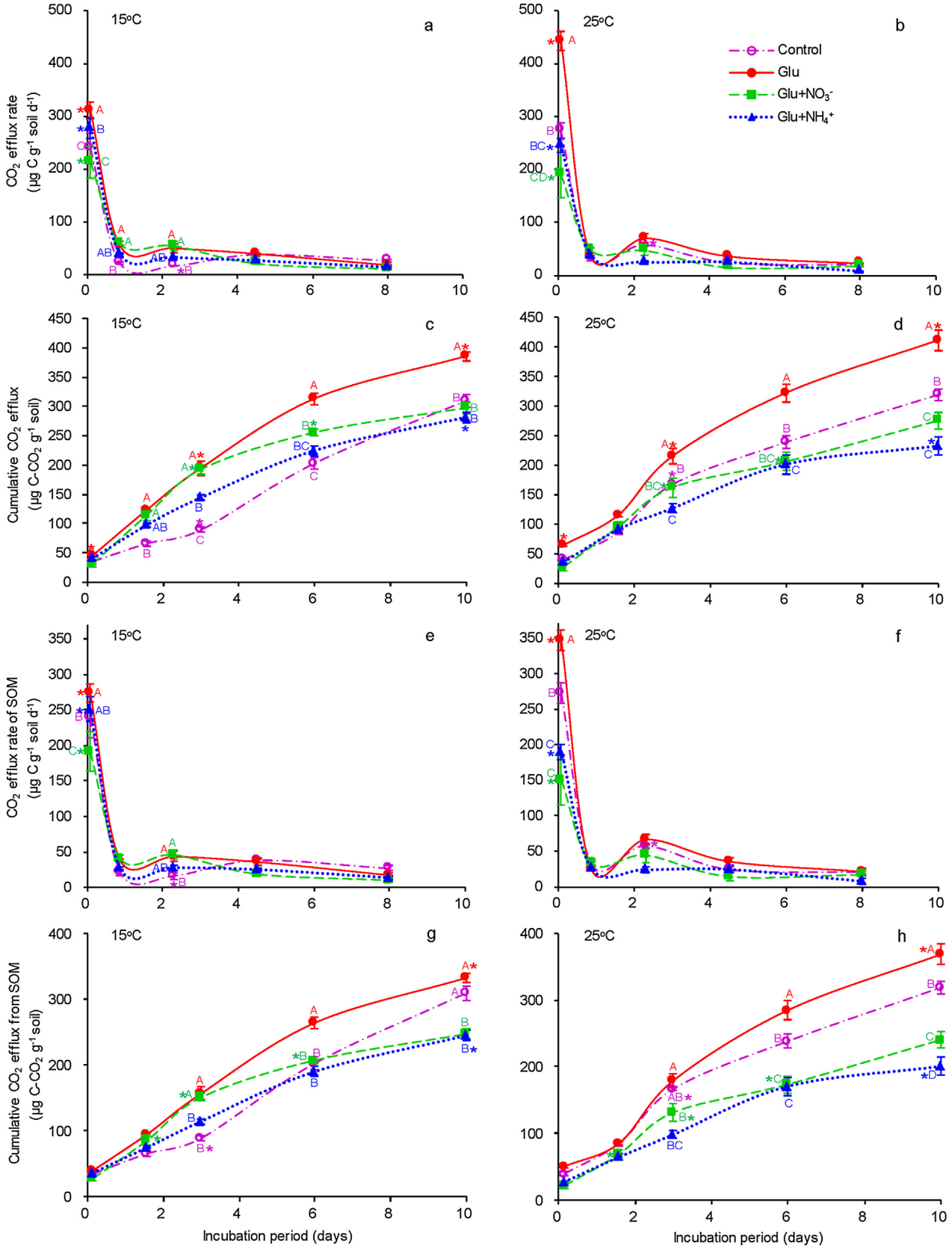

Fig. 1. CO_2 efflux rates (a, b), cumulative total CO_2 efflux (c, d), CO_2 efflux rates of SOM (e, f) and cumulative SOM-derived CO_2 efflux (g, h) during a 10-day incubation period of a subtropical soil. Left panel indicates 15 °C while right panel shows 25 °C. Pink dash-dotted lines with empty circles indicate treatment with water only (Control), red solid lines with solid circles indicate glucose input (Glu), green dash lines with solid squares indicate glucose and NO_3^- input (Glu + NO_3^-), blue dotted lines with solid triangles indicate glucose and NH_4^+ input (Glu + NH_4^+). Values represent means ± SE of four replications. Capital letters indicate significant ($P < 0.05$) differences between treatments at the same incubation time and the same temperature. Asterisks indicate significant differences for the same treatment between two temperatures at the same incubation time. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.3. Substrate addition, incubation, and sampling

Four substrate addition treatments were performed: addition of ^{13}C-labeled glucose (Glu), ^{13}C-labeled glucose with nitrate (Glu + NO_3^-), ^{13}C-labeled glucose with ammonium (Glu + NH_4^+) and a control treatment (Control, water addition only). Fifty grams of sieved soil were weighed into 250-ml Schott jars. The soil water content was adjusted to 43% WHC. To minimize the effects of steep changes in temperature on microbial composition and activity, soil samples were pre-incubated at 15 °C or 25 °C for 7 days to allow the microorganisms to adapt. Four replicates were performed for each treatment. In total, we prepared 160 bottles of soil samples. After pre-incubation, substrates were added to the soils according to the experimental design described above. Glucose was added as 100% of microbial biomass C (112 mg C kg^{-1}). Nitrogen was added as NH_4^+ in the form $(NH_4)_2SO_4$ at 4.5 mg N kg^{-1}, or as NO_3^- in the form KNO_3 at 3.3 mg N kg^{-1} based on their native concentrations in soil. The same amount of distilled water was added to the control treatment. After addition, the soil water content was monitored through weighing the bottles every 3 days and maintained at 70% WHC during the incubation. Small cups filled with 5 ml of 1 mol L^{-1} NaOH were placed in the jars to trap the evolved CO_2, and replaced at 4 h, 38 h, 3 day, 6 day, and 10 day. Soils were harvested at the end of incubation.

2.4. Sample analysis

Before starting the incubation period, total C and N contents in the soil were analyzed using an Elemental Analyzer (EA 1112, CE Instruments, Milan, Italy). Soil water content was determined at 105 °C for 24 h. Soil pH was measured with an electrode in the supernatant with dry soil-water ratio of 1:2. Microbial biomass C was determined by the chloroform fumigation extraction method (modified after Vance et al., 1987). Briefly, 10 g of fresh soil sample was weighed in 100-ml plastic bottles and extracted with 40 ml 0.05 mol L^{-1} K_2SO_4. Another 10 g of the same sample was weighed in a 50-ml beaker and fumigated with chloroform for 24 h. After fumigation, the samples were extracted using the same procedure. Total dissolved organic C in the extracts was measured using TOC (Liqui TOCII, Elementar, Germany). NO_3^- and NH_4^+ were measured on an autoanalyzer (AA3, Bran Luebbe, Germany).

The respired CO_2 from soil was determined by titrating NaOH against 0.2 mol L^{-1} HCl after addition of 0.5 mol L^{-1} $SrCl_2$. To measure ^{13}C in the respired CO_2, the remaining NaOH solution was precipitated by adding 0.5 mol L^{-1} $SrCl_2$ in excess and then washed with distilled water until the pH was close to 7.0. $SrCO_3$ precipitates were then transferred to glass bottles and dried at 105 °C. They were weighed in tin capsules for δ^{13}C analysis in a continuous-flow gas isotope ratio mass spectrometer (MAT253, Finnigan MAT, Bremen, Germany), with a ConFlo III device (Finnigan MAT) coupled to the elemental analyzer.

2.5. PLFA analysis

PLFAs were extracted following the method described by Bossio et al. (1998). After fatty acid methyl esters were formed through mild alkaline methanolysis, samples were analyzed using a Thermo ISQ gas chromatography mass spectroscopy system (Thermo TRACE GC Ultra ISQ). The samples were dissolved in hexane and analyzed with a DB-5 column using this mass spectroscopy system.

Different biomarkers for PLFAs, corresponding to distinct functional microbial groups, were used. Gram-positive bacteria were represented by i15:0, a15:0, i16:0, i17:0, and gram-negative bacteria were represented by 16:1ω7c, cy17:0, cy19:0. The sum of the biomarkers specifically for gram-positive bacteria and gram-negative bacteria were considered to represent the total bacteria (Frostegård and Bååth, 1996). The PLFAs 10Me18:0 and 10Me16:0 were used as an indicator of actinomycetes, and 18:2ω6 and 18:1ω9c were used as biomarkers for fungi. The total amount of PLFA biomarkers was taken to be representative of the microbial biomass.

2.6. Calculations and statistics

CO_2 efflux rates, cumulative mean values, and standard errors (SEs) were calculated for each sampling time from four replicates. Flux-weighted δ^{13}C values were based on CO_2 fluxes.

The end-member mixing model was used to partition CO_2 sources: SOM (C_{SOM}) and added glucose (C_G) (Phillips and Gregg, 2001; Phillips et al., 2005). Based on the fractions of glucose-derived CO_2 and their SDs obtained by the model proposed by Phillips and Gregg (2001), primed C was calculated as follows (Qiao et al., 2014):

$$\text{Primed C} = C_T - C_G - C_C \quad (1)$$

where C_T is total C—CO_2 from the soil with glucose addition, C_G is C—CO_2 from added glucose in the glucose-treated soil, and C_C is total C—CO_2 from the control soil.

Temperature sensitivity (Q_{10}) of SOM decomposition was calculated using the following equation (Zhu and Cheng, 2011):

$$Q_{10} = (R_{25}/R_{15})^{(10/\Delta T)} \quad (2)$$

where R_{25} and R_{15} are the mean SOM decomposition rates at 25 °C and 15 °C, respectively. ΔT is the difference value between two temperature treatments.

Tukey's HSD was used to test the effects of C and N additions and temperature on SOM priming. Repeated measures ANOVAs (Pallant, 2007) were performed with SPSS 21.0 (SPSS Inc., Chicago, IL, USA) to evaluate the effects of temperature and C and N additions on CO_2 efflux rates. Correlations between microbial PLFAs and mineral N were also analyzed. All differences were tested and significance was set as $P < 0.05$.

3. Results

3.1. CO_2 efflux and nitrogen mineralization

Similar total CO_2 efflux patterns were observed for all treatments at both temperatures (Fig. 1). The highest CO_2 efflux rate appeared at 4 h after C and N addition, decreased considerably during up to 38 h, and then leveled off until the end of the incubation (Fig. 1a,b). Repeated-measures ANOVA showed that labile C and N additions, as well as their interaction with temperature, significantly affected CO_2 efflux rates (Table 1,

Table 1
Results of repeated-measures ANOVA over time to evaluate effects of temperature and the addition of glucose with or without inorganic nitrogen on CO_2 efflux rates.

Source of variation	df	*MS*	*F*	*P*
Between subjects				
Intercept	1	1045349	2319	<0.001
C—N addition	3	13417	29.76	<0.001
Temperature	1	1069	2.37	0.14
C—N addition × Temperature	3	3086	6.85	0.02
Error	24	451		
Within subjects				
Time	1.17	1289077	651.35	<0.001
Time × C—N addition	3.51	28443	14.35	<0.001
Time × Temperature	1.17	5386	2.71	0.11
Time × C—N addition × Temperature	3.51	8615	4.25	0.11
Error	28.06	1964		

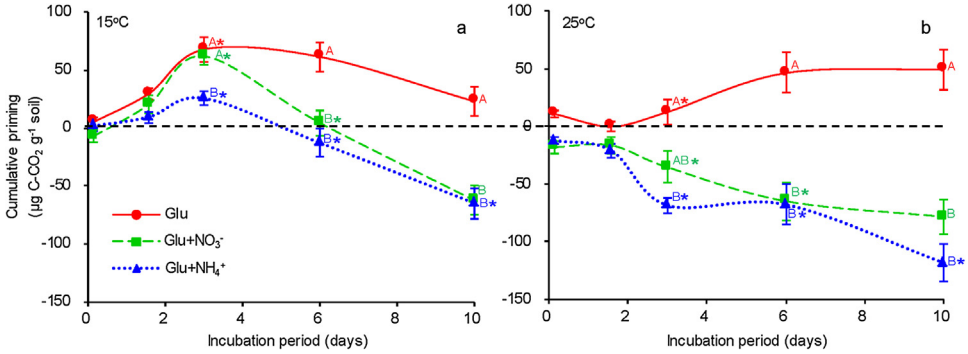

Fig. 2. Priming effects after addition of glucose (Glu), glucose with NO_3^- (Glu + NO_3^-) or with NH_4^+ (Glu + NH_4^+) at 15 °C (a) and 25 °C (b) during a 10-day incubation period in a subtropical soil. Red solid lines with solid circles indicate glucose input (Glu), green dash lines with solid squares indicate glucose and NO_3^- addition (Glu + NO_3^-), blue dotted lines with solid triangles indicate glucose and NH_4^+ addition (Glu + NH_4^+). Values represent means ± SE of four replications. Capital letters indicate significant ($P < 0.05$) differences between treatments at the same incubation time and the same temperature. Asterisks indicate significant differences between two temperatures at the same incubation time. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

$P < 0.05$), but temperature alone had no effect. Cumulative CO_2 efflux under labile C addition differed at both temperatures at the end of incubation (Fig. 1c,d, $P < 0.05$). At 15 °C, glucose addition increased total CO_2 efflux during the whole incubation period, whereas glucose with NO_3^- or NH_4^+ addition significantly increased total CO_2 efflux in the first 8 days (Fig. 1c, $P < 0.05$). Glucose addition increased total CO_2 efflux at 25 °C, whereas glucose with NO_3^- or NH_4^+ additions significantly reduced total CO_2 efflux (Fig. 1d, $P < 0.05$).

SOM-derived CO_2 efflux rates were highest at 4 h, then decreased rapidly and maintained stable (Fig. 1 e, f). Glucose addition significantly increased SOM-derived CO_2 efflux rates at both temperatures ($P < 0.05$), with stronger increase at 25 °C than at 15 °C (Fig. 1e,f). At 15 °C, cumulative SOM-derived CO_2 was significantly reduced by both N forms (Fig. 1g, $P < 0.05$). At 25 °C, glucose with NO_3^- or NH_4^+ additions significantly decreased SOM-derived CO_2 efflux by 24.6% and 37.1%, respectively (Fig. 1h, $P < 0.05$).

Soils amended with glucose demonstrated lower mineral N concentrations (11.4 ± 0.3 μg N g^{-1} soil, $P < 0.05$) than those added with glucose and NO_3^- (12.9 ± 0.3 μg N g^{-1} soil) or NH_4^+ (14.1 ± 0.4 μg N g^{-1} soil). The control soil had similar mineral N concentration as that amended with glucose and NO_3^-.

3.2. Priming effects and Q_{10} values

Glucose addition caused positive priming effects at both temperatures (Fig. 2), but its patterns differed during the 10-day period of incubation. At 15 °C, stronger priming occurred during the first 3 days and then decreased with time. Over 10 days, labile C input caused an additional CO_2 release of 23.1 μg C g^{-1} soil, corresponding to 6.9% of C release from the soil without addition (Fig. 2a). At 25 °C, labile C inputs slightly increased CO_2 emission from soil during the first 3 days and significantly so at 6 days, leading to positive priming at the end of incubation. Finally, labile C input caused an additional CO_2 release of 49.7 μg C g^{-1} soil at 25 °C, accounting for 13.5% of the amount released from the soil without addition (Fig. 2b). At both temperatures, additions of glucose together with NO_3^- or NH_4^+ resulted in significant negative priming at the end of incubation (Fig. 2, $P < 0.05$). Overall, the effect of temperature on priming depended on the addition of labile C or labile C plus N, and on the time of incubation (Fig. 2).

Q_{10} values of CO_2 fluxes from SOM in the four treatments fluctuated during the 10-day incubation period, ranging from 0.7 to 2. The effect of labile inputs on Q_{10} varied with time. Glucose addition reduced Q_{10} values between 38 h and 3 days ($p < 0.05$), exhibiting a contrasting pattern compared to the soil without addition. In contrast, glucose additions with NO_3^- or NH_4^+ caused a significant decrease in Q_{10} values especially in the first 3 days (Fig. 3, $P < 0.05$).

3.3. PLFAs

The effects of labile C and N additions on bacterial, fungal, and total PLFAs at the end of the incubation period were dependent on temperature (Fig. 4). At 15 °C, both bacterial and fungal PLFAs showed similar increases under labile additions (Fig. 4a). While at 25 °C, fungal PLFAs increased when only glucose was added, but bacterial PLFAs were reduced under both glucose and glucose with NH_4^+ amendments (Fig. 4b). Total PLFAs were higher at 25 °C than at 15 °C only after addition of glucose plus NO_3^- (Fig. 4, $P < 0.05$). Both bacterial and fungal PLFAs showed no correlation with NH_4^+ or NO_3^- at 15 °C. At 25 °C, bacterial PLFAs were positively correlated with NO_3^- (Fig. 5a), while fungal PLFAs were negatively correlated with NH_4^+ (Fig. 5b).

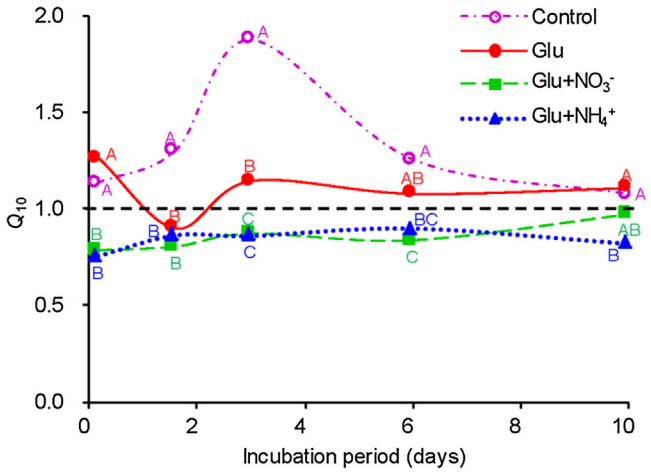

Fig. 3. Q_{10} values of four different treatments during a 10-day incubation period in a subtropical plantation soil. Pink dash-dotted lines with empty circles indicate treatment with water only (Control), red solid lines with solid circles indicate glucose input (Glu), green dash lines with solid squares indicate glucose and NO_3^- addition (Glu + NO_3^-), blue dotted lines with solid triangles indicate glucose and NH_4^+ addition (Glu + NH_4^+). Values represent means of four replications. Different capital letters indicate a significant difference between different treatments at the same incubation time at $P < 0.05$ level. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

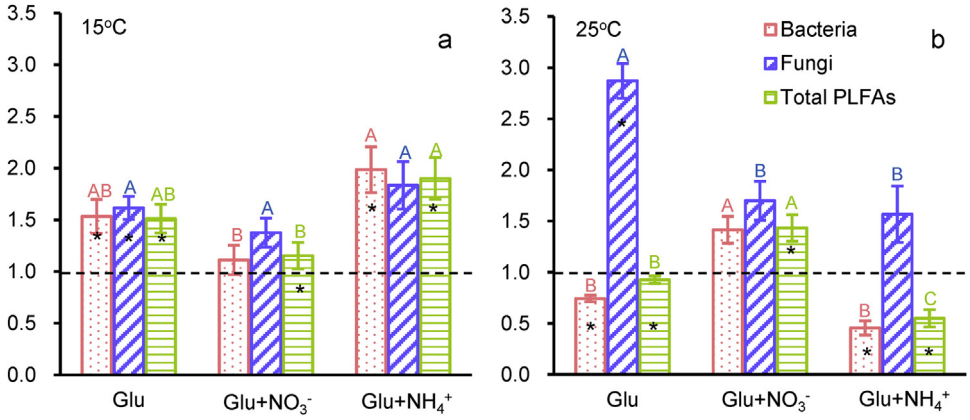

Fig. 4. Changes of phospholipid fatty acid (PLFA) profiles in soil after addition of glucose (Glu), or glucose with NO_3^- (Glu + NO_3^-), or with NH_4^+ (Glu + NH_4^+) in a subtropical soil at 15 °C (a) and 25 °C (b) at the end of the incubation (10 days). Changes in bacterial, fungal, and total PLFAs are presented relative to soil to which only water was added (Control soil = 1). Relative units of PLFAs are indicated in red dotted hatches (bacterial), blue diagonal hatches (fungal), and green horizontal hatches (total). Values represent means ± SE of four replications. Capital letters indicate significant ($P < 0.05$) differences between treatments for the same microbial composition at the same temperature. Asterisks indicate significant differences for the same microbial composition of the same treatment between two temperatures at the $P < 0.05$ level. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

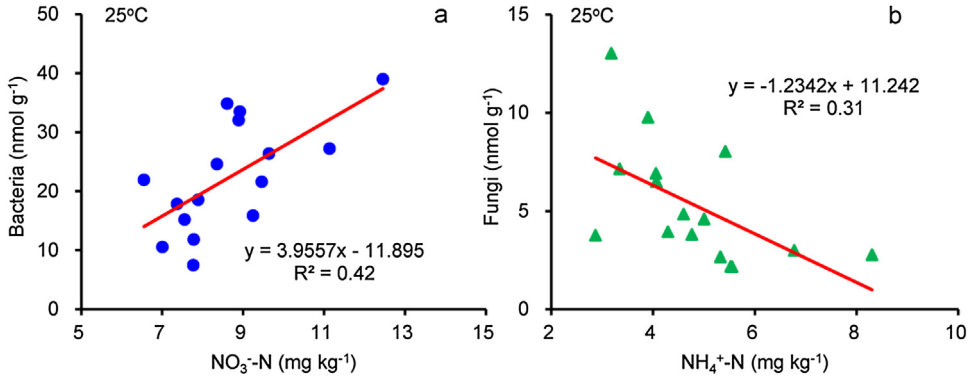

Fig. 5. At 25 °C, the correlation of bacterial PLFAs with soil NO_3^- concentration (a), and fungal PLFAs with soil NH_4^+ (b) at the end of incubation (10 days) of a subtropical soil.

4. Discussion

The addition of glucose alone or glucose together with inorganic N allowed us to assess the effects of labile C and N additions on priming of SOM decomposition and to examine the mechanism of "mining of nitrogen". Glucose addition (i.e. high C accessibility) induced positive priming in this subtropical plantation soil. Compared to the control, priming accelerated SOM decomposition by 13.5% at 25 °C and by 6.9% at 15 °C within 10 days. Such increases fall within the ranges reported previously (Fu and Cheng, 2002; Fontaine et al., 2004; Blagodatskaya et al., 2011, 2014), although they could largely be derived from the turnover of microbial biomass due to the short-term incubation (i.e. apparent priming, Blagodatskaya and Kuzyakov, 2008). In contrast, addition of glucose with NO_3^- or NH_4^+ produced negative priming (Qiao et al., 2016). The contrast between the positive priming seen after labile C addition and the negative priming after labile C and N addition is ascribed to the "mining of nitrogen" (Fontaine et al., 2003). Glucose addition is expected to increase C accessibility and to induce microbial growth or changes in the microbial community. As microbial growth continues, inorganic N is consumed and its concentration decreases. Microbes requiring N might be stimulated by the need for the element to produce extracellular enzymes to mine N from the SOM (Fontaine et al., 2003; Moorhead and Sinsabaugh, 2006), thus causing a positive priming effect (Fig. 6). This process is typically reduced by addition of N, which increases the available N concentration. Microbes requiring N could use the available N directly without the need to produce extracellular enzymes to mobilize N from SOM (Kuzyakov et al., 2000; Kuzyakov and Cheng, 2004). Microbes might prefer to use added C and N, leading to a negative priming effect (Fig. 6). Such negative priming effects have been further confirmed by Zang et al. (2016), who based their conclusion on 170 observations from 14 studies.

As expected, inorganic N addition induced negative priming, but NH_4^+ induced a larger negative priming effect than NO_3^-. This observation confirmed our first hypothesis. The difference in priming induced by NH_4^+ and NO_3^- is suggested to be a consequence of the additional energy needed by soil microbes to reduce NO_3^- to NH_4^+ before NO_3^- utilization for anabolism (Britto and Kronzucker, 2013; Gavrichkova and Kuzyakov, 2010). An additional explanation is that NH_4^+ addition could produce acidic conditions and thus affect microbial activity and priming intensity. Overall, this study confirms that priming can be modified by the C availability and the C/N ratio of the labile inputs.

Although temperature has been reported to accelerate SOM decomposition (Trumbore et al., 1996; Dalias et al., 2001; Knorr et al., 2005; Blagodatskaya et al., 2016; Razavi et al., 2017), we did not observe this effect in subtropical plantation soil over the 10-day incubation period despite an increase in SOM decomposition in the first 6 days at 25 °C (Fig. 1 c, d). Labile C and N addition altered the effect of temperature on SOM decomposition, e.g. high

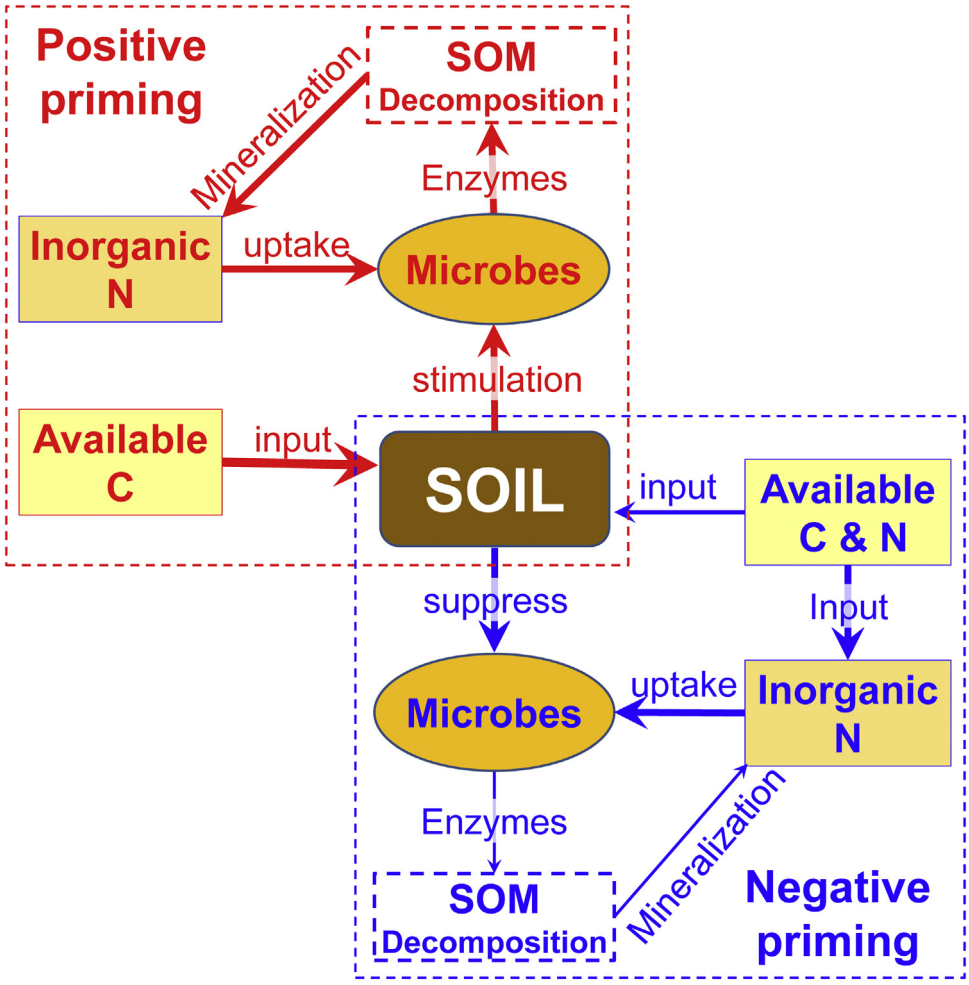

Fig. 6. A schematic diagram illustrating positive and negative priming effects caused by labile C and N additions to the soil. The red lines show processes for positive priming effects; the blue lines identify processes for negative priming effects. The thickness of the line indicates the magnitude of the process. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

temperature accelerated SOM decomposition under labile C addition, but strongly decreased SOM decomposition under labile C plus N addition (Fig. 1 g, h, $P < 0.05$). Q_{10} values for SOM decomposition were lower than the average Q_{10} of 2 (Davidson and Janssens, 2006), especially for soil with addition of labile C and N (Fig. 3). Compared to the control, the addition of C or C with NH_4^+ or NO_3^- considerably reduced Q_{10} values to less than 1.0. This suggests that soil CO_2 efflux depends mainly on the availability of the substrates (Dungait et al., 2012; Tian et al., 2015). The response of total CO_2 flux under labile C addition to temperature also indicates that C accessibility could control the temperature sensitivity of SOM decomposition. With respect to a priming effect, the response to temperature also relied on C and N accessibility, in contrast to a previous report (Ghee et al., 2013). After 10 days incubation, the addition of labile C had induced a higher priming effect at 25 °C than at 15 °C. By comparison, the addition of labile C and N strongly reduced priming at 25 °C compared to 15 °C. These findings provide important insights. First, subtropical plantation soil is not sensitive to temperature, while addition of labile C and N can strongly reduce temperature sensitivity with respect to SOM decomposition. Second, increased C accessibility has the potential to accelerate SOM decomposition through enhanced priming due to increased temperature, but labile C addition together with N could increase SOM sequestration via decreased priming due to high temperature. Our results partially supported our first hypothesis that priming of SOM decomposition could be enhanced by higher temperature in this subtropical soil.

PLFA analysis has previously been used to estimate the composition of living microbial communities (Dungait et al., 2011; Andresen et al., 2014). We found that increased temperature and labile inputs changed the PLFA profiles within the 10-day incubation. Bacterial and fungal PLFAs differed in responses to NO_3^- and NH_4^+ addition at the higher temperature (Fig. 5), indicating that bacteria and fungi might play different roles in N transformation (Wang et al., 2015; Balser and Firestone, 2005) and might alter N availability in the soil. However, we did not find any evidence that these changes were correlated with priming effects, thus negating our hypothesis that priming could be related to changes in microbial composition. One possible explanation is that PLFAs generally indicate living microbial groups, but they cannot distinguish living active from living inactive (dormant) microorganisms (Blagodatskaya and Kuzyakov, 2013). To investigate whether this was a contributory factor, the incorporation of ^{13}C into PLFAs need be analyzed to distinguish active microorganisms that utilize the added organics (Yao et al., 2014) or SOM (Hungate et al., 2015), together with the analysis of exoenzyme activities.

5. Conclusions

CO_2 efflux from subtropical plantation soil was not affected by temperature, but strongly dependent on labile C and N addition.

Within a 10-day incubation period, labile C addition induced positive priming, whereas the combination of labile C with mineral N resulted in negative priming. This priming reduction after N addition reflects the fact that microbial mining of N is an important mechanism responsible for priming of SOM decomposition in soil. Labile C and N addition altered the effects of temperature on SOM decomposition. High temperature increased priming after labile C addition, whereas priming was decreased under labile C and N addition. The microbial PLFA profile was altered by temperature and labile inputs, but was not correlated with priming in this soil. We conclude that temperature changes may have limited effects on SOM decomposition, but C accessibility could increase the effects of increased temperatures through priming of SOM.

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