Exogenous carbon addition reduces soil organic carbon: the effects of fungi on soil carbon priming exceed those of bacteria on soil carbon sequestration

Limin Zhang[1,2], Yang Wang[2], Jin Chen[2], Fangbing Li[2], Chengfu Zhang[1], Yang Cao[1], Guojun Cai[1], Lifei Yu[2*]

1. Institute of Mountain Resources, Guizhou Academy of Sciences, Guiyang 550001, Guizhou, China

2. Colleges of Life Sciences and Institute of Agro-Bioengineering, Key Laboratory of Plant Resources Conservation and Germplasm Innovation in Mountainous Regions (Ministry of Education), Research Center of Karst Ecological Environment, and Collaborative Innovation Center for Mountain Ecology and Agro-Bioengineering, Guizhou University, Guiyang 550025, Guizhou, China

*Corresponding author. Lifei Yu. E-mail address: lfyu@gzu.edu.cn

Abstract: Soil organic carbon (SOC) forms the largest terrestrial organic C pool, which is regulated by the complex connections between exogenous C input, microbial activity, and SOC conversion. Few studies have examined the changes in SOC due to microbial activity after exogenous C inputs in karst lime soils in China. In this research, the ^{13}C isotope tracer technique was employed to investigate the priming effect on typical lime soil of ^{13}C-litter and ^{13}C-$CaCO_3$ through a mineralization-incubation experiment. Samples were collected at 5, 10, 20, 40, 60, and 80 days of incubation and analyzed for SOC mineralization, SOC distribution across fractions (>250 μm, 53～250 μm, and <53 μm), and soil microbial diversity. A control consisting of no exogenous C addition was included. SOC mineralization and SOC priming were considerably higher (15.48% and 61.00%, respectively) after litter addition compared to $CaCO_3$. The addition of either litter or $CaCO_3$ reduced the total organic C (TOC) and macroaggregate (>250 μm) and microaggregate (53～250 μm) C fractions by 2150.13, 2229.06, and 1575.06 mg C kg^{-1} C_{bulk} on average and increased the mineral particulate C fraction (<53 μm) by

1653.98 mg C kg^{-1} C_{bulk}. As the incubation time extended, a significantly positive correlation was apparent between SOC priming and soil fungal diversity, as well as between the mineral particulate C fraction and soil bacterial diversity. The effect of soil fungal diversity on SOC priming (R = 0.40, *P* = 0.003) significantly exceeded that of bacterial diversity on SOC sequestration (R = 0.27, *P* = 0.02). Our results reveal that after adding litter or $CaCO_3$, soil fungi stimulate SOC mineralization and decomposition and that soil bacteria enhance SOC sequestration, with the effects of fungi being more pronounced. These findings can provide a theoretical basis for understanding C sequestration and emission reduction in karst lime soils.

Keywords: lime soil; ^{13}C isotope tracing technique; exogenous carbon addition; soil organic carbon mineralization; soil microbial diversity

1. Introduction

China's terrestrial ecosystems have a carbon stock of approximately 350 billion tons of CO_2, of which vegetation and soils account for approximately 50 and 300 billion tons, respectively. Hence, soil organic carbon pools constitute a biologically important component of the global C cycle (Yu et al., 2021). Forest SOC pools harbor about 40% of organic C in the 0–1-m soil layers and play an irreplaceable role in maintaining the global climate system, regulating the global C balance, and slowing the rise of greenhouse gas concentrations in the atmosphere (Pan et al., 2011; Du et al., 2016; Zhu et al., 2017).

As an important process in the C cycle, SOC mineralization has a direct impact on SOC stability (Chow et al., 2004; Lal, 2004). Extensive research has found that SOC mineralization is predominantly affected by exogenous matter (Hamer and Marschner, 2005; Liu et al., 2020). When added to soil, exogenous C first activates soil microbial activity. This phenomenon is referred to as "microbial

activation." New organic matter (OM) that enters the soil provides sufficient C and energy for microbes, thereby rapidly promoting microbial metabolism and biomass growth (Kuzyakov et al., 2000; Liu et al., 2020). Bacteria and fungi are the two main taxa of microbial decomposer communities, capable of decomposing OM and directly involved in SOC conversion (Liu et al., 2012; Klink et al., 2022).

Recent advances in C cycle modeling suggest that soil C priming is a major factor for global C distribution prediction, as it plays a crucial role in determining C exchange between soils and the atmosphere (Sulman et al., 2014; Guenet et al., 2018). However, adding exogenous C can either promote or inhibit organic C mineralization through its impact on microbial activities, giving rise to a positive or negative priming effect (Guenet et al., 2010; Li et al., 2018; Huo et al., 2022) and, thus, enhancing or reducing soil C sequestration (Ye et al., 2018; Luo et al., 2020). Kuzyakov et al. (2000) found that fresh OM (e.g., litter) input accelerated microbial biomass turnover in the short term, with a significant positive priming effect, whereas adding active C decreased microbial biomass turnover, leading to a significant negative effect. Zheng et al. (2021) found that adding substrates such as glucose and cellulose resulted in a negative C balance and reduced soil C content, becoming a negative priming effect. In contrast, a meta-analysis by Liang et al. (2018) concludes that, even when adding glucose could lead to a negative priming effect, net soil C content may increase if the C released from the added C substrate exceeds the priming-induced C loss. Zhang et al. (2013) also found that adding fresh organic C can be beneficial by stimulating specific microbial populations and increasing organic C decomposition. Conversely, the negative effect of soil priming due to a decreased efficiency in the microbial substrates' use has been reported recently (Zhang et al., 2022). The microbial community structure and activity play a substantial role in SOC mineralization (Witzgall et al., 2021). At the early stages of the mineralization process, exogenous C can provide sufficient energy and C for microbes,

increasing their activity and thus stimulating C turnover. However, as the mineralization process proceeds, the microbial nutrient supply becomes insufficient, reducing the C turnover rate (Kristina et al., 2021).

In southwestern China, carbonatite forms the core of the East Asian karst region, one of the world's three major concentrated karst regions (Wang et al., 2019). Guizhou Province represents about 25.8% of China's karst area (Chen et al., 2021); this is a specific, subtropical, non-zonal region characterized by slow soil formation, the presence of calcium-rich and alkaline soils, and a distinct soil C cycle (Wang et al., 2020), differing markedly from the features of non-karst regions.

Litter and calcium carbonate ($CaCO_3$) are the principal sources of C in karst forests (Zhang et al., 2022). Some studies have concluded that adding litter or $CaCO_3$ can stimulate soil C mineralization, mainly because these substances increase soil microbial activity and thus affect the soil CO_2 respiration rate (Feng et al., 2016; Xiao et al., 2018). Moreover, the continuous addition of litter and $CaCO_3$ to soils was reported to improve soil fertility within a short period and enhance their C sink capacity (Fornara et al., 2011). Previous studies have shown that adding rice straw generally alters the composition of soil microbial communities and increases the SOC mineralization rate (Phillips et al., 2002; Zhang et al., 2013). Nevertheless, little is known about the effects of adding litter and $CaCO_3$ on C mineralization in karst forest soils in southern China, as well as the role of microbes in the C mineralization process (Fornara et al., 2011; Wang et al., 2014).

It is generally believed that exogenous C addition affects the composition of bacterial and fungal communities, and thus soil C mineralization (Xie et al., 2022). This research investigated the effects of adding litter or $CaCO_3$ to typical lime soil through a ^{13}C-isotope mineralization-incubation experiment. This article primarily examines the effects of adding litter or CaCO3 to lime soil in three key areas: (1)

SOC priming effect; (2) SOC content and distribution across size fractions; and (3) the effects of bacteria and fungi on soil C priming and sequestration after the addition of these C sources.

2. Materials and methods

2.1 Study site and soil sampling

The experimental soil was collected at the end of 2019 from a shrub forest in the Dashahe Nature Reserve (DNR) in the southern Chinese province of Guizhou. The DNR is in the transition zone between subtropical and temperate regions, encompassing a total area of 270 km^2 at 564-1940 m above sea level (average: 1252 m). This natural area is under a humid monsoon climate and has an average annual temperature of 12.1 ℃, an annual precipitation of 1194 mm, and an annual sunshine duration of 1134 h. Featuring a karst landscape on soluble carbonate rock formations, the DNR is extremely abundant in biological resources. With 3594 plant species (belonging to 1082 genera and 296 families) and 208 species of macrofungi (distributed in 95 genera and 47 families), the DNR constitutes one of the most precious gene pools of biological species in China's central subtropical region. Table 1 summarizes the basic information on the study area.

Eight soil cores were collected at depths of 0～20 cm using a soil auger (10 cm diameter) and mixed to form one composite sample (about 5.5 kg). After removing large roots, wood, and litter, the soil was distributed in aseptic plastic bags, sealed, and transported to the laboratory. 3.5 kg samples were sieved (< 2 mm), mixed completely, and stored at 4 °C for the subsequent incubation experiments. The remaining samples were air-dried and used for the soil's physical and chemical characterization, summarized in Table 2.

2.2 Preparation of ^{13}C-labeled litter and application of labeled ^{13}C sources to soil

Labeled litter was obtained by labeling with ^{13}C potted *Koelreuteria paniculata* (a major tree

species in karst forests) seedlings over the period of August–October 2020 using the pulse-labeling method. Ten pots containing *K. paniculate* seedlings were placed in a special hermetically sealed Plexiglas growth chamber, into which $^{13}CO_2$ produced by the reaction between $NaH^{13}CO_3$ and HCl ($NaH^{13}CO_3 + HCl = NaCl + H_2O + {}^{13}CO_2$) was injected periodically, to allow ^{13}C to become introduced into *K. paniculate* tissues through photosynthesis (Fig. 1.1). This procedure was performed every half-month. Each time, 5.0 g of $NaH^{13}CO_3$ and 0.1 M HCl were injected into the chamber to allow them to react fully with each other, producing 1.31 L of $^{13}CO_2$. Three months later, after 6 injections, *K. paniculate* stems and leaves were harvested. The collected samples were dried, crushed, and sieved through a 5 mm sieve to produce an enriched ^{13}C-labeled litter for subsequent use. $NaH^{13}CO_3$ (Cat number: IR-33294, Enrichment: 99 atom%) and $Ca^{13}CO_3$ (Cat number: IR-32318, Enrichment: 98 atom%) to be applied directly to the soil were purchased from Shanghai ZZBio Co., Ltd., China.

To remove the inorganic C from the soil samples, 0.01 M HCl was added until no bubbles were produced. Subsequently, the soil samples were dried in an oven and injected with distilled water to adjust moisture to approximately 60% of the field moisture capacity, followed by a pre-incubation at 25 ℃ for one week. Next, the soil samples were laid flat on a plastic film. Crushed ^{13}C-litter or ^{13}C-$CaCO_3$ was spread evenly on the soil samples at a rate of 0.1 g 50 g^{-1} soil (in amounts sufficient to achieve the individual experimental treatment plan). After the mixture was adequately stirred with a glass rod, the plastic film was folded and carefully shaken until the added substance was well blended with the soil. The soils thus obtained were used in the subsequent incubation analyses. The ^{13}C abundance in ^{13}C-litter (δ_{lit} ^{13}C) and in the commercial ^{13}C-$CaCO_3$ (δ_{CaC} ^{13}C) was 1221.05 and 99.00 relative to the Vienna Pee Dee Belemnite (V-PDB) standard, respectively.

2.3 Incubation experiment

2.3.1 Experimental design

Three treatments were included: (1) no addition of any exogenous substance (control, CL); (2) addition of 0.1 g of ^{13}C-litter 50 g^{-1} soil (LL); and (3) addition of 0.1 g of ^{13}C-$CaCO_3$ 50 g^{-1} soil (CCL) (Fig. 1.2). A total of 18 replicates per treatment were performed to allow the collection of three replicated samples at each time-point (5, 10, 20, 40, 60, and 80 days). A total of 54 mineralization-incubation microsystems were set (three treatments × six sampling times × three replicates per sampling time).

2.3.2 Mineralization-incubation experiment

To determine SOC mineralization, the alkali absorption method was applied. Over the period of March–May 2021, 50 g of each isotopically labeled soil sample was placed in a 50 mL beaker (with sufficient replicates to achieve the experimental treatment plan) and the water content was adjusted with deionized water to approximately 60 % of the field moisture capacity. The beaker was then placed at the bottom of a 1000 mL wide-mouth bottle and preincubated at 25 ℃ for 7 d. Subsequently, a 50 mL absorbent cup containing 10 mL of 0.1 M NaOH solution was placed at the bottom of the incubation bottle, which was then sealed and further incubated in the dark at 25 ℃. Three experimental units per treatment were taken out at each time point and analyzed together with a blank control (no exogenous C source). For some replicates, the absorbent cup was removed at 5, 10, 20, 40, 60, and 80 days of incubation, immediately after which the bottle was sealed to allow incubation to continue. Nine absorbent cups were taken out at each time point. After adding two drops of phenolphthalein indicator, the remaining NaOH solution was titrated with 0.1 M HCl. The following reaction was expected to occur inside the sealed microsystem:$^{13}CO_2 + 2NaOH = Na_2{}^{13}CO_3 + H_2O$ (Fig.

1.3). The amount of mineralized C released as CO_2 was indirectly estimated from the titration of the remaining NaOH solution. Then, the aqueous solution in each absorbent cup was transferred into a 10 mL centrifuge tube to determine the ^{13}C abundance. A total of 58 soil samples (the 54 samples already described and four additional samples not subjected to incubation) were subsequently processed. Each sample was divided into two parts. One part was placed in a –70 °C refrigerator for soil microbial determinations, whereas the other part was dried and ground finely for SOC fractionation.

2.4 SOC fractionation

SOC fractionation was carried out using the wet-sieve fractionation method developed by Six et al. (2002). After air drying and passing through a 2 mm sieve, soil samples were weighed and placed, together with 15 glass beads, on the top sieve of a microaggregate separator sieve set (top-sieve mesh size: 250 μm; bottom-sieve mesh size: 53 μm). The separator sieve set was allowed to vibrate vertically for 30 min; aggregates >250 μm remained on the top sieve, and microaggregates 53～250 μm remained on the bottom sieve, whereas clay and silt particles passed through the 53 μm sieve (Fig. 1.4). Then, 25 mL of a 0.25 M $CaCl_2$ solution was added to the bucket below the inferior sieve and centrifuged at 1,730×*g* for 15 min to separate the clay from the silt particle fraction. Each fraction was transferred to an aluminum box and then steam-dried using a water bath, followed by drying in an oven at 60 ℃ for 12 h and fine grinding and passing through a 0.25 mm sieve. The >250 μm, 53～250 μm, and <53 μm fractions consisted of macroaggregates, microaggregates and mineral particulate organic C, respectively. A stable isotope ratio mass spectrometer was used to determine the organic C content and ^{13}C abundance in all samples (Fig. 1.5).

2.5 Soil physical and chemical properties

Soil pH was determined using the potentiometric method with a soil–water ratio of 1:2.5, and bulk

density was measured using the ring-knife weighing method. The oil bath-heated potassium dichromate oxidation volumetric technique was applied to calculate the SOC content. Total nitrogen was determined by Kjeldahl distillation, while the molybdenum antimony colorimetric and the NaOH fusion–flame photometric methods allowed the estimation of total phosphorus and total potassium contents, respectively. The exchangeable Ca content was measured using the ammonium acetate exchange–atomic absorption spectrophotometry method. Soil enzyme activity was determined based on the methods described in Guan (1986). Soil urease, sucrase, and neutral phosphatase activity were determined using the phenol–sodium hypochlorite colorimetric method, the 3,5-dinitrosalicylic acid colorimetric method, and the sodium benzene phosphate colorimetric method, respectively.

2.6 Soil microbial analysis

2.6.1 DNA extraction and PCR amplification

Total DNA extraction was performed with the E.Z.N.A.®Soil DNA Kit (Omega Bio-tek, Norcross, GA, USA), according to the manufacturer's instructions. The concentration and purity of DNA were determined using a NanoDrop 2000 spectrophotometer. The DNA extraction quality was evaluated using 1% agarose gel electrophoresis. Two primers, 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'), were used to amplify the V3–V4 variable region of the bacterial 16S rRNA gene (Xu et al., 2016). Two other primers, ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS2R (5'-GCTGCGTTCTTCATCGATGC-3'), were used to amplify the fungal internal transcribed spacer (ITS1 region) (Adams et al., 2013). PCR products were purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and quantified using QuantiFluor™-ST (Promega, USA). The processed PCR amplification products were sequenced on the IlluminaMiSeq platform (Illumina, San Diego, USA) using the double-end

sequencing method developed by Shanghai Majorbio Bio-pharm Technology Co., Ltd., China.

2.6.2 Sequence data processing

Paired-end sequences were merged into a single sequence using FLASH v.1.2.11, followed by quality filtering using Trimmomatic v.0.33 (average quality score: > 20). Chimeric sequences were then identified and removed using the UCHIME algorithm. Valid reads were thus obtained. Operational taxonomic units (OTUs) were identified using Uparse v.7.0 based on a similarity threshold of 97% (Edgar, 2013). To reduce spurious OTUs, those with fewer than two representative sequences were removed. The most representative sequence of each OTU was ultimately selected. Taxonomic information was annotated using the Ribosomal Database Project classifier based on Silva 132 and Unite 8.0 databases for bacteria and fungi, respectively, with a confidence threshold of 70%. To minimize the effects of variations in readings from different samples, all the samples were normalized based on the minimum sequence.

2.7 Calculations and statistical analysis

2.7.1 Data calculation methods

Organic C content in each fraction (mg kg^{-1} soil) = organic C mass fraction in each fraction (%) × organic C content in each fraction (mg kg^{-1} fraction)

Litter C input (g m^{-2}) = litter of the standing crop per unit area (g m^{-2}) × C content in the litter (g kg^{-1}) / 1000

The cumulative amount of organic C mineralized (mg CO_2 kg^{-1}) refers to the total amount of soil CO_2 released over the period from the beginning of the incubation process to a certain time point.

The following equations allow the calculation of mineralized C originating from exogenous C (Q) and native SOC (Q_{soi}) in mg kg^{-1}:

$$Q = Q_{tot} \times (\delta_{tot} - \delta_{soi}) / (\delta - \delta_{soi})$$

$$Q_{soi} = Q_{tot} \times (\delta - \delta_{tot}) / (\delta - \delta_{soi})$$

where Q_{tot} is the amount of respiratory soil mineralization (mg kg^{-1}), δ_{tot} is the $\delta^{13}C$ value of the mineralized solution during mineralization, δ_{soi} is the $\delta^{13}C$ value of the soil during mineralization, and δ is the $\delta^{13}C$ value of the litter or $CaCO_3$.

The following equation describes the SOC priming effect:

$$PE = CO_{2\text{-}tre} - CO_{2\text{-}ck}$$

where $CO_{2\text{-}tre}$ is the organic C originating from the native soil in the LL or CCL treatment and $CO_{2\text{-}ck}$ is the organic C originating from the native soil in the CL treatment (both in mg kg^{-1}).

The following equations allow the calculation of the soil C originating from exogenous C and native SOC:

$$\delta\ ^{13}C_{after} = \delta\ ^{13}C_{tre} \times (1 - f) + f \times \delta\ ^{13}C_{before}$$

$$C_{tre} = (1 - f) \times C_{total}$$

$$C_{soi} = f \times C_{total}$$

where $\delta^{13}C_{after}$ is the $\delta^{13}C$ value of different soil C fractions after a certain time of incubation, $\delta^{13}C_{tre}$ is the $\delta^{13}C$ value of the litter or $CaCO_3$, $\delta^{13}C_{before}$ is the $\delta^{13}C$ value of different soil C fractions before a certain time of incubation, f is the proportion of native SOC after a certain time of incubation (%), C_{tre} is the amount of C derived from the litter or $CaCO_3$ (mg kg^{-1}), C_{soi} is the amount of C derived from native SOC (mg kg^{-1}), and C_{total} is the C content in the different soil fractions after a certain time of incubation (mg kg^{-1}).

2.7.2 Data processing and analysis

SPSS (16.0) was used to perform the statistical analysis. A two-way analysis of variance (ANOVA) was conducted to analyze the effects of exogenous matter addition and incubation time on

SOC mineralization. Means were compared by the least significant difference test and Student t-test, and the level of significance (α) was set at 0.05. Linear regressions in R (http://www.r-project.org/) (Yergeau et al., 2007) were performed to investigate whether soil microbial characteristics significantly predicted the SOC priming effect and the soil mineral particulate C (< 53 μm fraction C) estimate. The decision coefficient R^2 depended on the optimal simulation models. Path analysis (using the plspm package in R) was used to investigate the direct and indirect effects of microbial variables on SOC mineralization. To simplify the model, the Shannon index values for both bacterial and fungal communities were used because they were shown to affect significantly SOC mineralization in previous Pearson correlation analyses. We calculated the standardized total effects (direct plus indirect effects from the path analysis) of the bacterial and fungal Shannon index on SOC mineralization.

3. Results

3.1 Soil C mineralization and native soil C priming

By analyzing $^{13}CO_2$, we could differentiate between CO_2 derived from the native SOC and CO_2 derived from the exogenous compounds. In the ^{13}C-litter amended soil, native soil-derived CO_2 (3360.79 mg CO_2-C kg^{-1} C_{bulk}) and net litter-derived CO_2 (33.52 mg CO_2-C kg^{-1} C_{bulk}) were significantly higher than in the ^{13}C-$CaCO_3$ amended soil (native soil-derived CO_2: 2855.76 mg CO_2-C kg^{-1} C_{bulk} ; net $CaCO_3$-derived CO_2:13.04 mg CO_2-C kg^{-1} C_{bulk}), with $P < 0.01$ and $t = 6.003$; $P < 0.001$ and $t = 18.512$, respectively (Fig. 2a and b). Litter-derived CO_2 only accounted for 0.99% of the total respiration in the ^{13}C-litter treatment, and the $CaCO_3$-derived CO_2 for around 0.45% in the ^{13}C-$CaCO_3$ amended treatment. The ^{13}C-litter addition induced a higher priming effect than ^{13}C-$CaCO_3$ addition (Fig. 2b and d), accounting for a net release of 832.12 mg CO_2-C kg^{-1} C_{bulk} from the native SOC compared to 327.09 mg CO_2-C kg^{-1} C_{bulk} in the ^{13}C-$CaCO_3$ treatment ($P < 0.001$, $t = 80.689$).

3.2 Litter-derived and $CaCO_3$-derived C distribution across different size fractions

We assessed the contribution of OC derived from the litter and $CaCO_3$ to the different OM pools. The soil-derived C in mg kg^{-1} C_{bulk} was similarly distributed across OM fractions in both treatments (P = 0.348, t = −1.062), showing a not statistically significant tendency towards a decreasing contribution to the > 250 μm and 53～250 μm fractions as far as the incubation time progressed, with decreases of 2916.71 and 1841.06 mg C kg^{-1} C_{bulk}, respectively, in the ^{13}C-litter treated soil, and of 2507.18 and 1905.14 mg C kg^{-1} C_{bulk} in the ^{13}C-$CaCO_3$ treated soil, coinciding with a trend towards an increasing contribution to the < 53 μm fraction from the 5th day to the 80th day of incubation (with increases of 1396.99 and 1556.55 mg C kg^{-1} C_{bulk} in LL and CCL, respectively) (Fig. 3a).

Interestingly, a significant difference was found in the amount of litter-derived C compared to $CaCO_3$-derived C across OM fractions. While ^{13}C-litter addition led to 5535.91, 3205.87, and 1120.91 mg C kg^{-1} C_{bulk} in > 250, 53～250, and < 53 μm fractions, respectively, in ^{13}C-$CaCO_3$ amended samples, these values were much lower: 583.48, 321.06, and 97.11 mg C kg^{-1} C_{bulk}, respectively (P = 0.000, t = 330.683). However, in both treatments, most of the C supplied by the exogenous source was found in the > 250 μm fraction, followed by 53～250 and < 53 μm fractions, and a non-significant increasing trend with the extension of the incubation time was observed (for > 250 μm, 53～250 μm, and < 53 μm fractions, increases of 337.27, 336.46, and 329.87 mg C kg^{-1} C_{bulk} in the ^{13}C-litter treatment and of 588.49, 259.63, and 24.59 mg C kg^{-1} C_{bulk} in the ^{13}C-$CaCO_3$ treatment, respectively) (Fig. 3b). The evolution of total C in the < 53 μm fraction is shown in Fig. 3c. Slight differences and an increasing trend with the extension of the incubation time was detected for both treatments (6899 and 5951.07 mg C kg^{-1} C_{bulk} for soil amended with ^{13}C-litter and ^{13}C-$CaCO_3$, respectively) (P = 0.034, t = 15.564).

3.3 Soil microbial community characteristics and diversity

The effect of exogenous C additions on soil bacterial and fungal community composition is shown in Fig. 4. In the unamended soil (CL), bacteria (Fig. 4a) and fungi (Fig. 4d) comprising >1% of the community did not change significantly throughout the incubation period. However, several bacterial groups showed a decreasing trend in LL and CCL treatments (Fig. 4b and c), while unclassified_k_Fungi showed an increasing trend (Fig. 4e and f), both mainly at the expense of increases in minority taxa (those comprising <1% and grouped as "others"). *Massilia* decreased by 9.82%, 18.84%, and 10.88% with the extension of incubation time in the CL, LL, and CCL treatments, respectively, while unclassified_k_Fungi increased by 21.09%, 43.43%, and 34.52%. However, the relative abundance of bacterial and fungal communities comprising >1% in the samples not subjected to incubation did not show significant differences among treatments (Fig. 4g and h).

The Shannon diversity index of soil bacteria increased significantly with the extension of the incubation time in LL and CCL treatments (by 0.79 and 0.43, respectively), whereas that of fungi decreased significantly (by 1.72 and 1.32, respectively). Bacteria and fungi diversity changed most obviously in the LL treatment (19.36% and 45.38%). There was no significant difference in bacterial and fungal diversity among the three treatments (Table 3).

3.4 Relationship of soil microbial communities with native C priming and soil mineral particulate organic C

The addition of exogenous C sources led to defined correlations between Shannon diversity indexes and the priming effect on native soil C and between those indexes and soil mineral particulate C (< 53 μm). The addition of both C sources led to a negative correlation between the bacterial Shannon diversity index and the priming effect (LL and CCL, $R^2 = 0.62$, $P = 0.000$ and $R^2 = 0.48$, $P = 0.001$), and a positive correlation between the fungal Shannon diversity index and the priming effect

(LL and CCL, $R^2 = 0.57$, $P = 0.000$ and $R^2 = 0.39$, $P = 0.005$) (Fig. 5). On the contrary, the addition of both C sources resulted in a significant positive correlation between the bacterial Shannon diversity index and the soil mineral particulate C amount (LL and CCL, $R^2 = 0.54$, $P = 0.001$ and $R^2 = 0.51$, $P = 0.001$), and to a negative correlation between fungal Shannon diversity index and soil mineral particulate C (LL and CCL, $R^2 = 0.63$, $P = 0.000$ and $R^2 = 0.52$, $P = 0.001$) (Fig. 6).

4. Discussion

4.1 Effects of exogenous C sources on SOC mineralization

In this study, SOC mineralization was 15.48% higher in the presence of litter than $CaCO_3$ over 80 days of continuous incubation. Litter constitutes an additional C source in the soil. The organic C in the litter may be consumed by soil microbes, accelerating SOC mineralization (Xiao et al., 2018; Song et al., 2022). Previous studies reported that, comparatively, the mineralization stability of the Ca-bonded organic C in the added $CaCO_3$ was higher than that of total SOC (Huang et al., 2013). Ca^{2+} is expected to bind to SOC and form aggregates, thereby slowing SOC mineralization. In addition, it was informed that Ca^{2+} combines with the free radicals in the active humified soil OM, covering its surface with a calcium crust, thereby limiting the contact between organic C and microbes and thus reducing the organic C decomposition rate (Hu et al., 2012; Zornoza et al., 2016). The formation of Ca-bonded organic C after the addition of exogenous $CaCO_3$ led to a high level of SOC stability, which, in turn, slowed SOC mineralization.A net increase or decrease in the cumulative SOC mineralization rate due to the addition of exogenous C has been referred to as a positive or negative priming effect, respectively (Xiao et al., 2018). Whether a positive or negative priming effect occurs depends primarily on soil microbial activity. Most soil microbes remain dormant under natural conditions. Adding fresh OM activates dormant soil microbes, causing changes in soil microbial community structure and

stimulating SOC decomposition (Qiao et al., 2014; Liu et al., 2020).

In this study, adding either litter or $CaCO_3$ led to a positive priming effect on SOC mineralization, suggesting the following ideas: (1) adding an exogenous OM source stimulated the activity of native soil microbes and provided sufficient nutrients for microbial activity, thereby accelerating the decomposition of native SOC; (2) the exogenous OM source added to the soil contained some microbes, thereby altered the soil microbial community structure and stimulated SOC decomposition; (3) the addition of litter or $CaCO_3$ may have promoted the growth of soil fungi but inhibited the growth of soil bacteria. This result is consistent with many previous studies (Liu et al., 2020; Liu et al., 2021; Huo et al., 2022). However, some authors found that exogenous C addition caused a negative priming effect on SOC (Santruckova et al., 2004; Guenet et al., 2010; Xie et al., 2022). The priming effect occurs in most soils (Lloyd et al., 2016; Razanamalala et al., 2018; Xie et al., 2022) and is determined by nutrient effectiveness, climate, soil type, vegetation, and microbial properties (Kuzyakov, 2010).

In this research, the SOC priming effect was 61.00% greater in the LL treatment than in the CCL treatment. This may be the consequence of higher organic C and cellulose contents in the litter, which could be easily decomposed and utilized when added to the soil, providing soil microbes with essential nutrients and increasing microbial activity, thereby accelerating SOC mineralization (Lou et al., 2007; Bernard et al., 2022).

4.2 Fate of exogenous C

In this study, as the incubation time increased, there was a decrease in the total OC contained in the fraction >250 μm and 53～250 μm and an increase in that contained in the fraction <53 μm. Macroaggregates and microaggregates constitute the primary sites of exchange between the soil and its

surrounding environment; the compounds contained in these aggregates could have been utilized and decomposed by soil microbes during the incubation process, undergoing mineralization and releasing CO_2. Consequently, the SOC in macroaggregates and microaggregates (>250 μm and 53～250 μm) decreased. On the other hand, the mineral particulate organic C tends to interact with clay minerals, forming organic-inorganic complexes. Under the strong physical, chemical, and biochemical protection exerted by clay minerals (Stegarescu et al., 2020), the mineral particulate organic C was less susceptible to microbial consumption and was stored and sequestered in the soil, forming a SOC pool difficult to be degraded.

A SOC decrease after exogenous C addition is not a common phenomenon (Luo et al., 2019; Luo et al., 2020); however, some studies have reported similar findings. For example, Luo et al. (2017) also found a loss of 3.3% of organic C after 87 days of incubation in soil amended with biochar. In line with our findings, Chen et al. (2018) examined the distribution of red soil aggregates using ^{13}C as a marker and discovered a gradual decrease in the SOC in macro- and microaggregates and a gradual increase in mineral particulate organic C (<53 μm) with incubation time. Due to the short period assessed in the present study, our findings only reflect the short-term changes in SOC. Grandy et al. (2008) found that the organic C in soil that received exogenous C decreased by 20% after three years of continuous incubation, but an upward trend was detected after six years of continuous incubation. Therefore, the short-term decrease in SOC after adding exogenous C may be transient.

We found that soil C content in the different fractions originated primarily from the C present in the native soil C. The >250 μm fraction had the highest C content, derived from native and exogenous C, followed by the 53～250 μm and <53 μm fractions. This suggests that both native and exogenous C preferentially entered the macroaggregates, followed by the microaggregates and, finally, smaller

aggregates. This finding is in agreement with previous results (Ge et al, 2012; Stegarescu et al., 2020). During the 80-day incubation period, a decrease in the C content derived from the native C in the >250 μm and 53～250 μm fractions and an increase in C derived from the exogenous sources could be noticed. This result suggests that as the incubation time increased, the origin of C in the >250 μm and 53～250 μm fractions tended to shift to exogenous C, whereas the native soil C tended to undergo mineralization and decomposition and, thus, decreased. Comparatively, an increase in the C content derived from both native and exogenous C was found in the <53 μm fraction, a mineral particulate fraction where C was reported to be stable and less subjected to decomposition and consumption by microbes, constituting the functional SOC fraction (Witzgall et al., 2021). In addition, the exogenous C entering this fraction could have been sequestered, resulting in an increased stable SOC fraction. This phenomenon was particularly pronounced in the LL treatment, in line with previous findings (Xiao et al., 2014).

4.3 Effects of microbes on soil C priming and sequestration after exogenous C addition

In this study, soil bacteria diversity was higher than fungi diversity, both expressed through the Shannon index. Besides, as the incubation time progressed, the Shannon index of soil bacteria exhibited an upward trend, whereas the Shannon index of soil fungi showed a downward trend. Extensive research has found that adding exogenous C can increase soil bacterial diversity (Zhu et al., 2020; Keyvan et al., 2021) but has no significant impact on soil fungal diversity (Wang et al., 2018; Saskia et al., 2022), a finding that has been mainly linked to the rise in easily soluble organic C at the early stages of incubation after exogenous C addition. A large amount of available C and nitrogen stimulates soil microbes, which rapidly decompose these compounds resulting in larger numbers and, usually, increased diversity (Xiao et al., 2018; Song et al., 2022). As the incubation time advances, the

proportion of soil OM readily available to be degraded decreases, whereas the proportion of more resistant materials (e.g., lignin) increases, resulting in insufficient available nutrients for soil microbes. However, at the initial stages of substrates' utilization, fungi are the first to play a dominant role. Some fungi can decompose the substrates available in the soil matrix efficiently and grow actively. The fast-growing mycelia can pass through plant cell walls and break down substances less prone to be decomposed, providing energy for bacterial growth and reproduction at later stages of incubation (Silverman, 2001; Zhang et al., 2005; Xiao et al., 2018). These concepts explain why the bacterial diversity is maintained or increases during incubation, whereas fungi of different functional groups die successively when they have fulfilled their respective roles and their preferent substrates become exhausted (Feng et al., 2016; Han et al., 2021), resulting in decreasing fungal diversity with incubation time.

Of note is that the SOC priming effect of both treatments was significantly negatively correlated with soil bacterial diversity but significantly positively correlated with soil fungal diversity. In contrast, the mineral particulate C fraction was significantly positively correlated with the Shannon diversity index of soil bacteria and significantly negatively correlated with the Shannon diversity index of soil fungi. As a whole, these findings suggest that soil fungi increased SOC mineralization and decomposition and accelerated the SOC priming effect, whereas soil bacteria increased SOC stability and mineral particulate C, which tend to constitute the functional fraction of SOC, as described before (Witzgall et al., 2021; Andrew et al., 2022; Cotrufo et al., 2022).

In Fig. 7, we present a conceptual diagram illustrating the main findings of this research. The effects of soil fungi on SOC priming significantly exceeded the effects of bacteria on SOC sequestration ($R = 0.40$ vs. 0.27, $P = 0.003$ vs. 0.02), leading to an increase in SOC mineralization and

decomposition after the addition of exogenous C. This phenomenon was more pronounced in the LL treatment than CCL treatment. Sheng and Zhu (2018) found that adding biochar to soil increased the diversity of soil bacterial communities, reduced CO_2 emissions, and increased soil C sequestration. Gai et al. (2021) found a decreasing trend in fungal diversity and an increased risk of soil C loss in a subtropical Lei bamboo (*Phyllostachys praecox*) forest after adding OM to the soil. Similarly, by examining red soil added with ^{13}C-labeled $CaCO_3$ and rice straw, Xiao et al. (2018) found that fungal diversity was a crucial factor for SOC mineralization and that soil C sequestration was significantly and directly affected by bacterial diversity. The results of this study are in line with that finding.

5. Conclusion

In this study, we performed an 80-day continuous ^{13}C isotope-labeled mineralization-incubation experiment and found that adding litter had a considerably greater impact on SOC mineralization than adding $CaCO_3$, but both additions resulted in a positive SOC priming effect. Adding litter or $CaCO_3$ reduced the total organic C content in the soil and the C fraction associated with macroaggregates but increased that associated with the mineral particulate fraction (<53 μm), suggesting that mineral particulate C was the functional fraction of SOC. As the incubation time increased, a significantly positive correlation became apparent between SOC priming and fungal diversity, as well as between the mineral particulate C fraction and soil bacterial diversity. This finding suggests that SOC priming was mainly dependent on fungal diversity, whereas SOC sequestration was mainly dependent on bacterial diversity, and that the effects of soil fungi on SOC priming significantly exceeded the effects of bacteria on SOC sequestration, resulting in a decrease in total SOC.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal

relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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652 **Table legends**

653 **Table 1 Basic information of the environmental background of sample point**

Item	altitude (m)	precipitation (mm)	temperature (℃)	slope	dominant species	Soil bedrock	soil type	Sample size (m^2)
shrub	865	1083.80	17.29	northwest	*Pyracantha fortuneana, Viburnum dilatatum Thunb., R. setchuenensis, Wild persimmon*	carbonatite	black calcareous soil	4 × 10

654 **Table 2 Selected properties of the experimental soil**.BD: soil bulk density, SOC: soil organic carbon,

655 TN: total nitrogen, TP: total phosphorus , Lci: litter carbon input, Ca: exchangeable calcium, Ur: soil

656 urease, Npa: neutral phosphatase, Sa: soil sucrase

Item	pH	BD ($g\ cm^{-3}$)	SOC ($g\ kg^{-1}$)	TN ($g\ kg^{-1}$)	TP ($g\ kg^{-1}$)	Lci ($g\ m^{-2}$)	Ca ($g\ kg^{-1}$)	Ur ($mg\ g^{-1}\ 24h^{-1}$)	Npa ($mg\ g^{-1}\ 24h^{-1}$)	Sa ($mg\ g^{-1}\ 24h^{-1}$)
Tested soil	6.55± 0.04	1.18± 0.03	29.23± 3.05	2.33± 0.17	0.37± 0.01	29.25± 2.34	1.85± 0.13	0.35± 0.03	0.69± 0.03	4.67± 1.51

Table 3 Diversity of soil bacteria and fungi under different treatments and incubation stages.CL：not addition, LL：^{13}C-litter addition，CCL：^{13}C-$CaCO_3$ addition. All data are means, "±" displayed with the standard deviation, n = 3 independent replicates. The lowercase and uppercase letters denote significant differences in bacterial and fungal diversity in different treatments at different stages of incubation and in different treatments, respectively. Statistical significance was analyzed using an unpaired two-sided t-test.

Name	Treatments	5	10	20	40	60	80	Average
Bacteria	CL	4.44±0.16a	4.63±0.09a	4.51±0.13a	4.50±0.19a	4.54±0.09a	4.60±0.14a	4.53±0.05A
	LL	4.08±0.11c	4.47±0.26b	4.64±0.15a	4.74±0.16a	4.76±0.11a	4.87±0.06a	4.60±0.12A
	CCL	4.39±0.23c	4.67±0.09b	4.83±0.03a	4.87±0.06a	4.85±0.07a	4.82±0.05a	4.74±0.06A
Fungi	CL	3.85±0.11a	3.73±0.06a	3.46±0.03b	3.40±0.07b	3.24±0.15b	2.88±0.20c	3.43±0.16A
	LL	3.79±0.06a	3.63±0.12a	3.57±0.05a	3.55±0.08a	2.20±0.11b	2.07±0.16b	3.14±0.09A
	CCL	3.96±0.10a	3.89±0.06a	3.70±0.22a	3.32±0.14b	3.15±0.09b	2.64±0.07c	3.44±0.13A

Figure legends

Fig. 1 Experimental setup. (1) The pulse-labeling method was employed to label with ^{13}C *Koelreuteria paniculata* seedlings, from which ^{13}C-litter was obtained. Every half-month, $^{13}CO_2$ generated by the reaction between $NaH^{13}CO_3$ and HCl was injected into a closed chamber where *K. paniculata* seedlings were growing. After six $^{13}CO_2$ pulses (three-month-old plants), the stems and leaves were harvested, mixed, dried, and ground finely through a 5-mm sieve to produce enriched ^{13}C-litter. **(2)** The lime soil was subjected to three treatments: addition of ^{13}C-litter, addition of ^{13}C-$CaCO_3$, and no addition of exogenous C. After adding the C source, soil samples were thoroughly mixed and incubated for 80 days. **(3)** Mineralization (CO_2 release) was measured at 10, 20, 40, 60, and 80 days of incubation with three replicates per treatment. **(4)** Air-dried and finely ground soil samples were used to determine C fractionation using a sieve-based system (> 250 μm, 53～250 μm, < 53 μm). **(5)** A

small portion of each soil sample was used for microbial determinations; C content and ^{13}C abundance were determined in aqueous solutions; the distribution of labeled C across fractions was analyzed using an Elementar vario cube TOC-isoprime100 organic C analyzer–stable isotope mass spectrometer.

Fig.2 Cumulative heterotrophic respiration in ^{13}C-litter and ^{13}C-$CaCO_3$ addition soils. Respired CO_2-C mg kg^{-1} soil during the 80-day incubation in (**a/b**) **^{13}C-**litter and (**c/d**) **^{13}C-**$CaCO_3$ addition soil. The total respired CO_2-C in soil with (**b**) **^{13}C-**litter and (**d**) **^{13}C-**$CaCO_3$ addition is displayed on the right (means, SDs displayed with errors bars, n = 3 independent replicates), together with the total priming effect. We report CO_2-derived C per amount C in incubated samples to directly showcase the mechanistic process level. Asterisks represent significant differences between the textures (*** $P <$ 0.001, ** $0.001 \leq P < 0.01$). Statistical significance was analyzed using an unpaired two-sided t-test.

Fig.3 Allocation of soil-derived and litter-derived C to SOC fractions in ^{13}C-litter and ^{13}C-$CaCO_3$ addition soils. Content of > 250 μm, 53～250 μm, < 53 μm fraction C and in mg C kg^{-1} soil of (**a**) soil, (**b**) allothigene (^{13}C-litter, ^{13}C-$CaCO_3$), and (**c**) all origin in different incubation periods of ^{13}C-litter and ^{13}C-$CaCO_3$ addition soils (means, SDs displayed with errors bars, n = 3 independent replicates). Asterisks represent significant differences between the average values of different addition soils (*** P < 0.001, * $0.01 \leq P < 0.05$, ns $P \geq 0.05$). Statistical significance was analyzed using an unpaired two-sided t-test.

Fig.4 Characteristics of the soil microbial community composition at the genus level. (a), (b), and (c) show the composition of the soil bacterial community in the CL (no addition of exogenous carbon), LL (^{13}C-litter addition), and CCL (^{13}C-$CaCO_3$ addition) treatments at different stages of incubation, respectively. (d), (e), and (f) show the composition of the soil fungal community in the CL (no addition of exogenous carbon), LL (^{13}C-litter addition), and CCL (^{13}C-$CaCO_3$ addition) treatments at different

stages of incubation, respectively. (g) and (h) show the composition of the bacterial and fungal communities in different treatments, respectively. The composition of each community with an abundance greater than 1% is shown in the figure, whereas the communities with an abundance less than or equal to 1% are classified as others (means, n = 3 independent replicates).

Fig.5 Correlation of soil microbial diversity with native soil carbon priming effect. LL is ^{13}C-litter addition，CCL is ^{13}C-$CaCO_3$ addition. The two plots on the left show the linear regression models of bacterial diversity and the priming effect. The two plots on the right show the linear regression models of fungal diversity and the priming effect. The regression equations, R^2, and differences are provided in the table below the figure. n = 18, and asterisks represent significant differences between the average values of different addition soils (*** $P < 0.001$, ** $0.001 \leq P < 0.01$). Statistical significance was analyzed using an unpaired two-sided t-test.

Fig.6 Correlation of soil microbial diversity with soil mineral particulate C. LL is ^{13}C-litter addition, and CCL is ^{13}C-$CaCO_3$ addition. The two plots on the left show the linear regression models of bacterial diversity and soil mineral particulate **C**. The two plots on the right show the linear regression models of fungal diversity and soil mineral particulate **C**. The regression equations, R^2, and differences are provided in the table below the figure. n = 18, and asterisks represent significant differences between the average values of different addition soils (*** $P < 0.001$, ** $0.001 \leq P < 0.01$). Statistical significance was analyzed using an unpaired two-sided t-test.

Fig. 7 Main effects of ^{13}C-litter and ^{13}C-$CaCO_3$ addition on organic carbon decomposition and stabilization in lime soil. BD: bacterial diversity, FD: fungal diversity, PE: priming effect, FC: C in < 53 μm fraction. Short vertical blue and red arrows represent increases and decreases in soil C processes in response to ^{13}C-litter and ^{13}C-$CaCO_3$ additions. Oblique blue and red arrows indicate positive and

719 negative correlations, respectively. Numerical values correspond to results at day 80 of incubation. A
720 path analysis of the direct and indirect effects of bacterial diversity and fungal diversity on the priming
721 effect and C in the < 53 μm fraction is presented. The width of the arrows indicates the strength of the
722 standardized path coefficient. The blue lines indicate positive path coefficients, and the red lines
723 indicate negative path coefficients. Asterisks represent significant differences between the average
724 values (** $0.001 \leq P < 0.01$, * $0.01 \leq P < 0.05$). Statistical significance was analyzed using an unpaired
725 two-sided t-test.