

Article

The Influence of Different-Quality Litter Inputs on Soil Mineralization and Priming Effect in Subtropical Areas

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Abstract

The size and stability of the soil carbon pool depend on the balance between plant litter input and soil organic carbon mineralization. As an important source of soil organic carbon, litter input plays a key role in the decomposition of soil organic carbon through the priming effect and ultimately affects soil carbon balance. Litter quality is a key factor affecting the decomposition and transformation of soil organic carbon. However, uncertainties remain regarding the effects of different-quality litters on soil mineralization and priming. In this study, we used an indoor incubation method to label China fir leaf litter and Acacia leaf litter with the isotope ¹³C. The effects of different-quality litters on the intensity and direction of soil priming effects were studied by adding different-quality litters to soils in different subtropical regions. The results show that, compared with China fir (*Cunninghamia lanceolata*) litter (low-quality, high C/N), Acacia (*Acacia confusa*) litter input (high-quality, low C/N) promotes soil mineralization and decomposition. By contrast, low-quality litter produces higher quantities of litter source C, which returns to the atmosphere through CO₂. Different-quality litters could regulate the direction and intensity of the soil priming effect. The priming effect of the Acacia litter addition treatment was higher than that of the China fir litter addition treatment. The addition of high-quality litter promotes the generation of a positive soil priming effect. The addition of Acacia litter to different regions of soil had a positive priming effect, while the addition of China fir litter had a negative priming effect for a period of time. Furthermore, the correlation analysis results showed a significant connection between soil physical and chemical indicators and litter C/N and soil mineralization and priming effects. The random forest analysis showed that litter C/N ($p < 0.01$) is one of the main factors affecting the soil priming effect, indicating that litter quality has a higher relative importance to the priming effect. These findings underscore the critical role of litter quality in regulating subtropical soil carbon dynamics and providing pivotal datasets for predicting terrestrial carbon sink potential.



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1. Introduction

Soil organic carbon (SOC) pools are the largest carbon reservoirs in terrestrial ecosystems. They store more organic carbon than the sum of vegetation and atmosphere [1]. Therefore, the carbon cycle process of terrestrial ecosystems is clearly affected by dynamic changes in the soil carbon pool. Slight changes in the SOC pool may have a significant

impact on the concentration of CO₂ in the atmosphere [2]. The size and stability of the soil carbon pool depend on the balance between plant litter input and soil organic carbon mineralization. Litter is an important source of SOC, and it affects the dynamic balance between the input and output of soil organic carbon. Litter input may promote (or inhibit) the decomposition of soil organic carbon through a positive (or negative) excitation effect, and ultimately affect soil carbon balance [3]. Litter quality is a key factor affecting the decomposition and transformation of soil organic carbon. The effect of different-quality (C:N) litters on the soil priming effect needs to be strengthened. Previous studies have shown that high-quality litter (such as high nitrogen content, low C/N) inputs are more likely to be mineralized into CO₂ and released into the atmosphere, while low-quality litter (with a low nitrogen content, high C/N) is more likely to be transformed into soil organic carbon in the form of litter residue carbon due to greater difficulty in decomposition [4]. High-quality litter is more likely to trigger a strong positive priming effect because it can provide high-quality carbon and nitrogen sources for microorganisms, which increase microbial biomass and extracellular enzyme secretion and accelerate the decomposition and transformation of original SOC [5]. Low-quality litter induces a strong negative priming effect. Due to the consumption of easy-to-decompose carbon sources in litter, low-quality litter becomes more difficult to decompose, and microorganisms need to invest more energy to degrade these components. As a result, microorganisms preferentially decompose the easily decomposed components in the original soil [6]. Some studies have shown that the duration of the excitation effect is relatively short, lasting only a few hours or weeks. However, others have found that for easily degradable organic matter to be completely decomposed, the soil priming effect caused by the input of exogenous organic matter can still last for several months, which leads to a continuous change in the soil carbon pool [7]. Therefore, when studying dynamic changes in SOC, the effect of different-quality litter inputs on the soil priming effect cannot be ignored. At present, most studies on the effect of litter quality on the priming effect use different plants (such as corn, wheat, masson pine, or Schima) or different parts of the plant (such as roots, stems, or leaves) for comparative study [8,9]. Some studies have shown that the soil mineralization and priming effect caused by plant leaf litter input is significantly stronger than that caused by root litter input in the short term. The litter C:N ratio is often regarded as a key indicator of plant quality. When faced with an elevated atmospheric CO₂ concentration and increased atmospheric nitrogen deposition, plant photosynthetic product distribution, nutrient absorption, and histochemical composition can change significantly, resulting in alterations in litter C/N [10]. Therefore, whether different-quality litter inputs induce different intensities of the soil priming effect remains to be studied.

The input of exogenous easily decomposed organic matter can accelerate or slow down the mineralization of SOC. It shows a positive or negative priming effect, which affects the process of soil carbon turnover [6]. In particular, the main view of the positive priming effect considers how the input of exogenous organic matter brings a large number of available carbon sources to soil microorganisms. This leads to nitrogen limitations for the microorganisms. Soil microorganisms accelerate the decomposition of soil organic matter in order to meet the nitrogen required for development, which is the “Microbial Nitrogen Mining Theory” [11]. By contrast, the input of exogenous organic matter promotes the growth and metabolism of soil microorganisms, accelerates the production of extracellular enzymes to decompose difficult components in the soil, and accelerates the decomposition and transformation of soil organic carbon, which is known as the “Co-metabolism hypothesis” [12]. However, the negative priming effect suggests that soil microbes preferentially utilize readily decomposable organic matter in exogenous additions, thereby reducing the decomposition of original SOC, which is known as the “Substrate Preference

Hypothesis” [13]. In addition, when the C/N ratio of exogenous organic matter is compatible with the soil microbial nutrient demand due to high microbial activity, it induces the strongest priming effect known as the “Stoichiometric Decomposition Theory” [14,15]. The difference in litter quality has an important impact on the community composition, biomass, activity of soil microorganisms, and other soil chemical properties, and it changes the intensity and direction of the soil priming effect. However, whether different-quality litter inputs can induce different soil priming effects and whether there is a consistent effect of different-quality litters on different soil environments still need to be strengthened through research.

In this study, we used isotope ^{13}C -labeled China fir (high C/N, low-quality) and Acacia (low C/N, high-quality) to study the effects of different-quality litters on the intensity and direction of the soil priming effect by adding different-quality litters to soils in different subtropical regions (Sanming, Taihe, Yongzhou, and Liupanshui). China fir and Acacia are widely distributed tree species in subtropical China. Their litter exhibits markedly divergent quality traits, representing the extremes of the litter quality spectrum, specifically, low-quality (high C/N) versus high-quality (low C/N) litter. Combined with soil microbial biomass carbon and nitrogen, soil enzyme activity, and other indicators were used to research the mechanism of litter quality on the soil priming effect. In addition, we hypothesized that the addition of high-quality litter promotes a positive soil priming effect, and the intensity and direction of the soil priming effect differ with changes in the soil environment. Compared with China fir litter, Acacia litter input can promote soil mineralization and decomposition. However, low-quality litter produces higher quantities of litter source C, which returns to the atmosphere through CO_2 . The findings of this study will help clarify the sensitivity of soil to litter inputs (high-quality vs. low-quality litter) in different regions and provide a scientific basis for studying the carbon cycle path and mitigating climate change.

2. Materials and Methods

2.1. Collection of Test Soil Samples

Soil samples for this study were collected from Sanming in Fujian, Taihe in Jiangxi, Yongzhou in Hunan, and Liupanshui in Guizhou (Figure 1). The four sampling sites are located in the typical subtropical climate zone of China, and different environmental factors lead to differences in the microenvironment. Sanming ($117^{\circ}37'–119^{\circ}39'$ E, $28^{\circ}14'–26^{\circ}17'$ N) has a mid-subtropical monsoon climate, with four distinct seasons and abundant rainfall. The mean average precipitation is about 1516–1608 mm, and the mean annual temperature ranges between 18.3 and 19.7 °C in a hilly landform valley. Taihe ($114^{\circ}17'–115^{\circ}20'$ E, $26^{\circ}27'–26^{\circ}58'$ N) has a subtropical humid monsoon climate, with abundant rainfall, warm and humid conditions, and four distinct seasons. The mean annual precipitation varies between 1550 and 1800 mm, the mean annual temperature is between 17.7 and 18.6 °C, and the landform is dominated by mountains and hills. Yongzhou ($111^{\circ}50'–111^{\circ}58'$ E, $26^{\circ}01'–26^{\circ}09'$ N) has a mid-subtropical monsoon humid climate, with four distinct seasons and concentrated precipitation. The mean annual precipitation varies between 1460 and 1607 mm, the mean annual temperature is between 15.2 and 17.5 °C, and the landform is mainly mountainous and hilly. Liupanshui ($104^{\circ}47'–104^{\circ}50'$ E, $25^{\circ}27'–26^{\circ}46'$ N) has a subtropical plateau monsoon warm climate. The mean annual precipitation varies between 1130 and 1350 mm, the mean annual temperature is between 17.7 and 18.6 °C, and the landform is dominated by karst hills and mountains. The soils at the four sites were derived from sandstone parent material, and the soil type was ultisol according to the United States Department of Agriculture (USDA) classification standards. Furthermore, the experimental soil in this study was collected from natural forests at the four locations. The natural forests

in Sanming were mainly composed of *Pinus massoniana*, *Schima superb*, *Castanopsis fargesii*, *Machilus pauhoi*, *Phoebe bournei*, *Eurya nitida*, *Cinnamomum camphora*, and *Cunninghamia lanceolata*. The natural forests in Taihe were mainly composed of *Cunninghamia lanceolata*, *Pinus massoniana*, *Taxus chinensis*, *Ginkgo biloba*, *Michelia macclurei*, and *Schima superb*. The natural forests in Yongzhou were mainly composed of *Cunninghamia lanceolata*, *Pinus massoniana*, *Taxus chinensis*, *Ginkgo biloba*, *Michelia macclurei*, and *Schima superb*. The natural forests in Liupanshui were mainly composed of *Pinus massoniana*, *Cunninghamia lanceolata*, *Davidia involucreata* var. *vilmoriniana*, *Cryptomeria fortune*, *Pinus yunnanensis*, and *Quercus aliena* var. *acuteserrata*. Three plots with a size of 20 m × 20 m and a spacing of 10 m were set up in the forest land. Before soil samples were collected, the litter covering the soil surface was removed. Five points were selected according to a “Z” shape in the sample plot. Soil at 0–20 cm was drilled using a soil drill and mixed evenly. After removing animal and plant residues and stones, the fresh soil was sieved to 2 mm and divided into two parts [16]. One of the soil samples was used to determine the basic physical and chemical properties, and the other part of the sample was placed in a 4 °C refrigerator for cultivation experiments. The basic physical and chemical properties of the soil are shown in Table 1.

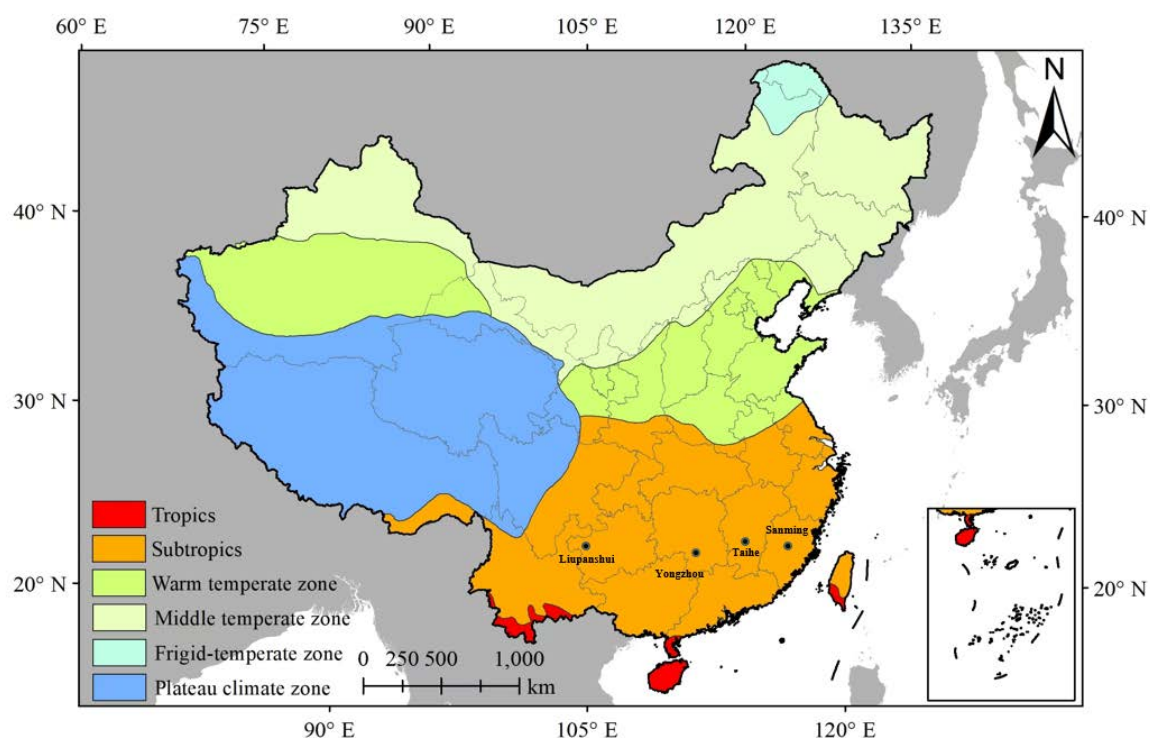


Figure 1. Sampling location of the test soil. Note: Different color regions in the figure represent different climatic zones, and black markers represent the locations of the sample plots.

Table 1. Chemical properties of the experimental soil.

Region	N (g·kg ^{−1})	C (g·kg ^{−1})	C/N(g·kg ^{−1})	δ ¹³ C (‰)	pH	SMC (%)
Sanming	3.12 ± 0.01 C	54.99 ± 0.71 C	17.65 ± 0.31 A	−27.86 ± 0.03 A	5.64 ± 0.23 B	16.06 ± 0.29 B
Taihe	1.37 ± 0.02 D	17.92 ± 0.49 D	13.11 ± 0.47 C	−23.44 ± 0.11 C	5.95 ± 0.18 AB	18.07 ± 0.81 A
Yongzhou	4.42 ± 0.03 B	77.48 ± 2.15 B	17.54 ± 0.35 A	−25.92 ± 0.02 B	6.05 ± 0.32 AB	16.73 ± 0.42 B
Liupanshui	7.54 ± 0.04 A	111.23 ± 0.64 A	14.75 ± 0.05 B	−26.19 ± 0.03 B	6.29 ± 0.15 A	16.24 ± 0.35 B

Note: SMC represents soil moisture content. Uppercase letters represent significant differences between different regions of soil ($p < 0.05$).

2.2. Plant ^{13}C Isotope Labeling

The seedlings of China fir and Acacia were placed in a glass labeling box (length $\times$ width $\times$ height of 1.5 m $\times$ 1.5 m $\times$ 1.8 m) and were isotope-labeled using a continuous pulse labeling method. $^{13}\text{CO}_2$ was generated by the reaction of Na_2CO_3 (99% atomic ^{13}C) with HCl. During the labeling process, a CO_2 detector (S8601A, Soonda, Beijing, China) and air conditioning were used to maintain the concentration of $^{13}\text{CO}_2$ in the labeling chamber at 600 ppm, with the room temperature set at 25 °C. In addition, a small fan was installed inside the labeling box to ensure that the $^{13}\text{CO}_2$ gas was evenly distributed throughout the labeling box. Labeling was performed every 10 days, 3 days each time, and a total of 15 isotope labeling tests were performed. After the end of labeling, the seedlings were treated at a low temperature with drying to allow the leaves to fall off naturally. Leaf litter was collected and dried at 50 °C for indoor culture experiments [17]. The basic physical and chemical properties of the litters are shown in Table 2.

Table 2. Chemical properties of different-quality litters.

Species	N ($\text{g}\cdot\text{kg}^{-1}$)	C ($\text{g}\cdot\text{kg}^{-1}$)	C/N($\text{g}\cdot\text{kg}^{-1}$)	$\delta^{13}\text{C}$ (‰)
<i>Cunninghamia lanceolata</i> Hook	11.37 $\pm$ 2.09 B	467.85 $\pm$ 5.15 A	35.27 $\pm$ 0.39 A	113.39 $\pm$ 1.81 B
<i>Acacia confusa</i> Merr	35.55 $\pm$ 0.13 A	474.65 $\pm$ 1.93 A	13.35 $\pm$ 0.05 B	317.37 $\pm$ 0.87 A

Note: *Cunninghamia lanceolata* Hook and *Acacia confusa* Merr represent the China fir and Acacia, respectively. Uppercase letters represent the significant differences between different litters ($p < 0.05$).

2.3. Experimental Design

In this study, the experiment included leaf litter of two tree species, four subtropical natural forest soils, and a no-addition control (CK). The experiment set up 12 treatments, each treatment had 4 replicates, and there was a total of 48 samples. Firstly, the fresh soil equivalent to 200 g of air-dried soil quality was weighed via an electronic balance and loaded into a 1000 mL glass culture bottle. Deionized water was added via the syringe to adjust to 60% of the saturated water holding capacity, and the soil was placed in a constant temperature incubator at 25 °C for 14 days to achieve soil microbial activity and environmental stability. Subsequently, the leaf litter of ^{13}C -labeled China fir and Acacia was cut into 2 mm-sized fragments and evenly covered the soil surface. In the experiment, the litter addition content was added to the bottle based on 10% of the SOC content. Finally, the culture bottle cap was sealed with a breathable film and cultured in a 25 °C incubator. (The culture temperature was beneficial to the activity of soil microorganisms and the decomposition of litter.) In the process of culture, deionized water was supplemented by the weighing method to keep the soil moisture constant [18].

2.4. Gas Sample Collection and Index Determination

The gas collector was used to collect CO_2 gas in the culture bottle on days 1, 3, 7, 14, 21, 28, 42, 56, 70, and 84 during the culture process. Before each collection of gas, high-purity air was introduced into a 1000 mL culture bottle with a connecting tube for 4–5 min (no CO_2), and the bottle was opened for 25–30 min to replace the residual CO_2 gas in the bottle. The CO_2 gas in the culture flask was collected at 0 h and 1 h, respectively, and the gas was injected into a gas chromatograph (GC-2014C, Shimadzu, Kyoto, Japan) through a gas collection device to determine the amount of CO_2 released from the soil and calculate the cumulative release amount. After gas collection was complete, it was placed in an alkali absorption bottle (20 mL 0.5 mol/L NaOH solution) for 24 h. After 24 h, the lye bottle was removed, and a 10 mL solution was transferred from the lye absorption bottle to the centrifuge tube. We added a 2 mL SrCl_2 (0.5 mol·L $^{-1}$) solution to form SrCO_3 with

CO₂. Subsequently, the solution was placed in a centrifuge tube, and deionized water was added for filtration (3000 r/min, 10 min). After three repeated operations, the precipitated samples were dried in a constant-temperature oven at 60 °C, and the δ¹³C of the precipitate was determined by a stable isotope ratio mass spectrometer, (Isoprime 100, Elementar, Langenselbold, Germany)

In addition, soil samples were collected on day 84 to determine soil microbial biomass (MBC, MBN), soil enzyme activity (cellulase, polyphenol oxidase, and peroxidase), and other indicators. MBC and MBN were fumigated with chloroform and extracted with K₂SO₄ [18]. The extract was filtered with a 0.45 μm filter membrane and determined using a total organic carbon analyzer (Shimadzu TOC, Kyoto, Japan). SOC and TN were determined using an elemental analyzer (Vario Isotope Cube, Elementar, Germany) [19]. Soil enzyme activity was determined using a double antibody sandwich method [20].

Index calculation of soil mineralization and priming effect

The related calculation formula of CO₂ release rate is given as follows:

$$F = \frac{dc}{dt} \times \frac{M}{m} \times \frac{V}{V_0} \times \frac{273}{273 + T}$$

F represents the release rate of CO₂, dc/dt represents the change in CO₂ concentration within 1 h in a 1000 mL culture bottle, m represents the dry weight of soil in a 1000 mL culture bottle, and M represents the molar mass of CO₂. In addition, V represents the capacity of the culture bottle; V₀ represents the gas molar volume of the standard atmosphere, which is 22.4 L · mol⁻¹; T represents the actual temperature (25 °C) during the culture process; the cumulative release of CO₂ is the cumulative release of CO₂ during the culture time.

The distinction between soil-derived and litter-derived CO₂ is given as follows:

$$C_L = C_T \times (\delta_T - \delta_S) / (\delta_L - \delta_S)$$

C_T (C_T = C_L + C_S) represents the total CO₂ emission of soil after litter addition, C_L represents the amount of CO₂ released by exogenous litter, δ_T represents the δ¹³C of CO₂ released by the culture soil after litter addition, δ_L represents the δ¹³C of the added litter, and δ_S represents the δ¹³C of CO₂ in the culture bottle of the CK.

The soil priming effect is calculated as follows:

$$PE = (C_1 - C_2) / C_2 \times 100\%$$

PE represents the soil priming effect, C₁ represents the CO₂ emission from the soil after adding litter, and C₂ represents the CO₂ emission from the soil without added litter.

2.5. Statistical Analysis

Two-way ANOVA was used to test the interaction effects of incubation time and treatment on soil cumulative release, the CO₂ release rate, cumulative release of CO₂ from different sources, and the soil priming effect. A one-way ANOVA test with Tukey's HSD multiple comparison was performed to reveal the differences in mean values of the CO₂ emission rate, total cumulative CO₂ emission, total cumulative CO₂ emission from soil sources, total cumulative CO₂ emission from litter sources, cumulative priming effect of soil mineralization, and mean values of the excitation effect of soil mineralization rate in different treatments and regions. Furthermore, the one-way ANOVA test with Tukey's HSD multiple comparison was performed to reveal the differences in soil variables for different treatments. Pearson correlation analysis was used to research the relationship between soil mineralization, priming effects, litter quality, and soil biochemical indicators. Moreover, a

random forest analysis was conducted using the R(4.4.2) Programming Language to test the relative importance of different factors on the priming effect. This study used Origin 2024 to plot the relevant data.

3. Results

3.1. Effects of Litter Addition on SOC, TN, SMC, and pH

The addition of litter significantly affected the physical and chemical properties of the soil. The SMC, TN, and SOC of litter added to soil in different regions showed that XS was significantly higher than SM and CK ($p < 0.05$). By contrast, the difference in pH between different litter addition treatments was not significant (Table 3).

Table 3. Effects of litter addition on soil physical and chemical properties.

Region	Treatment	TN ($\text{g}\cdot\text{kg}^{-1}$)	SOC ($\text{g}\cdot\text{kg}^{-1}$)	SMC (%)	pH
Sanming	SM	3.09 ± 0.51 B	55.79 ± 0.34 B	15.74 ± 0.79 B	5.71 ± 0.54 A
	XS	3.51 ± 0.89 A	57.76 ± 0.49 A	18.93 ± 0.62 A	5.74 ± 0.47 A
	CK	2.94 ± 0.43 C	54.45 ± 0.47 C	15.07 ± 0.58 B	5.75 ± 0.41 A
Taihe	SM	1.39 ± 0.12 B	17.51 ± 0.74 B	17.92 ± 0.19 B	6.01 ± 0.63 A
	XS	1.72 ± 0.37 A	19.72 ± 0.58 A	19.05 ± 0.54 A	6.06 ± 0.57 A
	CK	1.34 ± 0.18 B	16.85 ± 0.21 B	17.96 ± 0.26 B	5.98 ± 0.46 A
Yongzhou	SM	4.36 ± 0.11 B	76.01 ± 0.33 B	17.47 ± 0.43 B	6.04 ± 0.12 A
	XS	4.93 ± 0.39 A	80.45 ± 0.42 A	18.72 ± 0.69 A	6.01 ± 0.32 A
	CK	3.99 ± 0.25 C	71.61 ± 0.89 C	17.65 ± 0.64 AB	6.02 ± 0.58 A
Liupanshui	SM	7.54 ± 0.56 B	108.23 ± 0.24 B	16.87 ± 0.55 AB	6.51 ± 0.11 A
	XS	7.73 ± 0.35 A	112.91 ± 0.78 A	17.86 ± 0.82 A	6.48 ± 0.27 A
	CK	7.42 ± 0.63 B	104.59 ± 0.41 C	16.47 ± 0.58 B	6.43 ± 0.19 A

Note: SM, XS, and CK represent China fir litter addition treatment, Acacia litter addition treatment, and no treatment, respectively. SMC represents soil moisture content. Uppercase letters represent significant differences between different litters with the addition treatment ($p < 0.05$).

3.2. Effects of Litter Addition on CO₂ Release Rate and Cumulative Release Amount

Treatments and incubation time significantly affected the release rate of soil CO₂, and there was a significant interaction effect of treatment $\times$ incubation time. The CO₂ release rate of soil under different treatments showed a steadily increasing trend, followed by a gradual decrease. Different treatments significantly affected the average CO₂ release rate, and the average CO₂ release rate of the two litter addition treatments was higher than that of CK. Soil in different regions showed the same rule: China fir (SM) litter addition treatment > Acacia (XS) litter addition treatment > CK. In addition, the average CO₂ release rate of soils in Liupanshui and Yongzhou was significantly higher than in Sanming and Taihe (Figure 2).

The treatment and incubation time significantly affected the cumulative release of soil CO₂, and there was a significant interaction effect of treatment $\times$ incubation time. The total cumulative release of soil CO₂ in Acacia and China fir litter addition treatments was significantly higher than CK. The soil in different regions showed similar rules: China fir litter addition treatment > Acacia litter addition treatment > CK. The total cumulative release of soil CO₂ in Liupanshui and Yongzhou was significantly higher than in Sanming and Taihe (Figure 2).

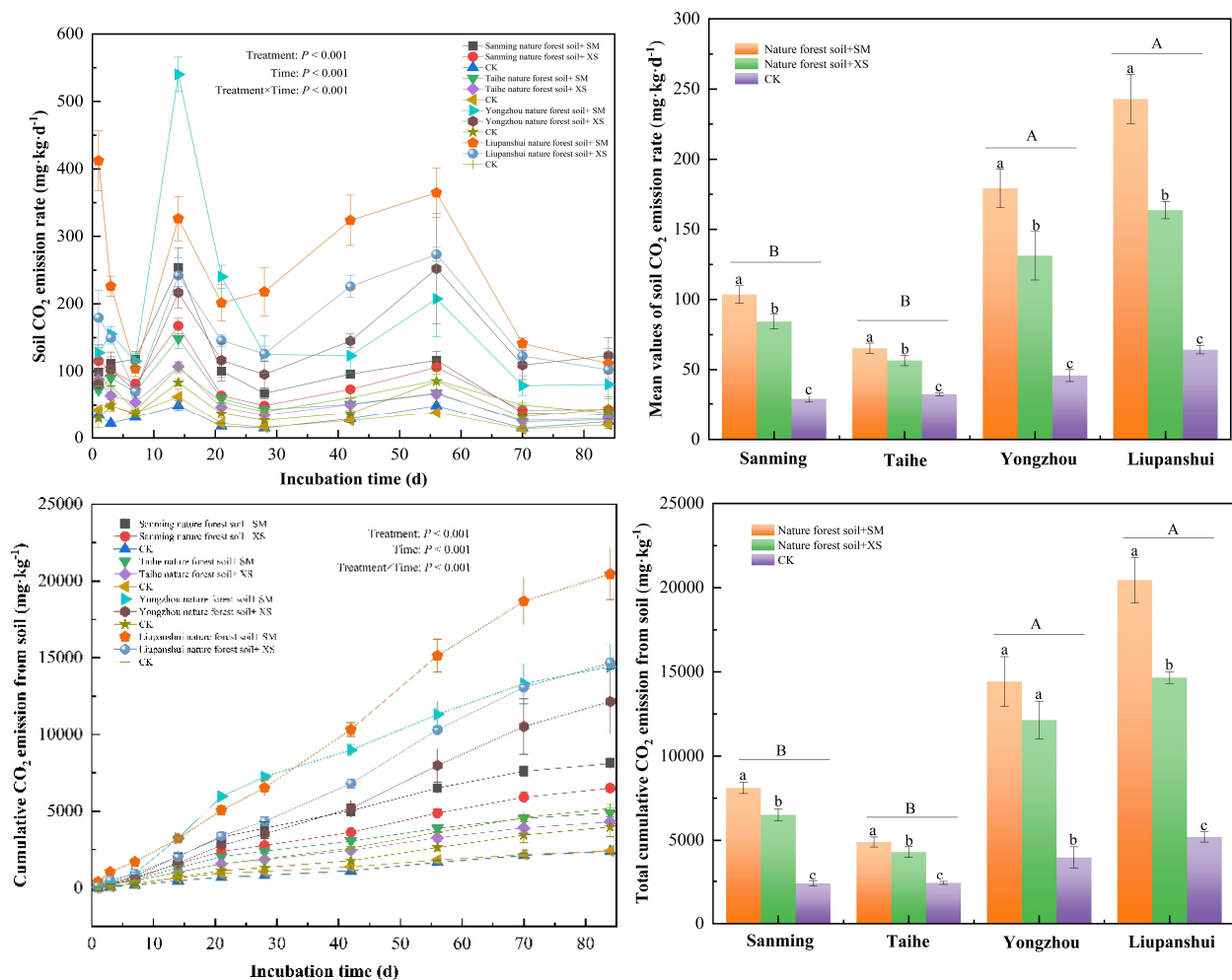


Figure 2. Effect of litter addition on soil CO₂ release rate and cumulative release. Note: SM and XS represent China fir and Acacia, respectively. Different lowercase letters represent significant differences between leaf apoplast addition treatments of different quality in the same soil environment, and different uppercase letters represent significant differences between soil environments for different apoplast addition treatments ($p < 0.05$). The picture below is the same.

3.3. Effects of Litter Addition on the Cumulative Release of CO₂ from Different Sources

The treatment and incubation time affected the cumulative release of CO₂ from the litter source, and there was a significant interaction effect of incubation time $\times$ treatment. The cumulative release of CO₂ from litter sources under different litter addition treatments showed a first increasing then slowly decreasing trend. The total cumulative release of CO₂ from litter sources was significantly affected by the treatment. The total release for China fir litter addition treatments (Sanming: 3528.58 mg·kg⁻¹, Taihe: 1225.03 mg·kg⁻¹, Yongzhou: 7051.29 mg·kg⁻¹, Liupanshui: 15,339.76 mg·kg⁻¹) in different regions was significantly higher than the Acacia litter addition treatment (Sanming: 1895.39 mg·kg⁻¹, Taihe: 576.62 mg·kg⁻¹, Yongzhou: 3877.96 mg·kg⁻¹, Liupanshui: 6297.32 mg·kg⁻¹) (Figure 3).

The treatment and incubation time significantly affected the cumulative release of CO₂ from soil, and there was a significant interaction effect of treatment $\times$ incubation time. In addition, the total cumulative release of soil-derived CO₂ in the Acacia litter addition treatment (8359.63 mg·kg⁻¹) was significantly higher than the China fir litter addition treatment (5113.52 mg·kg⁻¹). The total cumulative release of CO₂ from soil sources in Liupanshui and Yongzhou was significantly higher than in Sanming and Taihe (Figure 3).

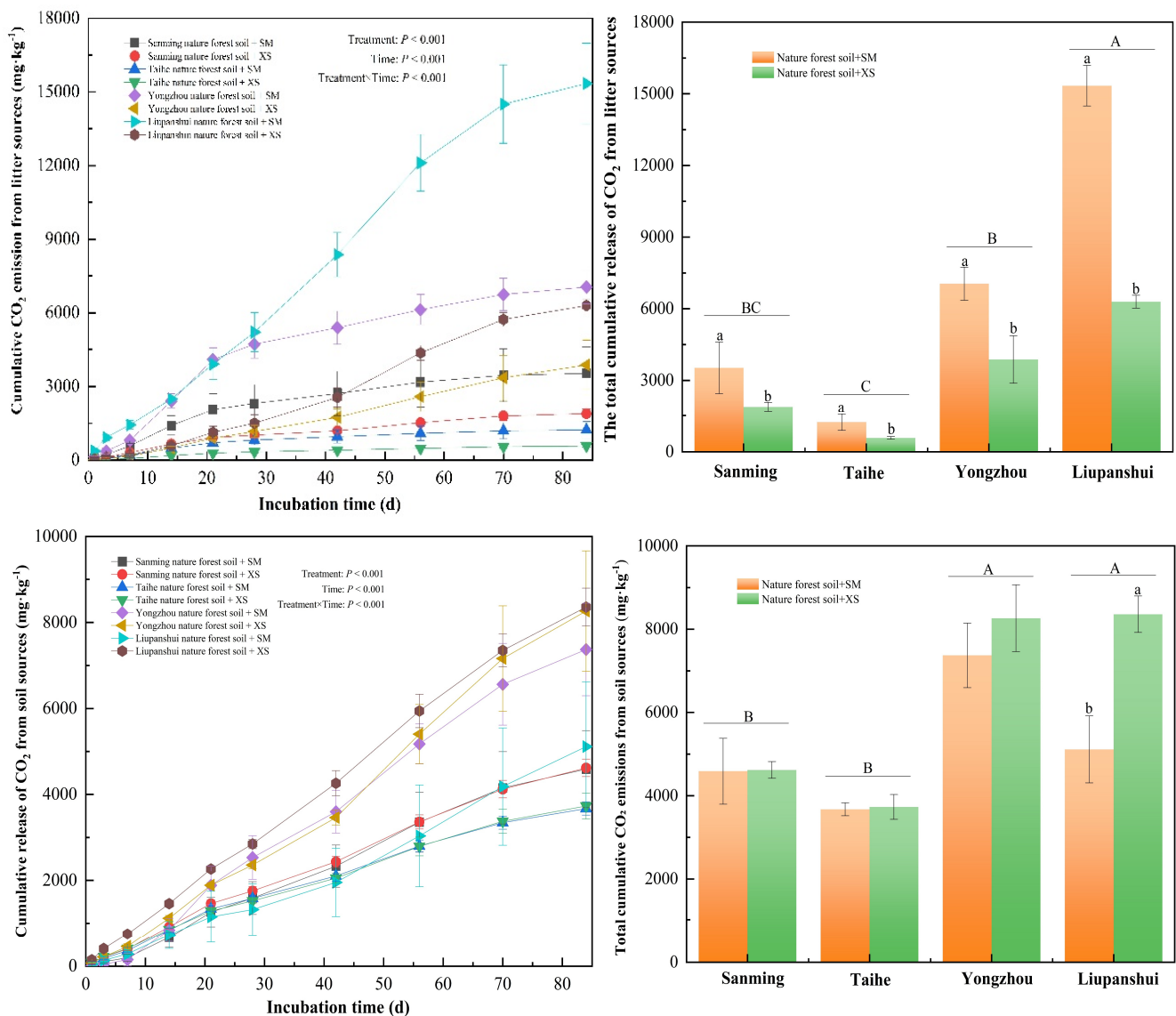


Figure 3. Effect of litter addition on cumulative release of soil CO₂ from different sources.

3.4. Effects of Litter Addition on Soil Priming Effect in Different Regions

The treatment and incubation time significantly affected the priming effect of soil mineralization rate, and there was a significant interaction effect of treatment method $\times$ incubation time. The mean value of the soil mineralization rate priming effect of *Acacia* litter addition treatment was generally higher than that of the *China fir* litter addition treatment. The average priming effect of the soil mineralization rate showed that values in Sanming and Yongzhou were higher than those in Taihe and Liupanshui. Furthermore, the treatment and incubation time significantly affected the cumulative priming effect of soil mineralization, and there was a significant interaction effect of treatment $\times$ incubation time. The mean cumulative priming effect of the *Acacia* litter addition treatment in Liupanshui soil was significantly higher than that of the *China fir* litter addition treatment. The cumulative priming effect of soil mineralization in Sanming and Yongzhou was significantly higher than in Taihe and Liupanshui (Figure 4).

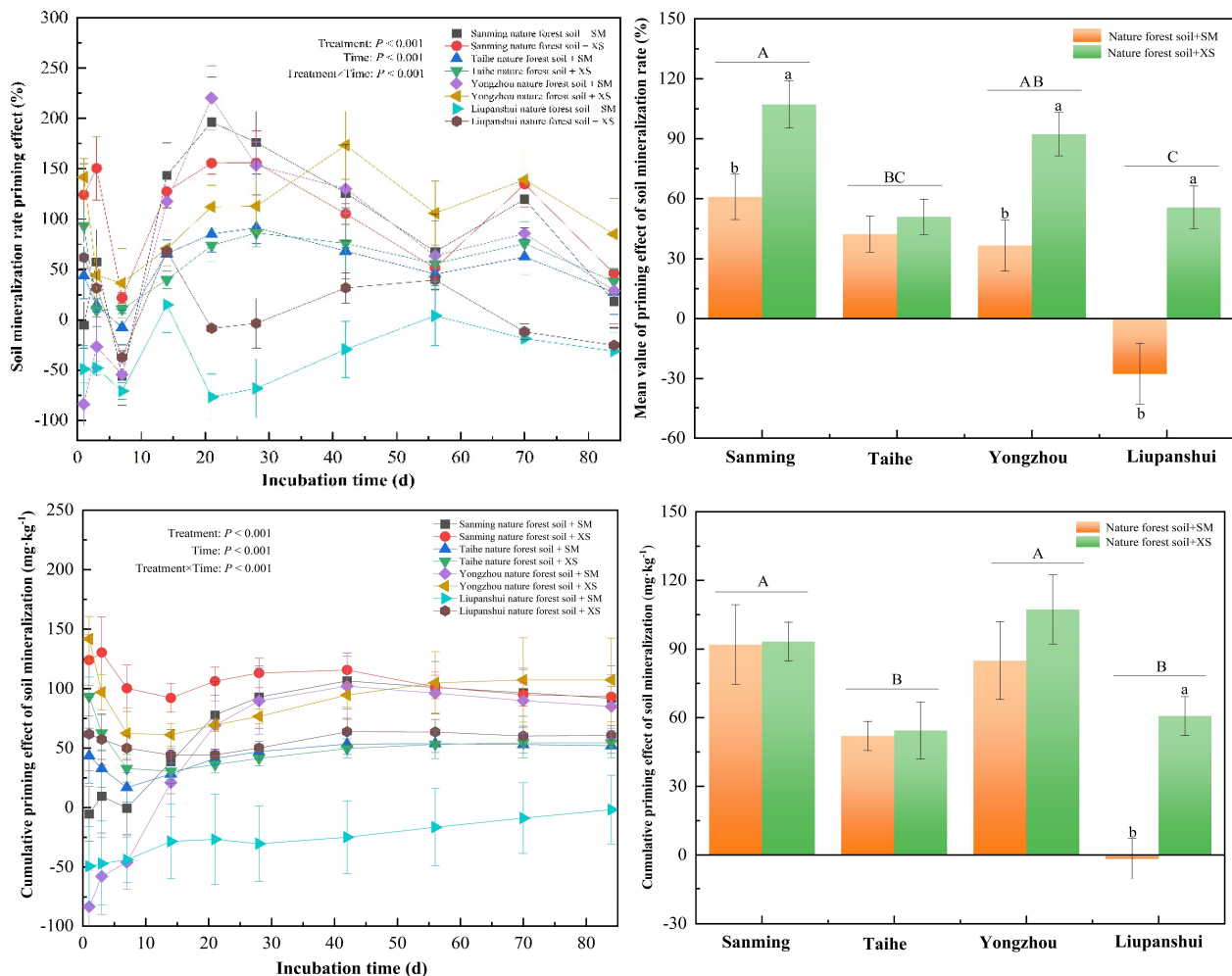


Figure 4. Effects of litter addition on SOC mineralization rate, priming effect, and SOC mineralization cumulative priming effect.

3.5. Effects of Litter Addition on Soil Microbial Biomass Carbon and Nitrogen and Enzyme Activity

Different litter addition treatments significantly affected soil microbial biomass carbon, soil microbial biomass nitrogen, and enzyme activity, but the results varied in different regions. The soil values in Yongzhou and Liupanshui areas were generally higher than those in Sanming and Taihe. In addition, the soil microbial biomass carbon, soil microbial biomass nitrogen, cellulase, peroxidase, and polyphenol oxidase after litter addition in the four regions showed similar patterns: The *Acacia* litter addition treatment was significantly higher than the *China fir* litter addition treatment and CK (Figure 5).

3.6. The Mechanism of the Effect of Litter Addition on Soil Priming Effect

Pearson correlation analysis identified the relationship between litter C/N and the physical and chemical properties of soil, soil mineralization, and soil priming effects. Cellulase, polyphenol oxidase, peroxidase, S-CO₂, and MBC were significantly positively correlated with PE ($p < 0.001$), and MBN and litter C/N were significantly positively correlated with PE ($p < 0.01$). By contrast, SMC and pH were significantly negatively correlated with PE ($p < 0.05$) (Figure 6). Moreover, the random forest model showed that MBC, cellulase, litter C/N, polyphenol oxidase, SOC, TN, pH, and MBN were the main factors affecting the soil priming effect. MBC, cellulase, and litter C/N have a higher relative importance to the soil priming effect than other factors, and their IncMSEs were 18.89% ($p < 0.01$), 15.41% ($p < 0.01$), and 12.31% ($p < 0.01$), respectively (Figure 7).

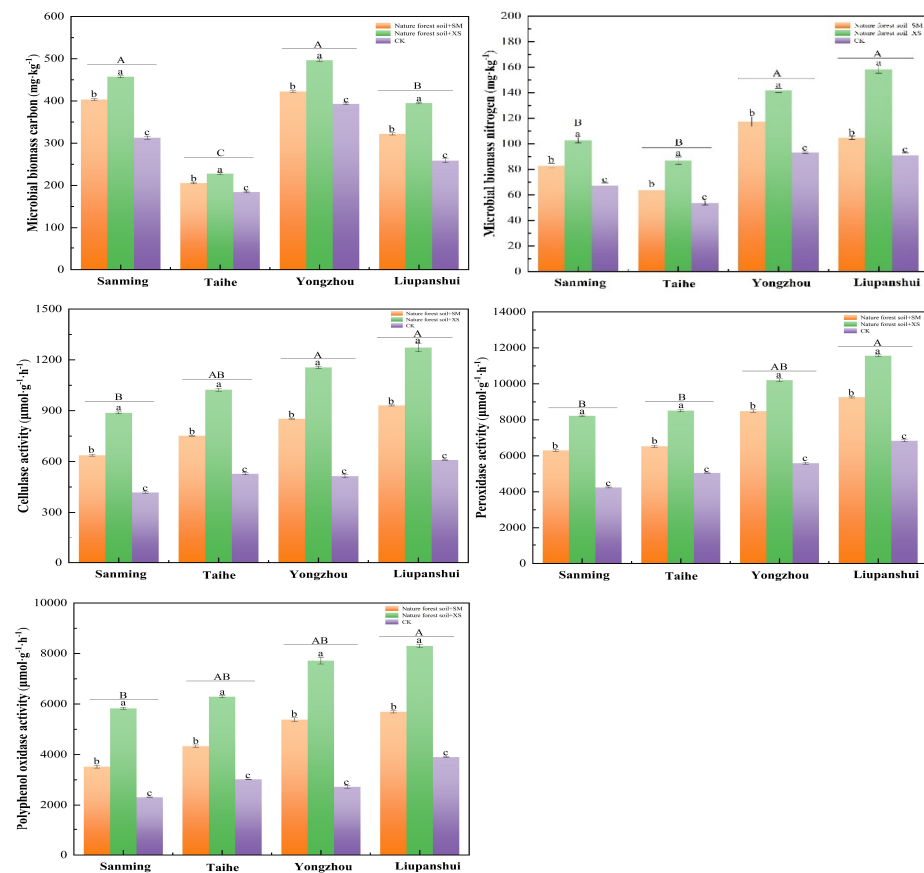


Figure 5. Effects of litter addition on microbial biomass carbon, microbial biomass nitrogen, and enzyme activities.

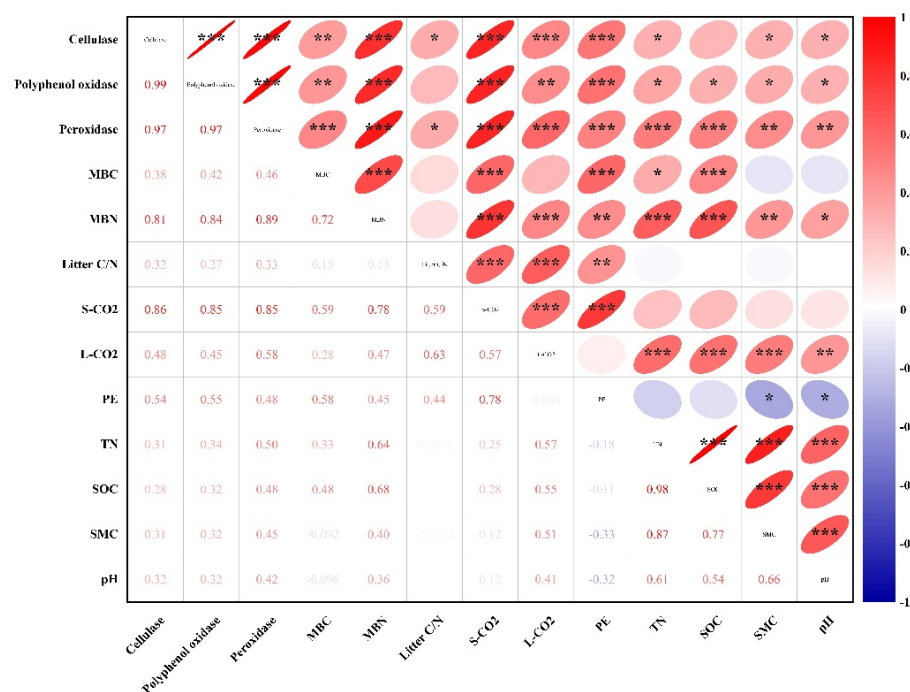


Figure 6. Correlation analysis of soil mineralization and priming effect with soil properties. Note: *, **, and *** represent $p < 0.05$, $p < 0.01$, and $p < 0.001$. MBC, MBN, S-CO₂, L-CO₂, PE, TN, SOC, and SMC represent microbial biomass carbon, microbial biomass nitrogen, cumulative releases of CO₂ from soil sources and litter sources, priming effect, total nitrogen, soil organic carbon, and soil moisture content.

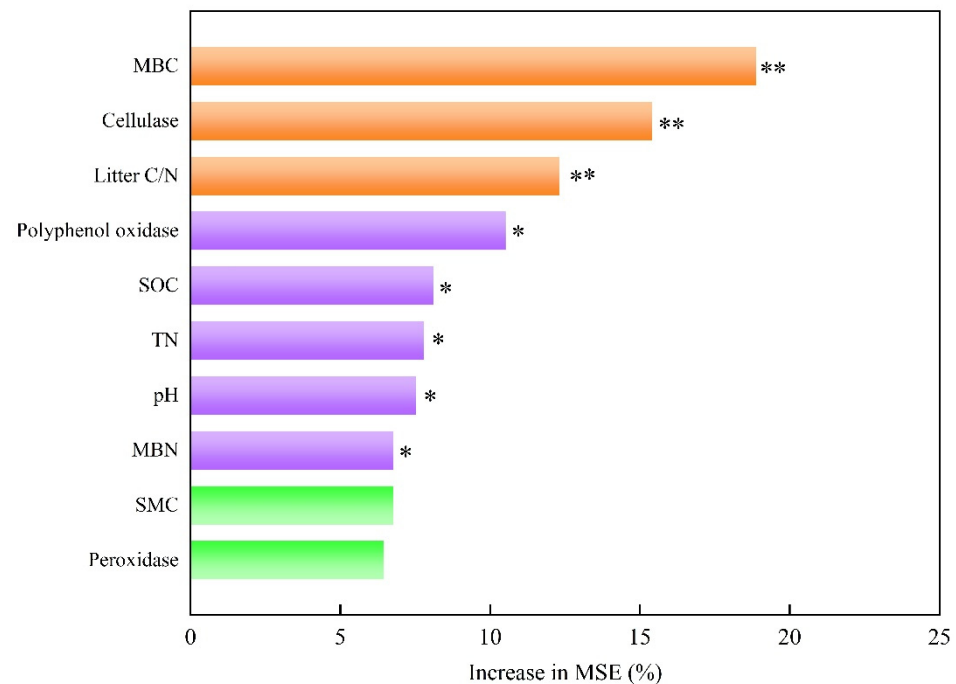


Figure 7. Relative importance of different environmental factors on soil priming effect. Note: * and ** represent $p < 0.05$ and $p < 0.01$. Increase in MSE represents increase in mean squared error. MBC, MBN, SOC, TN, and SMC represent microbial biomass carbon, microbial biomass nitrogen, soil organic carbon, total nitrogen, and soil moisture content.

4. Discussion

4.1. Effects of Different-Quality Litter on CO₂ Release Rate and Cumulative Release Amount

Litter decomposition and transformation are key processes in the C and N cycles in forest ecosystems. They provide an important link between the energy flow and material cycle of the atmosphere, plants, and soil [20]. Previous studies have shown that plant litter input can significantly increase the SOC mineralization rate and CO₂ cumulative release in forest, grassland, and farmland ecosystems. By experimenting with litter additions in subtropical evergreen broad-leaved forests, researchers found that the addition of leaf litter increased the cumulative amount of soil CO₂ release by 3–5 times in different forest types [21]. At the same time, the researchers found that the SOC mineralization rate and cumulative release after litter addition were positively correlated with litter amount. In addition, through field experiments, leaf litter of *Schima* and *Ormosia hosiei* was added to subtropical forest soils at different growth stages, and the soil mineralization rate increased by 7.5–41.5% [22]. On the one hand, plant litter releases a large amount of CO₂ during microbial decomposition and transformation. On the other hand, litter input can change the mineralization rate of the original SOC. Therefore, litter input can significantly increase the soil mineralization rate and CO₂ cumulative release. However, the results of studies differ, which may be related to variations in environmental factors such as microbial community structure, microbial activity, and soil properties. Forest litter source C input can provide necessary nutrients for the growth and development of various types of microorganisms, which accelerate the development and activity of soil microorganisms, thereby increasing the release rate and cumulative release of CO₂ in soil [23]. The results of this experiment showed that the addition of high-quality and low-quality litter could significantly increase the cumulative release rate and amount of CO₂. At the same time, the average release rate and total cumulative amount of soil CO₂ in Yongzhou and Liupanshui were significantly higher than in Sanming and Taihe. The cumulative release rate and amount for different-quality litter addition treatments caused significant dynamic changes. The CO₂ release

rate showed a trend of first increasing and then gradually decreasing. This occurs because the litter is mostly composed of complex organic compounds such as protein, cellulose, lignin, hemicellulose, and tannin. In the early stage of litter decomposition, a large number of soluble monosaccharides and other components are easily decomposed and utilized to release nutrients during the transformation process. This improves and changes the community composition, biomass, and activity of microorganisms [24]. Microorganisms meet their developmental needs using exogenous organic carbon. The demand for nutrients further increases the rate of CO₂ release, leading to a rapid accumulation of CO₂ in the soil in the early stages of culture. In the late stage of litter decomposition, the difficulty experienced by soil microorganisms in decomposing the refractory components leads to a decrease in the soil CO₂ release rate and the cumulative release amount, which tends to be stable [25]. The decomposition and transformation of organic matter in litter change the soil microbial community structure, soil enzyme activity, and other soil environmental conditions, resulting in regular dynamic characteristics of soil mineralization rate and release [26]. In addition, the results of this experiment showed that the soil release rate and cumulative release of the China fir litter addition treatment were higher than those of the Acacia litter addition treatment, as China fir litter usually has a higher C/N, while the Acacia litter may contain higher N and P contents. High C/N litter increases the difficulty for microorganisms to obtain nitrogen in the early stage of decomposition. As a result, microorganisms consume more carbon sources to meet metabolic needs by enhancing respiration and releasing more CO₂ [21]. Due to the high content of available nutrients (such as N and P), high-quality litter is more conducive to the direct production of soil microorganisms during the decomposition process. In the decomposition process of low-quality China fir litter, the fungal-dominated decomposition pathway is more active, and its metabolic efficiency is lower. Acacia litters with low C/N may be dependent on bacterial-driven rapid nutrient turnover [27].

4.2. The Influence of Different-Quality Litters on Soil Mineralization and Priming Effect

Litter is a key factor affecting the decomposition and transformation of soil organic matter, which is of great significance for balancing the global carbon cycle [26,27]. In this study, isotope labeling of plants was carried out by a continuous pulse labeling method, and the ¹³C isotope was used to distinguish the cumulative release of soil and plant litter sources. The results showed that there was significant interspecific heterogeneity in the release of CO₂ from litter sources with different-quality litter addition treatments. Litter quality is a key factor regulating the decomposition rate. High-quality litter (high N content, low C/N) has a relatively faster decomposition rate than low-quality litter (low N content, high C/N) [28]. Previous studies have found that the contribution rates of soil mineralization after litter input of *Cunninghamia lanceolata* and *Castanopsis eyrei* were 15.1% and 34.4%, respectively [29]. At the same time, previous studies have also shown that the contribution rate of adding coniferous litter to CO₂ release is more than 58% [25]. The quality of litter can significantly affect the accumulation of CO₂ released by soil mineralization. Our study found a significant difference in the cumulative release of litter-derived CO₂ and soil-derived CO₂ between high-quality and low-quality litter addition treatments due to the high proportion of soluble sugar, protein, and other easily decomposed components in Acacia litter, which directly stimulates the activity of microorganisms in the soil, resulting in a simultaneous increase in soil microbial community composition and enzyme activity [30]. In addition, the release of organic acids (such as oxalic acid and citric acid) during the decomposition of Acacia litter improved the availability of microorganisms, which accelerated the mineralization rate. Litter releases a large amount of organic matter during the decomposition process to activate microorganisms, which secrete extracellular

enzymes, thereby increasing the rate of soil organic matter decomposition. The results of this experiment showed that the Acacia litter (high-quality, low C/N) was more conducive to decomposition and utilization by microorganisms, promoting soil mineralization. In this process, the nutrient limitation of microorganisms was reduced, and the decomposition efficiency of organic matter was enhanced. Furthermore, the total cumulative release of litter and soil sources in Yongzhou and Liupanshui after litter addition was higher than in Sanming and Taihe. China fir litter contains a large amount of plant carbon sources such as cellulose, hemicellulose, and lignin. These substances rapidly release CO₂ through microbial hydrolysis in the early stage of decomposition. The released soluble sugars and organic acids stimulate an increase in microbial activity and accelerate the soil mineralization rate [31]. Therefore, low-quality litter is likely to release higher quantities of litter source C in the form of CO₂, which then returns to the atmosphere. This resulted in a higher cumulative release of litter source CO₂ from the China fir litter addition treatment. However, high-quality litter is more conducive to decomposition and transformation of microorganisms and has higher microbial utilization efficiency, which is conducive to the formation and accumulation of new SOC, thereby reducing the loss rate of litter-derived C in the form of CO₂ [16,26].

Litter quality is an important factor in regulating the direction and intensity of the soil priming effect and plays an important role in the process of the ecosystem carbon cycle. Litter input can change the mineralization rate and accumulation while promoting original SOC decomposition in soil, which has a positive priming effect; otherwise, there is a negative priming effect. The factors influencing the priming effect are closely related to environmental factors such as soil texture, litter quantity and quality, soil microbial community structure, soil pH, soil temperature, and humidity [32]. We found that the addition of China fir litter and alder litter can promote the decomposition of SOC and produce a significant positive priming effect [27]. Previous studies have pointed out that the positive priming effect appeared after the addition of China fir litter in the early stage, and gradually changed to the negative priming effect with an increase in incubation time [33]. Compared with related research results on litter, it was found that high-quality litter was beneficial to the production of a stronger soil priming effect. The addition of high-quality litter can provide nutrients for soil microorganisms to produce extracellular enzymes to decompose soil organic matter. High-quality exogenous carbon input can promote the decomposition and mineralization rate of soil organic matter and inhibit the accumulation of SOC, while low-quality exogenous carbon input slows down the decomposition and transformation of organic matter, resulting in soil C accumulation [34]. The results of this experiment showed that the addition of Acacia and China fir litter changed the priming effect, and litter quality was an important factor affecting this. The priming effect of the Acacia litter addition treatment (high-quality, low C/N) was significantly higher than that of China fir litter, and the cumulative priming effect and mineralization rate in Sanming and Yongzhou were higher than those in Taihe and Liupanshui. Observing the whole incubation time, the Acacia litter addition treatment showed a positive soil priming effect in different regions. Litter input provides the necessary nutrient elements for the growth and development of soil microorganisms and releases a large number of easily degradable carbon components (such as soluble sugar and amino acids) and available nitrogen during the decomposition process, which directly stimulates the activity of microorganisms [25,32]. The input of easily decomposed components increases the common metabolic demand for microorganisms for the original organic matter, which is conducive to the decomposition and mineralization of organic matter in the original soil by different types of microorganisms. Microorganisms preferentially use exogenous carbon as an energy source and secrete a large number of extracellular enzymes to accelerate the decomposition process of complex organic matter,

resulting in a positive priming effect [35]. On the other hand, the nitrogen in the soil cannot meet microbial growth; soil microorganisms need to decompose the original organic matter before using exogenous carbon, which results in a positive priming effect [24]. In this study, the China fir litter had a strong negative soil priming effect in the early decomposition stage in different regions. The reason for this is that China fir litter has a high C/N (low-quality litter), and soil microorganisms are faced with nitrogen limitations in the early stage of decomposition. Therefore, microorganisms give priority to easily decomposed exogenous carbon (such as soluble sugars in litter) to reduce the demand for soil organic matter, resulting in a negative priming effect [27]. Metabolites such as phenols and terpenoids released in the early stage of decomposition may inhibit microbial activity and delay the decomposition rate of soil organic matter [35]. With the increase in incubation time, the nitrogen limitation of microorganisms was alleviated, which promoted the decomposition of litter and the release of available nutrients and activated the “co-metabolism mechanism”. This process drove the decomposition and mineralization of soil organic matter and changed to a positive priming effect in the later stage of the experiment [36]. In addition, the addition of China fir litter to Liupanshui soil has a negative priming effect throughout the incubation time. The reason for this is that a large amount of nitrogen is required in the early stage of decomposition, leading to the preferential use of exogenous nitrogen by soil microorganisms rather than the original organic matter in the soil. This process inhibited decomposition. At the same time, metabolites such as phenols and terpenes released during the decomposition of China fir litter inhibit microbial activity and reduce the demand for soil organic matter [37]. Another reason is that the N content in Liupanshui soil is higher than in other soils. Under the condition of high-nitrogen soil, microorganisms give priority to litter rather than the original nutrients of the soil, reducing the demand for “excavation” of soil organic matter, which maintains a negative priming effect [38]. Notably, this study primarily characterized litter quality using the C/N ratio. Although the C/N ratio serves as a robust proxy for litter decomposability, cellulose, hemicellulose, and lignin are fundamental regulators of litter decomposition and soil priming effects. Future studies should incorporate detailed plant component analyses to elucidate how plant chemical composition mediates soil priming effects.

5. Conclusions

In conclusion, the soil CO₂ release rate and cumulative release of the China fir litter addition treatment were significantly higher than the Acacia litter treatment and CK. Differences were found in the cumulative release of CO₂ from litter and soil sources under different-quality litter addition treatments. Acacia litter was beneficial in the decomposition process and utilization by microorganisms, which promotes soil mineralization and decomposition. Low-quality litters return a greater quantity of litter source C to the atmosphere through CO₂, resulting in a relatively higher cumulative release of CO₂ from low-quality litter sources. The priming effect of the Acacia litter addition treatment was significantly higher than that of the China fir litter addition treatment. The addition of Acacia litter to different regions of soil had a positive priming effect, while the addition of China fir litter to different regions of soil had a negative priming effect for a period of time. Furthermore, the results of correlation analysis showed that there was a significant relationship between soil physical and chemical indicators, litter C/N, soil mineralization, and the priming effects. The random forest model also showed that litter quality was the key factor influencing the priming effect. These findings advance our understanding of the soil carbon cycling process.

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