

Article

Effects of Microbial Inoculant Application on the Decomposition of Organic Materials and the Priming Effect in Sandy Soils

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Abstract

The priming effect (PE) plays a critical role in regulating soil organic carbon (SOC) sequestration. Whether microbial inoculant combined with organic materials (e.g., crop straw) can reduce PE and enhance the fertility of coarse-textured sandy soils by accelerating straw decomposition remains poorly understood. We conducted a 90-day incubation experiment, applying ¹³C-labeled maize straw and/or microbial inoculant to coarse-textured sandy and fine-textured clay loam soils. Our results showed that microbial inoculant accelerated straw mineralization in both soils. Notably, microbial inoculant suppressed PE by 8.98% and 43.98% in the clay loam and sandy soil compared to straw addition alone, driven by adjusted soil available, C:N:P stoichiometry, and a shift in microbial community, as evidenced by a decrease in the ¹³C-G⁺ to ¹³C-G[−] ratio from 2.68 to 2.60 in clay loam soil and from 3.2 to 1.9 in sandy soil. Microbial inoculant further increased net SOC gain (clay loam soil: +22.1%, sandy soil: +641%), with sandy soils exhibiting superior responsiveness due to reduced organo-mineral associations and enhanced microbial carbon use efficiency. In conclusion, our study provides evidence for the mechanism by which microbial inoculants suppress the PE, particularly in sandy soils. This finding establishes a theoretical basis for further exploring microbial regulation strategies in soil carbon cycling under conditions of low organo-mineral associations.

Keywords: microbial inoculant application; sandy soil; ¹³C isotope; priming effect; soil carbon balance



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1. Introduction

Soil organic carbon (SOC) is a critical measure of soil quality and is of great importance in enhancing soil fertility, sustaining agroecosystems, and mitigating climate change [1,2]. Labile organic carbon inputs from organic materials (e.g., litter and rhizodeposits) rapidly stimulate microbial growth and activity. This acceleration induces the priming effect (PE), a phenomenon in which native SOC mineralization is either enhanced (positive PE) or reduced (negative PE) relative to soil without residue input [3]. Decomposable organic materials added to the soil can also form plant- and microbial-derived SOC through processes such as ex vivo modification and in vivo turnover. These processes contribute to SOC accumulation [4]. Consequently, the overall impact of organic materials on the SOC pool is mainly determined by the net balance between native SOC loss and new SOC accumulation [5,6]. Accelerated native SOC mineralization driven by the positive PE can

partially or fully offset the beneficial effects of organic materials on SOC sequestration. This offset causes minimal net increases or even reductions in SOC storage [7]. Therefore, both the PE and the fate of added organic carbon must be evaluated simultaneously when investigating the overall effect of SOC sequestration to organic materials.

The PE is governed by complex interactions between soil nutrient availability and microbial metabolic strategies [8,9]. Two key concepts based on the microbial physiological responses to nutrient demands have been mentioned: “Microbial nutrient mining” and “Microbial stoichiometric decomposition” [10]. Specifically, K-strategists preferentially decompose SOM when available soil nutrients are insufficient to meet microbial demands, generating a positive PE [11]. Conversely, in the case where nutrient availability aligns with the microbial stoichiometric ratios, r-strategists proliferate and accelerate native SOC decomposition, similarly inducing a positive PE [12]. Additionally, Cui et al. [13] proposed that under extreme C:N imbalance, N limitation can be alleviated through the reuse of microbial necromass. This process also led to a positive PE. Thus, soil nutrient availability can drive diverse PE outcomes, including enhancement [14,15], suppression [16], or negligible effects [17]. These discrepancies arise from the extent of soil nutrient limitation and the adaptive microbial strategies used to balance carbon and nutrient demands.

The incorporation of crop straw, a common agricultural by-product, into soil serves as a vital agricultural management practice for enhancing SOC sequestration and soil nutrient availability [18,19]. However, crop straw, rich in labile C, stimulates microbial activity and accelerates SOC mineralization through co-metabolism [20,21]. Numerous studies have shown that combining straw with mineral nutrients can retard SOC mineralization and promote SOC sequestration [14,22]. Notably, crop straw itself supplies a range of nutrients, including nitrogen, phosphorus, and other medium or trace elements. These nutrients can boost soil fertility and facilitate microbial metabolism [21,23]. The rate of nutrient release is largely controlled by the decomposition of straw fractions such as lignin, cellulose, and hemicellulose [24]. To efficiently utilize crop residue and promote nutrient release, microbial inoculants comprising diverse and specialized microorganisms are generally applied. These inoculants typically include bacteria (e.g., *Bacillus subtilis*), and fungi (e.g., *Aspergillus oryzae* and *Trichoderma viride*) [25], as they accelerate crop straw breakdown into simpler compounds [26]. The combined application of straw and microbial inoculants is widely adopted to promote straw decomposition and increase soil nutrient availability [27,28]. Meanwhile, this practice also alters the structure of the indigenous soil microbial community, thereby modifying the functional processes of microorganisms [27,29]. Soil nutrient availability and microbial metabolic strategies are known to jointly modulate the PE [9]. However, it remains unclear whether the elevated soil nutrient availability caused by microbial inoculants meets microbial nutrient demands in the presence of organic materials, and how this altered nutrient condition may influence SOC sequestration by regulating the direction and magnitude of the PE.

This knowledge gap holds particular significance for sandy soils. Globally, sandy soils cover approximately 900 million hectares, of which about 200 million hectares are employed for agricultural use [30]. Owing to their low silt and clay content, these soils exhibit a poorly developed structure, limited moisture retention, and low nutrient availability [31], and consequently contain less SOC than fine-textured soils [7]. Enhancing SOC sequestration is therefore essential for increasing the productivity of agricultural sandy soils [32]. To date, some evidence indicates that organic amendments can enhance SOC levels in sandy soils [33,34]. However, the efficiency of SOC sequestration in these soils is constrained not only by the elevated native SOC priming due to inadequate organo-mineral associations, but also by the slow decomposition of organic materials resulting from low microbial abundance and activity [35,36]. While microbial inoculants are known to accelerate the

decomposition of exogenous organic materials and promote nutrient release in fine-textured soils, their role in sandy soils remains unclear. Specifically, whether microbial inoculants can favorably influence SOC dynamics by modulating nutrient release and microbial metabolic strategies, particularly in soils with minimal organo-mineral associations, remains an unresolved question.

In this study, we hypothesized that (1) compared with straw addition alone, microbial inoculant application reduces the intensity of the PE by increasing nutrient availability; (2) microbial inoculants greatly enhance SOC sequestration in coarse-textured sandy soil; and (3) microbial inoculants alter soil nutrient availability, thereby modulating microbial nutrient demands and influencing the cumulative PE. Our purposes were to (1) determine how microbial inoculants influence the intensity of the PE and the fate of straw-derived carbon in sandy soils; (2) quantify how microbial inoculants influence the net SOC balance, and (3) elucidate how soil nutrient availability and microbial traits jointly regulate the cumulative PE and net SOC balance. To test these hypotheses, we sampled fine- and coarse-textured soils and performed a 90-day incubation experiment by combining ^{13}C -labeled maize straw and microbial inoculant. CO_2 emissions from different carbon sources were measured, alongside assessments of soil available nutrients, extracellular hydrolase activity, and ^{13}C -phospholipid fatty acids (^{13}C -PLFAs). This study provides valuable insights into straw decomposition and SOC sequestration in sandy soils.

2. Materials and Methods

2.1. Study Site Characterization, Soil Sampling and ^{13}C -Labeled Maize Straw Preparation

The experimental soils were collected from two sites: (i) Clay loam soil was sampled from a long-term experimental site, without straw-returning practice, located at Northwest A&F University, Shaanxi, China ($34^{\circ}17'38''$ N, $108^{\circ}04'02''$ E, ~525 m above sea level). This region experiences a continental monsoon semi-humid climate, characterized by mean annual temperatures around 13°C and annual precipitation of approximately 635 mm. The soil, developed from loess parent material, falls under the category of Earth-cumulic Orthic Anthrosols under the Chinese Soil Classification System (analogous to Udic Haplustalf in the USDA system). The long-term experimental site was a winter wheat monoculture agroecosystem. (ii) Sandy soil was collected from Yulin City, Shaanxi, China ($38^{\circ}22'50''$ N, $109^{\circ}45'27''$ E, ~1090 m above sea level). This region features a temperate semi-arid continental monsoon climate, characterized by mean annual temperatures around 10.7°C and annual precipitation of approximately 412.4 mm. The Chinese Soil Classification System classifies the soil as Aeolian soil (corresponding to Entisols in the USDA system). This site is under a maize mono-cropping system.

At each site, topsoil (0–20 cm) was collected from three randomly selected sampling points within a representative field in June 2021 using a stainless-steel spade. This provided three independent soil samples per soil type. Upon transportation to the laboratory, fresh soil samples were obtained after manually taking out fine roots and plant residues (>2 mm), then processed through a sieve (<2 mm) and thoroughly homogenized. Subsequently, the three samples from the same site were combined and homogenized to form a single composite sample. Each composite sample was partitioned into two subsamples: one portion was preserved at 4°C for analyzing physical and chemical properties, whereas the remaining portion was air-dried for use in the incubation experiment.

The ^{13}C -labeled maize straw was obtained from a continuous $^{13}\text{CO}_2$ pulse-labeling experiment (99.0 atom% ^{13}C), conducted based on the methodology of An et al. [37], with some adjustments. In brief, maize plants were pulse-labeled with $^{13}\text{CO}_2$ at the jointing stage. The $^{13}\text{CO}_2$ was generated by reacting H_2SO_4 (0.5 M) with $\text{Na}_2^{13}\text{CO}_3$ (99% atom% ^{13}C , Sigma Aldrich, St. Louis, MO, USA). Following the daily 7 h labeling process, the

maize plants were transferred to a ventilated area to prevent the reabsorption of $^{13}\text{CO}_2$ from aboveground respiration. At maturity, maize plants were harvested, washed with deionized water, and subjected to enzyme deactivation at 105 °C for 30 min. The aboveground residues were dried and pulverized into particles <2 mm in size by using a mill prior to incorporation into the soil. A representative part of the straw was pulverized into fine particles using a hybrid grinder (MM 200, Retsch GmbH, Haan, Germany) to be analyzed. Detailed procedures are provided in Text S1. The basic properties of the two soils and maize straw are summarized in Table 1.

Table 1. Basic properties of two soils and ^{13}C -labeled maize straw.

	Clay Loam Soil	Sandy Soil	^{13}C -Labeled Maize Straw
Total organic C (g C kg^{-1})	9.38	6.54	440.6
Total N (g N kg^{-1})	0.88	0.64	8.07
$\delta^{13}\text{C}$ (‰)	−22.514	−20.131	3215.943
Sand (%)	28.62	92.51	-
Silt (%)	40.06	6.74	-
Clay (%)	31.33	0.75	-

2.2. Experimental Design and Soil Incubation

The incubation experiment was conducted using distinct straw-incorporation and microbial inoculant application methods in two soil types (clay loam and sandy soil): no maize straw or microbial inoculant addition (CK), microbial inoculant alone (A), maize straw alone (M) and maize straw combined with microbial inoculant (MA). Thus, eight treatments were performed, each with three replicates. The amount of ^{13}C -labeled maize straw added was 12 g kg^{-1} soil, equivalent to $15,000 \text{ kg ha}^{-1}$ under field applications. The microbial inoculant used was “Ams” (Beijing Ammst Bio-Technology and Science, Beijing, China), which contains *Aspergillus oryzae*, *Saccharomyces cerevisiae*, and *Bacillus subtilis*, with an effective viable count of $\geq 2 \times 10^8 \text{ CFU g}^{-1}$. The microbial inoculant was applied at a dosage of 5% (w/w) of the straw mass, equivalent to 0.6 g kg^{-1} soil. To prevent resource competition or antagonism between the indigenous microbiota and the introduced microorganisms caused by nutrient limitations [29,38] and to promote the conversion of straw carbon into stable SOC [39], we supplemented additional nutrients. According to the C:N:P:S ratio of 10,000:833:200:143 reported by Kirkby et al. [39], we additionally applied 5 kg N, 2 kg P, and 1.3 kg S per ton of maize straw. The NPS nutrient solution was prepared from urea ($\text{CH}_4\text{N}_2\text{O}$), ammonium dihydrogen phosphate ($\text{NH}_4\text{H}_2\text{PO}_4$), and potassium sulfate (K_2SO_4). The pH of the NPS solution was set to 7 using a 10 M NaOH solution.

Soil samples (200 g fresh weight) were placed in 250 mL sealed jars, adjusted to 50% field water capacity with deionized water, and subsequently pre-incubated at 25 °C for 7 d to stabilize microbial activity. After pre-incubation, ^{13}C -labeled maize straw, in the presence or absence of microbial inoculant, was gently mixed into the soils to ensure uniform distribution according to the treatment. Deionized water and NPS nutrient solution were then added to achieve 70% field water capacity. To avoid masking the effect of the microbial inoculant or introducing confounding effects from nutrient addition, the same volume of NPS solution was applied to all treatments. All samples were incubated in the dark at 25 °C for 90 days. Throughout the incubation period, deionized water was added as needed to sustain 70% of field water content.

2.3. Soil CO_2 Sampling and Analyses

The sealed plastic jars containing soil samples were equipped with 50 mL beakers filled with 1 M NaOH (20 mL) to capture released CO_2 . The NaOH solution, which reacts to form Na_2CO_3 , was refreshed with new NaOH on days 1, 2, 3, 4, 6, 8, 11, 14, 18, 22, 28, 35, 42,

49, 59, 75, and 90. To quantify total CO₂ concentration, 0.5 M BaCl₂ was added in excess to the collected Na₂CO₃, causing precipitation of BaCO₃. The residual NaOH in the solution was titrated with 0.5 M HCl to determine the CO₂ concentration. The precipitated BaCO₃ was washed repeatedly with deionized water and purified using centrifugation at 5000× g rpm for 10 min. The purified BaCO₃ was dried, subsequently pulverized, and portioned into tin capsules for isotopic analysis [7]. We used an isotope ratio mass spectrometer (DELTA V Advantage, Thermo Fisher Scientific, Bremen, Germany) to measure the δ¹³C value of BaCO₃. The jars were ventilated for 30 min each time the NaOH trap was replaced to keep aerobic conditions.

2.4. Soil Property Analysis

Upon completion of the incubation, we collected soil samples destructively and partitioned them into three parts: a portion was kept at −80 °C for microbiological analyses, while another was preserved at 4 °C, and the third was air-dried for the assessment of soil properties. We determined SOC via the potassium dichromate oxidation method. An isotope ratio mass spectrometer (DELTA V Advantage, Thermo Fisher Scientific, Germany) was used to assess soil carbon isotopes (δ¹³C). Soil inorganic nitrogen (Min-N) was extracted with 1 M KCl (soil-to-solution ratio of 1:10 *w/v*) and quantified using a continuous analyzer (AA3, SEAL Analytical GmbH, Norderstedt, Germany). Min-N is defined as the total of NH₄⁺-N and NO₃[−]-N. For the determination of available phosphorus (AP), samples were extracted with 0.5 M K₂SO₄ (1:10 *w/v*), followed by analysis with the same continuous analyzer. The determination of microbial biomass carbon (MBC) and nitrogen (MBN) was carried out through chloroform fumigation, followed by extraction [40]. In brief, fresh soil was fumigated with ethanol-free chloroform in a vacuum oven at 25 °C for 24 h in the dark. Fumigated and non-fumigated samples were extracted using 0.5 M K₂SO₄ (1:10 *w/v*) and filtered. A TOC analyzer (vario MACRO cube, Elementar Analysensysteme GmbH, Langenselbold, Germany) and a continuous analyzer were used to measure total organic carbon (TOC) and nitrogen (N) concentrations, respectively. Of these, TOC in the K₂SO₄ extract of non-fumigated samples was used to assess dissolved organic carbon (DOC). We calculated the difference between fumigated and non-fumigated samples and applied conversion coefficients (*k*_{EC} = 0.45, *k*_{EN} = 0.54) to assess MBC and MBN. Furthermore, microbial biomass phosphorus (MBP) was determined in fresh soil using the same fumigation extraction method as employed for MBC and MBN [41]. The same fumigation method employed for MBC and MBN was utilized for fresh soil. All samples were carried out with 0.5 M NaHCO₃, and TP was quantified through the colorimetric analysis. MBP was calculated similarly to MBC and MBN, with conversion coefficients set at 0.40.

2.5. Soil Extracellular Hydrolase Activities

Upon completion of the incubation experiments, six extracellular hydrolase activities were measured using the methodology presented by [42]. These enzymes included carbon (C)-acquisition enzyme (β-glucosidase [BG], cellobiosidase [CBH], and xylosidase [XYL]), nitrogen (N)-acquisition enzyme (glucosaminidase [NAG] and aminopeptidase [LAP]), and phosphorus (P)-acquisition enzyme (phosphatase [PHOS]). All enzyme activities were determined using a 96-well microplate (Thermo Fisher Scientific, Waltham, MA, USA). Text S2 provides a description of specifics.

2.6. Extraction and Analysis of Phospholipid Fatty Acids

Soil phospholipid fatty acids (PLFA) were extracted, isolated, and purified to evaluate the microbial community composition, based on the methods presented by Yuan et al. [43]. Text S3 provides a description of measured specifics. The PLFAs served as biomarkers for identifying bacteria (B) and fungi (F): Firmicutes (i14:0, i15:0, i16:0, i17:0, i18, a15:0, a16:0,

a17:0, a18:0, and a19:0) and Actinomycetes (10Me16:0, 10Me17:0, and 10Me18:0) together were employed as indicators of Gram-positive bacteria (G^+). Gram-negative bacteria (G^-) were identified by cy17:0, cy19:0, 16:1 ω 7, 16:1 ω 9, 17:1 ω 8, and 18:1 ω 7. Arbuscular mycorrhizal fungi (AMF) were represented by 16:1 ω 5c, saprophytic fungi by 18:1 ω 9c, and Ascomycota and Basidiomycota by 18:2 ω 6c. Non-specific PLFAs included 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, and 20:4 ω 6,9,12,15. Bacterial biomarkers were determined by summing the G^+ and G^- PLFAs, whereas fungal biomarkers encompassed AMF, saprophytic fungi, and non-specific fungi (18:3 ω 6,9,12), as described by Joergensen [44]. PLFA content functioned as a proxy for determining soil microbial abundance, while the F/B ratio and the G^+/G^- ratio served as indicators of microbial community structure [21].

2.7. Calculations

- (1) The $\delta^{13}C$ (‰) was converted to ^{13}C atom-% according to Wu et al. [5]:

$$\text{atom}\%^{13}CO_2 = \frac{100 \times 0.0111802 \times \left(\frac{\delta^{13}CO_2}{1000} + 1 \right)}{1 + 0.0111802 \times \left(\frac{\delta^{13}CO_2}{1000} + 1 \right)} \quad (1)$$

- (2) The CO_2 from various carbon sources ($CO_{2 \text{ straw}}$, $CO_{2 \text{ SOC}}$) were determined by the following equations [5]:

$$CO_{2 \text{ straw}} = CO_{2 \text{ total}} \times \frac{\text{atom}\%^{13}CO_{2 \text{ total}} - \text{atom}\%^{13}CO_{2 \text{ soil}}}{\text{atom}\%^{13}C_{\text{straw}} - \text{atom}\%^{13}CO_{2 \text{ soil}}} \quad (2)$$

$$CO_{2 \text{ SOC}} = CO_{2 \text{ total}} - CO_{2 \text{ straw}} \quad (3)$$

where $CO_{2 \text{ total}}$, $CO_{2 \text{ straw}}$, and $CO_{2 \text{ SOC}}$ refer to the amount of total CO_2 , CO_2 derived from straw, and SOC-derived CO_2 in the straw-amended soil, respectively. $\text{Atom}\%^{13}CO_{2 \text{ total}}$ and $\text{atom}\%^{13}CO_{2 \text{ soil}}$ indicate the ^{13}C atom-% values of the values of CO_2 from the soil amended with straw and the unamended control soil; and $\text{atom}\%^{13}C_{\text{straw}}$ represents the ^{13}C atom-% values of the labeled maize straw. The cumulative amount of CO_2 derived from straw and SOC was obtained by summing the values of straw-derived CO_2 and SOC-derived CO_2 emissions at each measurement interval.

- (3) The PE was computed based on the following equation:

$$PE = CO_{2 \text{ SOC}} - CO_{2 \text{ soil}} \quad (4)$$

where $CO_{2 \text{ soil}}$ denotes the total CO_2 from the straw-unamended soil.

- (4) Straw-derived SOC (SOC_{straw}) was calculated according to Equation (5):

$$SOC_{\text{straw}} = SOC_{\text{total}} \times \frac{\text{atom}\%^{13}C_{\text{total}} - \text{atom}\%^{13}C_{\text{soil}}}{\text{atom}\%^{13}C_{\text{straw}} - \text{atom}\%^{13}C_{\text{soil}}} \quad (5)$$

where SOC_{straw} and SOC_{total} denote the amount of straw-derived SOC and total SOC from straw-amended soil, respectively. $\text{Atom}\%^{13}C_{\text{total}}$ and $\text{atom}\%^{13}C_{\text{soil}}$ represent the ^{13}C atom-% values of the SOC in the soil with added straw and the matching soil without straw addition, respectively; and $\text{atom}\%^{13}C_{\text{straw}}$ refers to the ^{13}C atom-% values of the labeled maize straw.

- (5) The net SOC balance was defined by subtracting the SOC loss (PE) from the remaining straw-derived SOC (SOC_{straw}) after straw amendment [6]:

$$\text{Net C balance} = SOC_{\text{straw}} - PE \quad (6)$$

- (6) The $\delta^{13}\text{C}$ value of individual PLFAs was corrected by following equations [45,46]:

$$\delta^{13}\text{C}_{\text{PLFA}} = \frac{(N + 1)\delta^{13}\text{C}_{\text{FAME}} - \delta^{13}\text{C}_{\text{MeOH}}}{N} \quad (7)$$

$$\text{atom}\%^{13}\text{C}_{\text{PLFA}} = \frac{100 \times 0.0111802 \times \left(\frac{\delta^{13}\text{C}_{\text{PLFA}}}{1000} + 1 \right)}{1 + 0.0111802 \times \left(\frac{\delta^{13}\text{C}_{\text{PLFA}}}{1000} + 1 \right)} \quad (8)$$

where N denotes the number of carbon atoms, $\delta^{13}\text{C}_{\text{PLFA}}$ refers to the $\delta^{13}\text{C}$ value of the specific PLFA; and $\delta^{13}\text{C}_{\text{MeOH}}$ and $\delta^{13}\text{C}_{\text{FAME}}$ refer to the $\delta^{13}\text{C}$ values of the methanol derivatizing agent (methanol) and individual FAME, respectively. The $\delta^{13}\text{C}$ abundance of methanol is 41.78‰.

Microbial PLFAs incorporating ^{13}C was determined using Equation (9) [47]:

$$\text{PLFA}_{\text{straw}} = \text{PLFA}_{\text{total}} \times \frac{(\text{atom}\%^{13}\text{C}_{\text{PLFA-total}} - \text{atom}\%^{13}\text{C}_{\text{PLFA-control}})}{(\text{atom}\%^{13}\text{C}_{\text{straw}} - \text{atom}\%^{13}\text{C}_{\text{PLFA-control}})} \quad (9)$$

where $\text{atom}\%^{13}\text{C}_{\text{PLFA-total}}$ and $\text{atom}\%^{13}\text{C}_{\text{PLFA-control}}$ are the ^{13}C atom-% values of PLFA in the soil with added straw and the matching soil without straw addition, respectively; and $\text{atom}\%^{13}\text{C}_{\text{straw}}$ refers to the ^{13}C atom-% values of the labeled maize straw.

- (7) Carbon use efficiency (CUE) provides an indicator of quantifying the fraction of carbon assimilated by microorganisms. The straw-derived microbial biomass was estimated using ^{13}C -enriched PLFAs, equating to the biomass determined via the fumigation-extraction method with a conversion coefficient of 2.4 [48]. Thus, CUE was computed using Equation (10) [47]:

$$\text{CUE} = \frac{\text{PLFA}_{\text{straw}} \times 2.4}{\text{PLFA}_{\text{straw}} \times 2.4 + \text{CO}_2 \text{ straw}} \quad (10)$$

2.8. Statistical Analysis

Three-way ANOVA was conducted to examine the main and interactive effects of the soil type, maize straw addition and microbial inoculant application on cumulative total CO_2 emissions, soil chemical and microbial properties, soil available C:N:P stoichiometry, and extracellular hydrolase activities. For variables measured only in the presence of maize straw, two-way ANOVA was conducted to evaluate the main and interactive effects of the soil type and microbial inoculant application on cumulative CO_2 emissions derived from SOM and straw, the cumulative PE, straw-derived SOC, net SOC balance, CUE and soil ^{13}C -PLFAs. The least significant difference test was applied to compare the means and determine whether the differences were significant at $p < 0.05$. In the two-way ANOVA, when both soil type and microbial inoculant application showed significant effects, η^2 was employed as a measure of each factor's contribution on the total effect size.

$$\eta^2 = \text{SS}_A / \text{SS}_{\text{total}}$$

where SS_A denotes the variance between treatments due to factor A, and SS_{total} is the total sum of squares. All statistical analyses were performed using IBM SPSS version 19.0. All Bar charts were produced with Origin 2021.

To identify factors contributing to variation in the cumulative PE, straw-derived SOC, and net SOC balance, random forest regression analysis was performed to assess the relative importance of predictor variables, including CUE, soil available and microbial C:N:P stoichiometries, extracellular hydrolase activities, ^{13}C -PLFA, the ratios $^{13}\text{C}\text{-F}/^{13}\text{C}\text{-B}$, and $^{13}\text{C}\text{-G}^+ / ^{13}\text{C}\text{-G}^-$ (using the *RandomForest* package, version 4.7.1.2). Model significance

was tested using the *rfUtilities* package (version 2.1.5). Linear regression was used to explore the relationship between the cumulative PE, straw-derived SOC, net SOC balance and the most important predictors identified by the random forest model. Pearson correlation analysis (using the *psych* package, version 2.4.12) was employed to evaluate relationships involving the cumulative PE, straw-derived SOC, net SOC balance and all other observed variables. Partial correlation analysis (using the *ppcor* package, version 1.1) was then conducted to further assess the potential interconnections and correlations among variables influencing the cumulative PE. This analysis determined whether the significant effects of other variables on the cumulative PE were independent.

3. Results

3.1. Dynamics of CO₂, Straw-Derived CO₂, and Soil-Derived CO₂ Emission

The maximum emission rates of total CO₂ and straw-derived CO₂ were observed on day 1 in clay loam soil, whereas in sandy soil, the peak occurred on day 2 (Figure 1a,c). The emission rates of total CO₂, straw- and SOM-derived CO₂ declined progressively throughout the incubation period (Figure 1a,c,e).

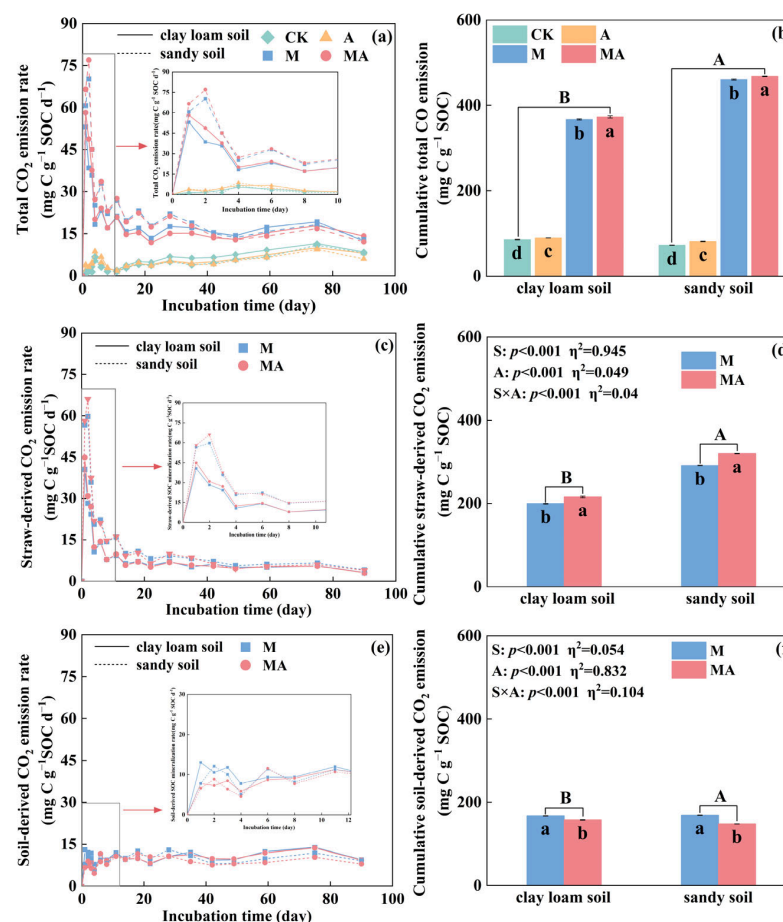


Figure 1. Trends of the emission rates of total, straw-derived and SOM-derived CO₂ (a,c,e), and cumulative total CO₂, straw-derived and SOM-derived CO₂ emission (b,d,f) in two soils. Error bars on the bars denote the standard errors of the mean values ($n = 3$). Within the same group, different lowercase letters signify significant variations among treatments in the same soil. Different uppercase letters denote significant variations in the examined variable between soil types. $p < 0.05$. CK: only soil, A: microbial inoculant application, M: straw addition, MA: straw plus microbial inoculant application. Two-way ANOVA, S: soil type, A: microbial inoculant application. η^2 , each factor's contribution on the total effect size. Results of three-way ANOVA for cumulative total CO₂ emission are provided in Table S1.

Over the 90-day incubation period, straw addition alone or in combination with microbial inoculant increased cumulative total CO₂ emissions by 278.36–284.32 mg C g^{−1} SOC in clay loam soil and by 383.53–390.93 mg C g^{−1} SOC in sandy soil compared with CK (Figure 1b). The sole application of the microbial inoculant also increased cumulative total CO₂ emissions relative to CK. Notably, compared with straw addition alone, combining with microbial inoculant significantly enhanced cumulative straw-derived CO₂ emissions in both soils, while reducing cumulative SOM-derived CO₂ emissions (Figure 1d,f). In general, cumulative straw-derived and SOM-derived CO₂ emissions were 47.34% and 2.65% higher in sandy soil than in clay loam soil (Figure 1d,f).

3.2. Priming Effect on SOC Mineralization

Straw addition induced a lower cumulative positive PE in clay loam soil than in sandy soil, irrespective of microbial inoculant application (Table 2). However, microbial inoculant reduced the cumulative positive PE by 8.98% in clay loam soil and by 43.98% in sandy soil compared with straw addition alone (Table 2).

Table 2. Straw-derived C, soil cumulative PE, net SOC balance and CUE in two soils in reaction to straw addition and microbial inoculant application.

Treatments		Straw-Derived SOC (mg C g ^{−1} SOC)	Cumulative PE (mg C g ^{−1} SOC)	Net SOC Balance (mg C g ^{−1} SOC)	CUE
Clay loam soil	M	111.77 ± 0.34 Aa	77.66 ± 0.57 Ba	34.11 ± 0.52 Ab	0.12 ± 0.00 Ab
	MA	112.31 ± 1.25 Aa	70.69 ± 1.29 Bb	41.62 ± 1.24 Aa	0.13 ± 0.00 Aa
Sandy soil	M	100.65 ± 1.62 Ba	96.66 ± 0.48 Aa	3.99 ± 0.69 Bb	0.04 ± 0.00 Bb
	MA	104.22 ± 4.80 Ba	74.60 ± 0.23 Ab	29.62 ± 1.35 Ba	0.05 ± 00 Ba
Two-way ANOVA					
Soil type (S)		***	***	***	***
Microbial inoculant (A)		ns	***	***	***
S × A		ns	***	***	**

Note: Values are the mean ± standard error ($n = 3$). Two-way ANOVA was used to assess the differences among these variables (ns, not significant; ** and *** represent $p < 0.01$ and $p < 0.001$, respectively). Within the same group, different lowercase letters signify significant variations among treatments in the same soil. Different uppercase letters denote significant variations in the examined variable between soil types. A: microbial inoculant application, M: straw addition, MA: straw plus microbial inoculant application, CUE: Carbon use efficiency.

3.3. Net SOC Balance

Utilizing microbial inoculant did not affect straw-derived SOC (Table 2). Clay loam soil contained 23% more straw-derived SOC than the sandy soil (Table 2). Adding straw, with or without microbial inoculant, generated a positive net SOC balance (net SOC gain) in both soils (Table 2). This is because straw-derived SOC exceeds native SOC losses from the PE. Microbial inoculation further enhanced the net SOC gain relative to straw alone, with a particularly pronounced effect in sandy soil, leading to a 6-fold net SOC gain (Table 2). Correspondingly, straw combined with microbial inoculant significantly enhanced SOC content by 0.17 g kg^{−1} in clay loam soil and by 0.41 g kg^{−1} in sandy soil, compared with straw addition alone (Table 3). However, applying microbial inoculant alone failed to alter SOC in two soils (Table 3). Furthermore, the results of CUE indicated that the utilization of straw carbon by microorganisms was less than 0.15, while microbial inoculant significantly increased CUE. The CUE in sandy soil was substantially lower than in clay loam soil (Table 2).

Table 3. Soil organic carbon, available nutrient and soil microbial biomass in two soils in reaction to straw addition and microbial inoculant application.

Treatment		SOC (g kg ⁻¹)	DOC (mg kg ⁻¹)	Min-N (mg kg ⁻¹)	AP (mg kg ⁻¹)	MBC (mg kg ⁻¹)	MBN (mg kg ⁻¹)	MBP (mg kg ⁻¹)
Clay loam soil	CK	9.25 ± 0.01 Ac	77.96 ± 0.64 Bc	100.27 ± 0.21 Bd	29.20 ± 0.18 Bc	155.00 ± 2.34 Ad	8.02 ± 0.01Ac	6.70 ± 0.01 Ac
	A	9.29 ± 0.03 Ac	81.49 ± 3.44 Bc	186.13 ± 0.62 Bb	39.19 ± 0.42 Bb	161.49 ± 2.19 Ac	7.97 ± 0.06Ac	6.74 ± 0.03 Ac
	M	10.88 ± 0.01Ab	139.61 ± 1.79 Bb	164.37 ± 0.33 Bc	26.78 ± 0.04 Bc	217.63 ± 2.19 Ab	14.01 ± 0.25 Ab	8.29 ± 0.01 Ab
	MA	11.04 ± 0.01Aa	165.9 ± 8.01 Ba	206.23 ± 0.60 Ba	41.81 ± 0.45 Ba	239.78 ± 2.39 Aa	14.96 ± 0.01 Aa	9.22 ± 0.01 Aa
Sandy soil	CK	6.26 ± 0.03 Bc	331.99 ± 0.15 Ab	386.79 ± 0.54 Ad	36.53 ± 0.59 Ac	6.09 ± 0.26 Bd	1.58 ± 0.02 Bb	3.24 ± 0.02 Bc
	A	6.30 ± 0.10 Bc	329.07 ± 0.08 Ab	413.74 ± 0.17 Ac	53.81 ± 0.07 Aa	20.25 ± 0.42 Bc	1.60 ± 0.00 Bb	3.24 ± 0.03Bc
	M	7.42 ± 0.02 Bb	428.68 ± 1.03 Aa	422.47 ± 0.22 Ab	37.87 ± 0.29 Ac	53.56 ± 2.25 Bb	5.13 ± 0.02 Ba	3.57 ± 0.01 Bb
	MA	7.82 ± 0.06 Ba	434.93 ± 0.25 Aa	456.47 ± 0.10 Aa	53.06 ± 0.12 Ab	64.55 ± 0.58 Ba	5.32 ± 0.22 Ba	4.95 ± 0.05 Ba
Three-way ANOVA								
Soil type (S)		***	***	***	***	***	***	***
Maize Straw (M)		***	**	***	ns	***	***	***
Microbial inoculant (A)		***	**	***	***	***	**	***
S × M		***	***	***	ns	***	***	***
S × A		ns	**	***	***	ns	***	***
M × A		***	**	***	**	*	*	***
S × M × A		ns	ns	***	***	**	**	***

Note: Values are the mean ± standard error ($n = 3$). Three-way ANOVA was used to assess the differences among these variables (ns, not significant; *, ** and *** represent $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively). Within the same group, different lowercase letters signify significant variations among treatments in the same soil. Different uppercase letters denote significant variations in the examined variable between soil types. CK: only soil, A: microbial inoculant application, M: straw addition, MA: straw plus microbial inoculant application. Abbreviations: SOC, soil organic carbon, DOC, dissolved organic carbon, Min-N, NH_4^+ -N and NO_3^- -N, AP, available P, MBC, microbial biomass C, MBN, microbial biomass N, MBP, microbial biomass P.

3.4. Soil Properties

Compared with straw addition alone, combined microbial inoculant significantly increased the DOC, Min-N and AP content in both soils (Table 3). Overall, soil nutrients levels and the stoichiometric ratios of DOC, Min-N, and AP were significantly lower in clay loam soil than in sandy soil (Table 3, Figure 2a–c). Microbial inoculant application reduced the DOC/Min-N, DOC/AP and Min-N/AP ratios in the presence of straw in both soils (Figure 2a–c).

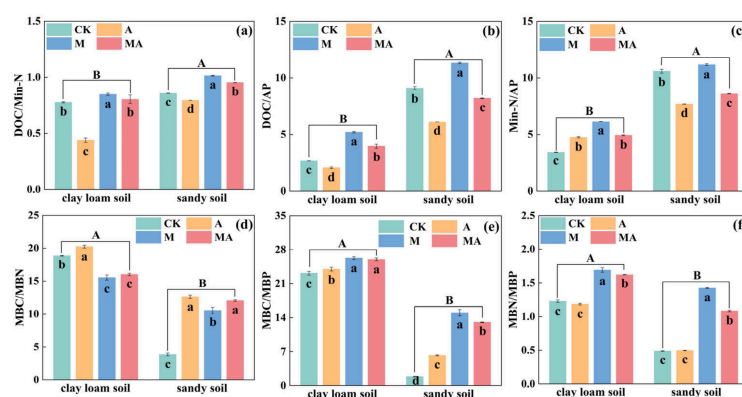


Figure 2. Soil available nutrient ratios (a–c), and soil microbial biomass ratios (d–f) in two soils. Error bars denote the standard errors of the mean values ($n = 3$). Within the same group, different lowercase letters signify significant variations among treatments in the same soil. Different uppercase letters denote significant variations in the examined variable between soil types. $p < 0.05$. CK: only soil, A: microbial inoculant application, M: straw addition, MA: straw plus microbial inoculant application. Results of three-way ANOVA for soil available nutrient ratios and soil microbial biomass ratios are provided in Table S1.

Compared to clay loam soil, sandy soil had lower microbial biomass (MBC, MBN, MBP) (Table 3). Straw combined with microbial inoculant significantly increased MBC, MBN, and MBP in both soils (Table 3), while it had minimal impact on the stoichiometric ratios of MBC/MBN and MBC/MBP in clay loam soil (Figure 2d,e). In sandy soil, microbial inoculant application increased the MBC/MBN ratio (Figure 2d) and reduced the MBC/MBP and MBN/MBP ratios (Figure 2e,f).

3.5. Soil Enzyme Activities

Overall, higher enzyme activities were observed in clay loam soil (Figure 3). Compared with straw addition, combining with microbial inoculant significantly enhanced the activities of C-acquisition enzymes in both soils (Figure 3a), whereas it inhibited N-acquisition enzyme activities in both soils and P-acquisition enzyme activities in sandy soil (Figure 3b,c).

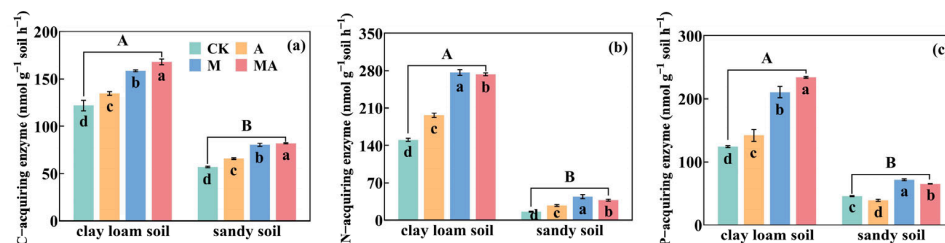


Figure 3. Activities of carbon-acquiring enzymes (a), nitrogen-acquiring enzymes (b), and phosphorus-acquiring enzymes (c) in two soils. Within the same group, different lowercase letters signify significant variations among treatments in the same soil. Different uppercase letters denote significant variations in the examined variable between soil types. $p < 0.05$. CK: only soil, A: microbial inoculant application, M: straw addition, MA: straw plus microbial inoculant application. Carbon-acquiring enzymes is defined as the total of BG, CBH and XYL. Nitrogen-acquiring enzymes is defined as the total of NAG and LAP. Results of three-way ANOVA for enzyme activities are provided in Table S2.

3.6. Incorporation of ¹³C into PLFAs and Composition of Soil Microbial Community

Microbial inoculant increased the incorporation of straw-derived C into PLFAs by 14.25% in clay loam soil and by 25.38% in sandy soil (Table 4). Sandy soil exhibited markedly lower values compared to the clay loam soil (Table 4). In clay loam soil, ¹³C was primarily utilized by G⁺ and G[−] bacteria, whereas in sandy soil, it was predominantly utilized by G[−] bacteria (Table 4). Relative to straw addition alone, the synergistic addition of straw and microbial inoculant decreased the ¹³C-G⁺ to ¹³C-G[−] ratio in sandy soil from 3.2 to 1.9 (Table 4). A significant enrichment of *Firmicutes*, *Ascomycota* and *Basidiomycota* were observed in both soils following the introduction of microbial inoculant (Figure S3). Notably, in scenarios with or without straw addition, microbial inoculant application increased the total bacterial and fungal PLFAs in both soils (Figure S3).

Table 4. Incorporation of ¹³C into phospholipid fatty acids (PLFAs), ¹³C-fungi/¹³C-bacteria and ¹³C-G⁺/¹³C-G[−] in reaction to straw addition and microbial inoculant application.

Treatments		¹³ C-Bacterial-PLFAs (nmol g ^{−1} Soil)	¹³ C-G ⁺ (nmol g ^{−1} Soil)	¹³ C-G [−] (nmol g ^{−1} Soil)	¹³ C-Fungal-PLFAs (nmol g ^{−1} Soil)	¹³ C-Total PLFAs (nmol g ^{−1} Soil)	¹³ C-F/ ¹³ C-B	¹³ C-G ⁺ / ¹³ C-G [−]
Clay loam soil	M	31.47 ± 0.03 Ab	22.21 ± 0.64 Ab	8.60 ± 0.08 Ab	10.30 ± 0.10 Ab	41.77 ± 0.11 Ab	0.33 ± 0.00	2.68 ± 0.03 Ba
	MA	36.73 ± 0.27 Aa	26.52 ± 1.17 Aa	10.21 ± 0.21 Aa	10.98 ± 0.20 Aa	45.81 ± 0.38Aa	0.31 ± 0.00	2.60 ± 0.00 Bab
Sandy soil	M	14.78 ± 1.76 Bb	11.35 ± 1.38 Ba	3.43 ± 0.39 Bb	4.92 ± 0.06 Bb	19.70 ± 1.80Bb	0.34 ± 0.00	3.23 ± 0.02 Aa
	MA	19.38 ± 0.23 Ba	12.31 ± 0.54 Ba	5.40 ± 0.24 Ba	6.45 ± 0.05 Ba	25.83 ± 0.28Ba	0.33 ± 0.00	1.92 ± 0.11 Ab
Two-way ANOVA								
Soil type (S)		***	***	***	***	***	ns	*
Microbial inoculant (A)		**	*	**	***	**	ns	***
S × A		ns	ns	*	**	ns	ns	***

Note: Values are the mean ± standard error ($n = 3$). Two-way ANOVA was used to assess the differences among these variables (ns, not significant; *, ** and *** represent $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively). Within the same group, different lowercase letters signify significant variations among treatments in the same soil. Different uppercase letters denote significant variations in the examined variable between soil types. M: straw addition, MA: straw plus microbial inoculant application. Abbreviations: ¹³C-G⁺: Incorporation of ¹³C into Gram-positive bacteria-PLFAs, ¹³C-G[−]: Incorporation of ¹³C into Gram-negative bacteria-PLFAs, ¹³C-F/¹³C-B: ¹³C-fungi/¹³C-bacteria.

3.7. Drivers of the Cumulative PE and Net SOC Balance

Random forest analysis identified that the $^{13}\text{C-G}^+ / ^{13}\text{C-G}^-$ ratio and CUE emerged as the most dominant factors regulating the cumulative PE, followed by DOC/Min-N and C-acquisition enzyme activities (Figure 4a). CUE and $^{13}\text{C-PLFA}$ were the primary predictors of straw-derived SOC, followed by DOC/Min-N and C-acquisition enzyme activities (Figure 4b). Moreover, the net SOC balance was primarily regulated by CUE and MBC/MBN (Figure 4c). The cumulative PE was positively correlated with the $^{13}\text{C-G}^+ / ^{13}\text{C-G}^-$ ratio, but negatively correlated with CUE (Figure 4d,e). By contrast, straw-derived SOC and net SOC balance were both positively correlated with CUE (Figure 4f,h). Additionally, straw-derived SOC showed a significant positive correlation with $^{13}\text{C-PLFA}$ (Figure 4g), and net SOC balance was positively correlated with MBC/MBN (Figure 4i).

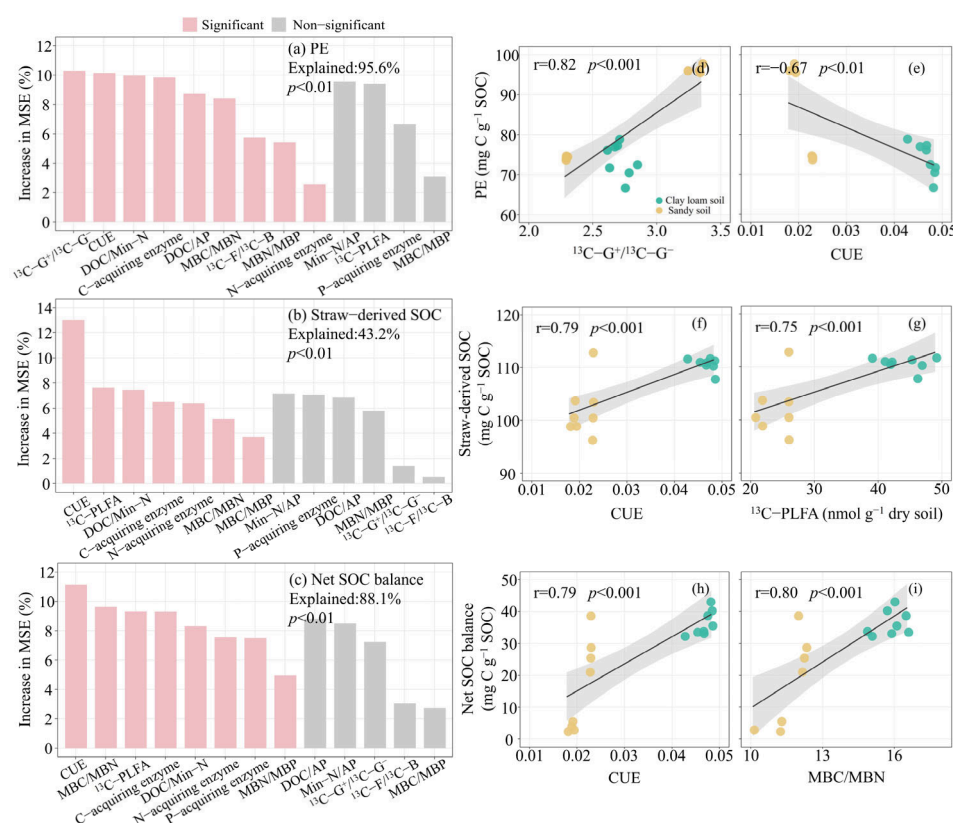


Figure 4. Random forest regression modeling indicated the relative significance of the examined variables in influencing PE (a), straw-derived SOC (b) and net SOC balance (c). Linear regressions between the PE (d,e), straw-derived SOC (f,g) and net SOC balance (h,i), and the most important factors.

Microbial inoculant affected the net SOC balance in both soils mainly by affecting the cumulative PE (Table 2), highlighting the need for studies on how microbial inoculant modulate the cumulative PE. Partial correlation analysis revealed that the effects of some variables on the cumulative PE were not entirely independent. The influence of $^{13}\text{C-G}^+ / ^{13}\text{C-G}^-$ on the cumulative PE remained a highly significant positive correlation, unaffected by other variables. In contrast, CUE and $^{13}\text{C-PLFA}$ showed no significant correlation with the cumulative PE after accounting for the influence of DOC/Min-N (Figure S4b).

4. Discussion

4.1. Soil PE in Response to Microbial Inoculant Application

Microbial inoculant alleviated PE compared to straw addition alone (Table 2). To our knowledge, this study first provides new evidence for how the combination of straw

and microbial inoculants influences SOC sequestration through the lens of soil PE. From a mechanistic perspective, the observed reduction in PE was associated with microbial nutrient acquisition strategies, which can be interpreted with the “microbial nutrient mining” hypothesis [10]. Specifically, microbial inoculant introduces functional microorganisms (e.g., yeast and *Bacillus subtilis*) that proliferate in soil (Figure S3). These microorganisms accelerated straw decomposition by increasing C-acquisition enzyme activities (BG and CBH) (Figures 3a and S2a,b) [27,49]. This process converted more straw-derived nitrogen and phosphorus into bioavailable forms [28,50]. Consequently, microbial inoculant elevated soil available nutrients content (Table 2), and reduced soil DOC/Min-N (from 0.85 to 0.80 in sandy soil; from 1.01 to 0.95 in clay loam soil; Figure 2a) and DOC/AP (from 11.32 to 8.20 in sandy soil; from 5.21 to 3.97 in clay loam soil; Figure 2b). The resulting adjustment of nutrient availability and the stoichiometric ratios of C, N and P alleviated soil nutrient imbalance, which subsequently reduced native SOM utilization and suppressed the cumulative PE (Table 2) [8,51]. The strong positive correlations between soil available C:N:P stoichiometry and the cumulative PE further support this mechanism (Figures 4a and S4a). Hence, our results suggest that microbial inoculants suppressed the cumulative PE by increasing soil nutrient availability, verifying our first hypothesis and aligning with theoretical predictions that soil nutrient availability governs microbial substrate prioritization [3,20,52]. Critically, the reduction in PE was further associated with a shift in microbial life-history strategy mediated by altered nutrient availability and the stoichiometric ratios of C, N and P [17]. This was evidenced by a decrease in the $^{13}\text{C-G}^+ / ^{13}\text{C-G}^-$ ratio following microbial inoculant application (Table 4), and by the fact that $^{13}\text{C-G}^+ / ^{13}\text{C-G}^-$ ratio served as the most important predictor of PE (Figures 4a and S4a). Upon microbial inoculant application, elevated soil nutrient availability increased the abundance of $^{13}\text{C-G}^-$ (*r*-strategists) (clay loam soil: +10%; sandy soil: +29%) (Table 4). Consequently, this promoted the preferential utilization of labile straw-derived C while reducing reliance on native SOM [53,54], which is consistent with the dominance of *r*-strategists in nutrient-rich environments [55]. Enhanced C-acquisition enzyme activities (clay loam soil: +38%; sandy soil: +52%; Figure 3a) further supported this shift, as *r*-strategists typically invest in hydrolytic enzyme for fresh labile C degradation [56]. Collectively, enhanced soil nutrient availability drove a functional community succession, supporting our third hypothesis, i.e., that microbial inoculants regulate PE intensity through shifts in *K/r*-strategy mediated by soil available C: N: P stoichiometry.

Notably, the decline in the cumulative PE was much lower in clay loam soil (8.98%) than in sandy soil (43.98%) following microbial inoculant application (Table 2). This difference primarily stems from distinct nutrient availability between soil types. Sandy soils are inherently low in available nutrients [32]. Limited resources are initially prioritized for allocation to stress-tolerant G^+ bacteria (*K*-strategists), thereby supporting their biomass maintenance and environmental adaptation, as reported in nutrient-poor soils [57]. However, microbial inoculant increased soil available nutrient content and triggered a rebalancing of soil C:N:P stoichiometry in sandy soils (Table 3, Figure 2). This likely alleviated microbial nutrient acquisition constraints, subsequently reducing N- and P-acquiring enzyme production and native SOC mineralization (Figure 3b,c). This drove a pronounced shift in microbial community toward *r*-strategists (G^- bacteria), as evidenced by a dramatic 29% increase in $^{13}\text{C-G}^-$ bacteria abundance and a sharp decline in the $^{13}\text{C-G}^+ / ^{13}\text{C-G}^-$ ratio from 3.2 to 1.9 (Table 4) [58]. Although the microbial inoculant contained abundant *B. subtilis* (G^+), sandy soils were selectively enriched in G^- -PLFAs and $^{13}\text{C-G}^-$ -PLFAs (Tables 4 and S3). These findings indicated that the combination of rapid nutrient cycling and a lack of stabilization mechanisms fosters an environment that temporarily favors *r*-strategists, leading to a pronounced suppression of PE in sandy soil. In clay loam soil, microbial inoculant also increased soil nutrient availability and reduced the positive PE

(Tables 2 and 3). However, compared with sandy soil, the lower available nutrients content in clay loam soil may constrain the rebalancing of available soil C: N: P stoichiometry, which in turn limits the restructuring of microbial life-history strategies [6,59]. Accordingly, the less pronounced decrease in the $^{13}\text{C}\text{-G}^+ / ^{13}\text{C}\text{-G}^-$ ratio resulted in a weaker suppression of the PE (Tables 2 and 4). Collectively, these findings indicated that soil texture regulates the intensity of the PE response to microbial inoculant by modulating the extent of nutrient rebalancing and microbial community strategy shift.

4.2. Fate of Straw Carbon in Response to Microbial Inoculant Application

Microbial inoculant significantly enhanced straw carbon mineralization in both soils (Figure 1d), indicating that it plays an essential role in labile carbon turnover. Our study aligns with the findings of Wang et al. [24], who observed that microbial inoculants substantially accelerated straw decomposition and carbon release in field experiments. However, our data show that most straw-derived carbon was mineralized and emitted as CO_2 (Figure 1d). This outcome likely resulted from the introduction of functional microorganisms capable of producing hydrolytic enzymes that degrade cellulose and lignin, thereby controlling the extent of straw mineralization [28,50]. Microbial inoculant significantly stimulated the preferential mineralization of labile straw-C as CO_2 (Figure 1d). This was attributed to a shift in microbial metabolism driven by improved soil nutrient situations, which led to the preferential utilization of more labile carbon sources [6]. Supporting evidence for this includes the elevated soil C-acquisition enzyme activities, higher MBC, and $^{13}\text{C}\text{-PLFA}$ levels in MA treatments compared to the M treatment (Figure 3a; Tables 2 and 4).

Straw addition promotes the microbial assimilation of straw carbon into new SOC by providing sufficient carbon along with other nutrients to fulfill microbial demands [21]. Nevertheless, in this study, microbial inoculant did not affect straw-derived SOC. Sandy soil exhibited 23% lower straw-derived SOC than clay loam soil (Table 2). This discrepancy may be due to the lower microbial biomass in nutrient-poor soils [34,60], as evidenced by the positive correlation between $^{13}\text{C}\text{-PLFAs}$ abundance and straw-derived SOC (Figures 4g and S4a). This positive correlation emphasizes the critical role of microbial turnover in accumulating microbial by-products (i.e., microbial necromass) [61,62]. As demonstrated by Duan et al. [63], microbial necromass accumulation in drylands is closely linked to microbial biomass utilizing exogenous carbon. The lower $^{13}\text{C}\text{-PLFA}$ levels in sandy soils indicate that a smaller proportion of straw carbon entered the microbial anabolic pathway. This likely contributed to the reduced straw-derived SOC by limiting the microbial biomass available for subsequent turnover and thereby reducing the formation of microbial necromass, an important precursor of newly formed SOC [4]. Taken together, microbial inoculant accelerated straw carbon turnover by increasing microbial abundance and C-acquisition enzyme activities in both soils. Consequently, this process enhanced straw mineralization compared with straw-only addition.

4.3. Microbial Inoculant Combined with Straw Increased Soil Net Carbon Gains

The net SOC balance is regulated by the trade-off between native SOC mineralization and newly formed SOC accumulation [6,20]. All treatments exhibited a net SOC gain because straw-derived SOC growth surpassed native SOC loss (Table 2). Our results showed that microbial inoculant increased net SOC balance not only by suppressing the intensity of the PE but also through elevated CUE, which was the primary predictor of net SOC gain (Figures 4c and S4a). Microbial inoculant improved soil nutrient availability (Table 3), thereby shifting microbial utilization of exogenous C away from the respiratory (CO_2 losses) toward biomass synthesis and consequently enhancing CUE (Table 2) [6,47]. This mechanism was further corroborated by the elevated $^{13}\text{C}\text{-PLFAs}$ (Figure 4c), reflect-

ing greater assimilation of straw-derived C into microbial biomass. Although microbial inoculant increased the abundance of *r*-strategists (G^- bacteria) (Table 4), which typically exhibit lower CUE, their rapid turnover accelerated the generation of microbial by-products (e.g., microbial necromass) [64,65]. These by-products serve as key precursors for stable SOC formation, particularly mineral-associated organic carbon (MAOC) [4,66], thereby ultimately enhancing the potential for SOC accumulation. Overall, microbial inoculant promoted SOC accumulation (Table 3), which is in agreement with Zhao et al. [27], who reported that microbial inoculants significantly stimulated the decomposition of organic materials and promoted SOC accumulation.

Furthermore, compared with straw-only treatments, microbial inoculant enhanced net SOC gains far more dramatically in sandy soil (by 619%) than in clay loam soil (Table 2), validating our second hypothesis that microbial inoculants hold greater potential to enhance SOC in coarse-textured soils. The enhancement primarily arose from the greater reduction in the PE (sandy soil vs. clay loam soil: 43.98% vs. 8.98%, Table 2) along with a significant increase in CUE (by 25.9% vs. 3.2%, Table 2). The facilitated assimilation of straw carbon was evidenced by the greater elevation in ^{13}C -PLFA abundance in sandy soil (25.4% vs. 14.3%) (Table 4). These findings demonstrated that functional microbial supplementation, rather than substrate quality alone, can directly optimize microbial metabolism efficiency. This approach provides a novel strategy for artificially regulating microbial metabolism in low-fertility soils. However, the inherently low clay content of sandy soils (0.75%, Table 1) limits the formation of organo-mineral complexes [32,67], thereby restricting the physical protection of microbial necromass and rendering it susceptible to degradation [67,68]. This limitation implies that the observed increase in SOC gain may be transient. Our results highlight the potential importance of integrating microbial metabolism management with physical protection mechanisms for sustainable SOC sequestration in sandy soils. For instance, applying clay minerals (e.g., montmorillonite) or biochar could stabilize microbial necromass through organo-mineral interactions [34,69], although their effectiveness under field conditions requires further investigation.

However, applying microbial inoculant alone increased cumulative CO_2 emissions in both soils compared to CK, yet this had no significant effect on SOC content (Figure 1b; Table 3). This finding implied that in the absence of external carbon inputs, microbes likely enhance extracellular enzyme secretion to degrade native SOM (Figure 3) [70]. This process may promote the formation of microbial by-products (e.g., necromass) that offset native SOC loss and maintain SOC stocks [49,71]. These observations emphasize the importance of co-applying crop straw with microbial inoculants to stimulate straw decomposition while mitigating native SOC mineralization, thereby optimizing SOC sequestration.

4.4. Implications and Limitations of Microbial Inoculants for SOC Accumulation

Collectively, our study revealed that in comparison to adding labile C (e.g., straw) alone, combined with microbial inoculant reduced native SOM mineralization and enhanced net SOC gain, with the extent of net SOC gains depending on soil type. This effect was closely associated with shifts in microbial life-history strategies, which were regulated by the optimization of available C:N:P stoichiometry induced by microbial inoculant (Figure 5). However, our study has certain limitations. We focused on how the co-application of microbial inoculant and crop straw influenced SOC sequestration under controlled conditions. In practice, field applications must account for complex environmental and biological factors. For instance, labile C inputs (e.g., root exudates) could disrupt microbial community dynamics [72], while environmental factors such as soil moisture fluctuations may compromise the survival and activity of microbial inoculants [73]. Despite these limitations, our findings provide mechanistic insights into the potential of

co-applying organic materials with microbial inoculants to enhance SOC sequestration. These results suggest that combining organic materials with microbial inoculants may serve as a promising strategy for improving SOC sequestration in sandy agroecosystems, although field-scale validation is required before any practical recommendations can be made. Notably, future work should focus on evaluating the efficacy of microbial inoculants under realistic field conditions, and on identifying site-specific conditions and complementary management practices that optimize the survival and functionality of the microbial inoculants across diverse agricultural ecosystems.

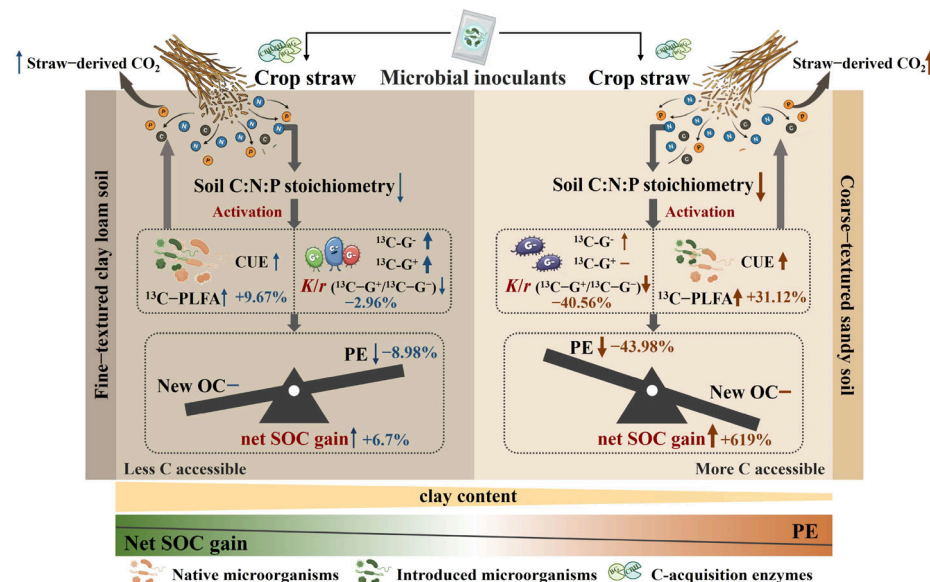


Figure 5. Conceptual framework illustrating how crop straw combined with microbial inoculant application affects microbial mechanisms controlling the priming effect and net carbon gain in two soils. The blue and brown arrows respectively represent the effects of straw combined with microbial inoculant on the measured variables in clay loam soil and sandy soil, compared to the addition of straw alone. The thickness of the arrows approximates the extent to which the application of straw along with microbial inoculant influenced the measured variables in the two soils. Arrow direction illustrates the impact direction of microbial inoculant application on the measured variable (upward denotes a positive effect, while downward signifies a negative effect), with the horizontal lines indicating no effect.

5. Conclusions

Our study demonstrated that microbial inoculant enhanced straw carbon mineralization in both fine-textured clay loam and coarse-textured sandy soils. Importantly, the intensity of the PE was reduced following microbial inoculant application, with a more pronounced reduction in the sandy soil than in the clay loam soil. This implies that nutrient-poor and coarse-textured soils are highly responsive to such microbial amendments. Microbial inoculant further increased net SOC gains in both soils. Notably, the net SOC gains in sandy soil were approximately six-fold higher than that in clay loam soil. This large enhancement is attributed primarily to the slow mineralization of native SOM and the increase in microbial CUE in sandy soils. Nevertheless, straw-derived SOC was consistently lower in sandy soil than in clay loam soil, regardless of microbial inoculant addition. Observations from our experiments highlight that soil microorganisms adjusted their metabolism and community composition in reaction to soil available C:N:P stoichiometry under microbial inoculant application, which dampened the intensity of the PE and promoted SOC accumulation. In summary, our findings provide mechanistic insights into the co-application of microbial inoculants and organic materials for enhanc-

ing SOC sequestration. For coarse-textured sandy soils, applying organic materials (e.g., straw) in combination with microbial inoculants is strongly recommended to optimize the utilization of organic materials and achieve substantial increases in SOC. To improve long-term SOC stabilization, it is advisable to combine this practice with supplementary strategies (e.g., clay amendments) to enhance SOC protection. In fine-textured clay loam soils, applying microbial inoculants can still serve as an effective approach to enhance soil fertility. Furthermore, when co-applying organic materials and microbial inoculants, it is essential to ensure an adequate supply of nutrients, particularly nitrogen and phosphorus, to enable the microbial inoculant to function properly and promote SOC sequestration. These practical recommendations are directly derived from our experimental findings, but field validation is necessary before large-scale adoption. Future studies should focus on evaluating the compatibility of microbial inoculants with different types of organic materials and examining its long-term agronomic outcomes. Upon validation, the combined use of microbial inoculants and organic materials could become a practical and sustainable management strategy for sandy agroecosystems.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy16141350/s1>, Text S1: ^{13}C -labeled maize straw. Text S2: Measurements of soil organic carbon functional pools. Text S3: Measurements of soil phospholipid fatty acids. Table S1: The effects of soil type, maize straw addition, microbial inoculant application and their interactions on the cumulative total CO_2 emission, $\text{DOC}/\text{Min-N}$, DOC/AP , $\text{Min-N}/\text{AP}$, MBC/MBN , MBC/MBP and MBN/MBP using three-way ANOVA. Table S2: The effects of soil type, maize straw addition, microbial inoculant application and their interactions on enzyme activities using three-way ANOVA. Table S3: Total phospholipid fatty acids (PLFAs) and composition of soil microbial community in reaction to straw addition and microbial inoculant application. Table S4: The effects of soil type, maize straw addition, microbial inoculant application and their interactions on each microorganism using three-way ANOVA. Table S5: The effects of soil type microbial inoculant application and their interactions on each incorporation of ^{13}C into microorganism using two-way ANOVA. Figure S1: Trends of cumulative CO_2 emission from total CO_2 (a), CO_2 derived from straw (b) and soil (c), and the priming effect (d) in two soils. Error bars on the bars denote the standard errors of the mean values ($n = 3$). $p < 0.05$. CK: only soil, A: microbial inoculant application, M: straw addition, MA: straw plus microbial inoculant application. Figure S2: Soil extracellular hydrolase activities in two soils. Error bars on the bars denote the standard errors of the mean values ($n = 3$). Significant variation among treatments in the same soil is represented by different lowercase letters within each group. Significant divergence in the examined variable between soil types is denoted by varying uppercase letters. $p < 0.05$. CK: only soil, A: microbial inoculant application, M: straw addition, MA: straw plus microbial inoculant application. Figure S3: The ^{13}C enrichment and total distribution of each microorganism in different treatments of clay loam soil and sandy soil, as well as significant differences in individual composition among diverse treatments. Significant variation among treatments in the same soil is represented by different lowercase letters within each group. Significant divergence in the examined variable between soil types is denoted by varying uppercase letters. CK: only soil, A: microbial inoculant application, M: straw addition, MA: straw plus microbial inoculant application. Figure S4: Results of correlation analysis (a) and partial correlation analysis (b). The circle size indicates the Pearson coefficient; blue refers to a negative correlation, red refers to a positive correlation. Asterisks represent the level of significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

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