Decomposition Process and CO2 Emission Characteristics of Fungal Residue in Paddy Soil under Indoor Constant Temperature Conditions: Postprint

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Abstract

Mushroom residue is the byproduct after cultivating edible fungi and can be reused as organic fertilizer. This study investigated the changes in organic carbon and total nitrogen under different treatments, explored the decomposition process of mushroom residue in paddy soil, and analyzed CO_2 release characteristics by incubating mixtures of mushroom residue and paddy soil at different ratios under laboratory conditions [no mushroom residue applied (TS), soil to mushroom residue mass ratios of 10:1 (SM1), 5:1 (SM2), and 2:1 (SM3), all mushroom residue (TM)], to provide a reference for the rational utilization of mushroom residue. The results showed that at the same incubation time, the organic carbon and nitrogen contents in treatments with different proportions of added mushroom residue were all higher than those in the TS treatment, with the TM treatment showing increases of 10.7-fold and 11.0-fold in organic carbon and total nitrogen, respectively, compared to the TS treatment. The increase in organic carbon and nitrogen content mainly depended on the amount of mushroom residue added. Overall, with the extension of incubation time, organic carbon and nitrogen in all treatments showed a downward trend due to carbon and nitrogen decomposition; after 35 days, the organic carbon and nitrogen in the TM treatment decreased more rapidly. The more mushroom residue added, the greater the organic carbon residual rate. After 63 days of incubation, the relationship equations between the decomposition residual rates of mushroom residue organic carbon (YC) and nitrogen (YN) and the amount of mushroom residue added (X) were: YC=71.26X 0.607 5, r^2=1.000 0** and YN=74.039X 0.413 3,r^2=0.999 9**. The CO_2 release rates from soil in all treatments showed a trend of first increasing, then decreasing, and then stabilizing. The higher the amount of mushroom residue, the higher the CO_2 release rate;

the CO_2 release rates of all treatments at different incubation times followed the order TM>SM3>SM2>SM1>TS. The CO_2 release rates of all treatments peaked on day 7, gradually entered a stable declining state by day 14, and after 35 days of incubation, the soil organic carbon mineralization intensity in all treatments was very low, with most organic carbon being sequestered in the soil, and the TM treatment showing the lowest organic carbon mineralization intensity. In summary, the more mushroom residue returned to the field, the more carbon was sequestered in the soil.

Full Text

Decomposition Process and CO_2 Release Characteristics of Spent Mushroom Substrate in Paddy Soils Under Constant Temperature Incubation

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Abstract

Spent mushroom substrate (SMS), the leftover material from mushroom cultivation, can be reused as organic fertilizer. This study investigated changes in organic carbon and total nitrogen content in mixtures of paddy soil and SMS at different ratios under laboratory incubation conditions. Five treatments were established: no SMS application (TS), soil to SMS mass ratios of 10:1 (SM1), 5:1 (SM2), and 2:1 (SM3), and pure SMS (TM). The decomposition process of SMS in paddy soil and associated CO_2 release characteristics were examined to provide a reference for rational SMS utilization. Results showed that at the same incubation time, organic carbon and nitrogen contents in all SMS-amended treatments were significantly higher than in the TS treatment. The TM treatment increased organic carbon and total nitrogen by 10.7-fold and 11.0-fold, respectively, compared to TS. The magnitude of increase in carbon and nitrogen content depended primarily on the SMS application rate. Overall, organic carbon and nitrogen decreased over time due to decomposition in all treatments, with a more rapid decline in the TM treatment after 35 days. Higher SMS application rates resulted in greater organic carbon residue rates. After 63 days of incubation, the relationships between residue rates of organic carbon (YC) and nitrogen (YN) with SMS application amount (X) were: $\mathrm{YC} = 71.26\mathrm{X}^{-0}.^{6075}$, $r^2 = 1.0000$ and $\mathrm{YN} = 74.039\mathrm{X}^{-0}.^{4133}$, $r^2 = 0.9999$, respectively. All treatments showed an initial increase in CO_2 release rate followed by a decrease and eventual stabilization. Higher SMS application rates produced higher CO_2 release rates, with the consistent ranking of TM > SM3 > SM2 > SM1 > TS across all sampling times. Peak CO_2 release occurred on day 7, followed by a gradual decline after day 14. After 35 days, soil organic carbon mineralization intensity was minimal in all treatments, with most organic carbon being sequestered in the soil, particularly in the TM treatment which showed the lowest mineralization intensity. In conclusion, higher SMS application rates resulted in greater carbon sequestration in soil.

Keywords: Spent mushroom substrate (SMS); Paddy soil; Organic carbon; Total nitrogen; Decomposition process; CO_2 release

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Introduction

China is the world's leading producer of edible mushrooms, generating enormous quantities of spent mushroom substrate (SMS) annually [1]. SMS, the residual material after mushroom cultivation, is rich in cellulose, lignin, vitamins, antibiotics, mineral elements, and other biologically active substances [2,4], making it suitable for reuse as organic fertilizer or soil amendment. It can also be used for plant hormone extraction, animal feed, energy production [3,5-6], crop seedling cultivation, and growth substrates [7-9].

Previous research on SMS application has focused primarily on changes in plant physiological indicators. Studies have shown that SMS application can increase leaf length, leaf number, leaf area, and plant height in pineapple [Ananas comosus (Linn.) Merr.] [6]. Agaricus blazei Murr. and Lentinus edodes SMS can promote lettuce (Lactuca sativa L.) growth and soil remediation [10], with 40% SMS application rate being optimal for melon (Cucumis melo L.) seedling growth [11]. SMS application also enhances soil microbial biomass carbon and glucose content [12-13], increases microbial diversity and enzyme activity [11], and can alter soil aggregate distribution [14]. However, few studies have examined the decomposition characteristics of SMS itself.

Soil CO_2 release constitutes an important component of ecosystem carbon budgets [15-16]. Organic material application promotes soil CO_2 emissions [17-21], which increase with soil organic carbon content [22-24]. After SMS and other organic materials are returned to fields, one portion becomes a source of soil organic carbon and is sequestered, while another portion of this fixed carbon is subsequently released as CO_2 through microbial turnover [25]. Medina et al. [26] found that SMS application increased soil respiration rates and phosphatase activity. However, other studies reported that SMS application did not significantly increase soil CO_2 release [13,16], possibly because soil respiration is constrained by soil type, moisture, temperature, and other factors [27], necessitating further investigation.

Soil organic matter is a crucial indicator for balanced fertilization, and its formation depends not only on the quantity of organic fertilizer input but also on its decomposition residue rate [28]. Understanding SMS decomposition patterns is theoretically and practically significant for scientifically replenishing and renewing soil organic matter and developing rational SMS fertilization strategies. Although organic fertilizer application significantly increases CO_2 emissions [17-18,29], different organic materials exhibit distinct carbon transformation characteristics. Research remains limited on the effects of SMS application in paddy soils, its decomposition process, the relationship between application rate and soil respiration, and CO_2 release characteristics.

Based on a long-term field experiment of SMS application in paddy fields, this study explored the decomposition process and CO_2 release characteristics of SMS in soil under laboratory conditions, aiming to establish quantitative relationships between SMS application rate and soil organic carbon decomposition. This research provides a theoretical foundation for understanding soil organic carbon cycling and CO_2 source-sink characteristics, offers scientific guidance for rational SMS application, and contributes to sustainable soil and agricultural development.

1.1 Study Site Description

The paddy soil was collected from a long-term monitoring station for SMS fertilization located in Longjiang Village, Jiaomei Taiwan Business Investment Zone, Longhai City, Fujian Province (117°53 46 E, 24°34 16 N). The site has cultivated double-cropping rice since 2007, using the hybrid rice variety ‘Fengliangyou 1’ . Initial soil chemical properties were: pH 6.07, organic carbon 9.66 $g \cdot kg^{-1}$, total nitrogen 2.70 $g \cdot kg^{-1}$, alkaline hydrolyzable nitrogen 101.2 $mg \cdot kg^{-1}$, available phosphorus 35.42 $mg \cdot kg^{-1}$, and available potassium 99.03 $mg \cdot kg^{-1}$.

The SMS was obtained from local Agaricus bisporus growers. After pretreatment and grinding, its organic carbon, total nitrogen, total phosphorus, and total potassium contents were 398.45 $g \cdot kg^{-1}$, 18.8 $g \cdot kg^{-1}$, 4.61 $g \cdot kg^{-1}$, and 6.37 $g \cdot kg^{-1}$, respectively, with a C/N ratio of 33.2.

1.2 Experimental Design and Sample Collection

The experiment consisted of five treatments: (1) no mushroom substrate (TS), 100% paddy soil; (2) soil to mushroom substrate ratio of 10:1 (SM1); (3) soil to mushroom substrate ratio of 5:1 (SM2); (4) soil to mushroom substrate ratio of 2:1 (SM3); and (5) pure mushroom substrate (TM). Each treatment was replicated three times.

Sieved (2 mm) paddy soil and SMS were weighed according to the above ratios and thoroughly mixed, with 200 g total per treatment, then placed in 1000 mL plastic bottles. Soil moisture was adjusted to 70% of field water-holding capacity and pre-incubated for 7 days under the same conditions as the main experiment to activate soil microorganisms. The bottles were sealed with plastic wrap to prevent rapid moisture loss while being perforated with small holes to ensure aeration, then incubated at 25°C in a constant temperature chamber. Moisture content was maintained through regular watering during the incubation period. Samples were collected on days 7, 21, 35, 49, and 63. At each sampling, the soil-substrate mixture in each bottle was thoroughly homogenized, and approximately 30 g was collected and refrigerated at 4°C for subsequent analysis, with three replicates.

For CO_2 measurement, additional mixtures of sieved (2 mm) paddy soil and SMS were prepared at the same ratios, with 50 g per treatment placed in 1000 mL incubation bottles and spread evenly at the bottom. Soil moisture was adjusted to 70% of maximum water-holding capacity. After 7 days of pre-incubation, a special vial containing 5 mL of 0.6 $\text{mol} \cdot \text{L}^{-1}$ NaOH solution was carefully placed inside each incubation bottle, which was then sealed and incubated at (28$±1)°C.Ondays1, 3, 7, 14, 21, 28, and35, thevialswereremoved, rinsedintoErlenmeyerflasks, mixedwith2m L^{-1}$ $BaCl_2$ solution and two drops of phenolphthalein indicator, then titrated with standard acid until the red color disappeared to calculate CO_2 release [30], with three replicates.

1.3 Measurement Methods

Soil carbon and nitrogen analysis: Collected samples were oven-dried, weighed, ground, and sieved through a 100-mesh screen before determination. Organic carbon was measured using the H_2SO_4-K_2CrO_7 external heating method, and total nitrogen was determined by the Kjeldahl method [31].

1.4 Data Processing and Statistical Analysis

The residue rates of organic carbon and nitrogen in SMS were calculated as:

$$rC = (g_1C/gC - g_2C/gC) \times 100\% \qquad (1)$$
$$rN = (g_1N/gN - g_2N/gN) \times 100\% \qquad (2)$$

where rC and rN represent the residue rates of organic carbon and nitrogen, respectively; g_1C and g_1N represent the carbon and nitrogen contents in the soil-SMS mixture after decomposition for a certain period; g_2C and g_2N represent the carbon and nitrogen contents in the control soil after decomposition for the same period; and gC and gN represent the carbon and nitrogen contents in the added SMS [32-33].

Soil CO_2 release rate $[\text{mg(C)} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}]$ was defined as the amount of carbon mineralized and released as CO_2-C per unit mass of soil (dry weight) per unit time. Cumulative CO_2 release $[\text{mg(C)} \cdot \text{kg}^{-1}]$ was defined as the total amount of carbon mineralized and released as CO_2-C per unit mass of soil (dry weight) during a specific incubation period. Soil organic carbon mineralization intensity (mineralization rate) was the ratio of cumulative CO_2 release to soil organic carbon content over a given time period.

Since the original soil organic carbon and nitrogen also decomposed during incubation and the mass of added SMS decreased due to mineralization, corrections were applied in calculations by subtracting the control soil values.

Data were processed using Microsoft Excel. Statistical analyses were performed using SPSS 16.0 and DPS (v3.01 Professional Edition) software.

2.1 Soil Organic Carbon Content After Adding Different SMS Ratios

As shown in [Figure 1: see original paper], at any given time, adding different proportions of SMS increased soil organic carbon content, with higher application rates resulting in greater organic carbon content. The ranking of soil organic carbon content was consistently TM > SM3 > SM2 > SM1 > TS, with the magnitude of increase depending primarily on the SMS application rate. At each sampling time, differences in soil organic carbon content among treatments were statistically significant.

After 63 days of incubation, soil organic carbon in treatments SM1, SM2, and SM3 increased by 86.7%, 171.4%, and 351.4%, respectively, compared to the TS treatment, while the TM treatment showed a 10.7-fold increase. Following day 35, the TM treatment exhibited a relatively rapid decline in organic carbon, decreasing by 9.8% from day 35 to day 49 (a significant difference). Overall, soil organic carbon tended to decrease over time in all SMS-amended treatments, though differences were not substantial (except for TM), primarily because carbon decomposition proceeded slowly.

2.2 Organic Carbon Decomposition Residue Rate After Adding Different SMS Ratios

As illustrated in [Figure 2: see original paper], at any given incubation time, higher SMS application rates resulted in greater soil organic carbon residue rates, with the consistent ranking of TM > SM3 > SM2 > SM1 > TS. Differences in organic carbon residue rates among treatments were statistically significant at each sampling time.

On day 7, the organic carbon decomposition residue rates for SM1, SM2, SM3, and TM treatments were 7.09%, 13.39%, 28.76%, and 94.33%, respectively. By day 63, these values had decreased to 5.73%, 11.34%, 23.25%, and 70.62%, respectively. After 63 days of incubation, the organic carbon decomposition residue rate showed an extremely significant positive correlation with SMS application amount ($\mathrm{YC} = 71.26\mathrm{X}^{-0}.^{6075}$, $\mathrm{r}^2 = 1.0000$).

2.3 Soil Total Nitrogen Content After Adding Different SMS Ratios

Soil total nitrogen content serves as an indicator of baseline soil nitrogen fertility and reflects nitrogen reserves. As shown in [Figure 3: see original paper], at any given time, adding different proportions of SMS increased soil total nitrogen content, with higher application rates yielding greater nitrogen content. The ranking was consistently TM > SM3 > SM2 > SM1 > TS, with the magnitude of increase depending primarily on the SMS application rate. Differences in total nitrogen content among treatments were statistically significant at each sampling time.

After 63 days of incubation, soil total nitrogen in treatments SM1, SM2, and SM3 increased by 95.3%, 186.7%, and 362.4%, respectively, compared to TS, while the TM treatment showed an 11-fold increase. Overall, soil total nitrogen tended to decrease over time in all treatments, though differences were not substantial due to slow nitrogen decomposition. The TM treatment, however, showed a relatively rapid decline after day 35, decreasing by 10.9% from day 35 to day 49 (a significant difference).

2.4 Soil Nitrogen Decomposition Residue Rate After Adding Different SMS Ratios

The nitrogen decomposition residue rate refers to the residual rate of organic nitrogen in organic materials after mineralization and decomposition for a certain period [33]. As shown in [Figure 4: see original paper], at any given incubation time, higher SMS application rates resulted in greater nitrogen decomposition residue rates, with the consistent ranking of TM > SM3 > SM2 > SM1 > TS. Differences in nitrogen decomposition residue rates among treatments were statistically significant at each sampling time.

The nitrogen decomposition trend was generally similar to that of organic carbon, with an initial rapid decomposition phase followed by a slower phase. On day 7, the nitrogen decomposition residue rates for SM1, SM2, SM3, and TM treatments were 7.17%, 13.40%, 27.05%, and 91.36%, respectively. By day 63, these values were 6.28%, 12.29%, 23.87%, and 73.70%, respectively. After 63 days of incubation, the nitrogen decomposition residue rate showed an extremely significant positive correlation with SMS application amount ($YN = 74.039X^{-0}.^{4133}$, $r^2 = 0.9999$).

2.5 CO_2 Release Rate and Cumulative Release After Adding Different SMS Ratios

The dynamic changes in soil CO_2 release rate after adding different SMS ratios are shown in [Figure 5: see original paper]. During the 35-day incubation period, all treatments exhibited an initial increase in CO_2 release rate followed by a decrease and eventual stabilization. Higher SMS application rates produced higher CO_2 release rates, with peak release occurring on day 7 in all treatments. The TM treatment showed the highest release rate at 67.23 $mg(C) \cdot kg^{-1} \cdot d^{-1}$ on day 7, followed by SM3 at 55.89 $mg(C) \cdot kg^{-1} \cdot d^{-1}$, with these values being significantly different from rates at other sampling times ($P < 0.05$). All treatments showed a gradual and steady decline after day 14. The ranking of CO_2 release rates across all sampling times was consistently TM > SM3 > SM2 > SM1 > TS.

Cumulative CO_2 release, representing the total amount of CO_2-C released per kilogram of dry soil over time, is an indicator of soil organic carbon mineralization rate [34]. Overall, cumulative CO_2 release in all treatments showed rapid initial growth followed by slower growth later in the incubation period [Figure 5: see original paper], consistent with the pattern of CO_2 release rates. After 35 days, the ranking of cumulative CO_2 release was TM > SM3 > SM2 > SM1 > TS, with significant differences among all treatments. The SMS-amended treatments (TM, SM3, SM2, SM1) showed cumulative CO_2 release 8.9, 6.4, 3.5, and 2.0 times higher than the TS treatment, respectively.

2.6 Soil Organic Carbon Mineralization Intensity After Adding Different SMS Ratios

Soil organic carbon mineralization intensity (mineralization rate) is the ratio of cumulative CO_2 release to soil organic carbon content over a given time period [34-35]. As shown in [Figure 6: see original paper], after 35 days of incubation, soil organic carbon mineralization intensity was minimal in all SMS-amended treatments, indicating that most organic carbon was sequestered in the soil. The mineralization intensity in treatments SM1, SM2, and SM3 was 57.61%, 49.08%, and 41.07% higher than in the TS treatment, respectively ($P < 0.05$), though differences among these three treatments were not significant. The TM treatment showed the lowest mineralization intensity, significantly different from all other treatments.

The decomposition of organic materials and their residue rates are important indicators for evaluating their effectiveness in maintaining and improving soil organic matter status and fertility. These processes are influenced by organic material type, chemical composition, soil type, and decomposition environment [36]. Clarifying decomposition patterns of organic fertilizers is essential for scientifically replenishing soil organic matter and developing appropriate fertilization strategies [28].

Different organic fertilizers from various plant and animal sources contain different qualities of carbon, nitrogen, and other elements, which may result in different soil CO_2 emissions when applied at equal rates. Soil CO_2 release is a major pathway of organic carbon output, directly affecting nutrient element release and supply as well as CO_2 gas emissions [34,37]. Soil organic carbon mineralization intensity reflects organic matter decomposition and nutrient supply status [37]. Numerous studies have demonstrated that organic material application promotes soil CO_2 release [17-18].

For example, Dai et al. [38] found that soils amended with manure and straw released significantly more CO_2 than unfertilized soils. Li et al. [39] reported that the ranking of potential organic carbon mineralization CO_2-C release in red soils under different fertilization treatments was: organic fertilizer > organic fertilizer plus chemical fertilizer > straw return plus chemical fertilizer > no fertilizer. The increased CO_2 emissions resulting from straw and organic fertilizer application do not directly contribute to atmospheric CO_2 increase; instead, they can enhance soil carbon sequestration and mitigate the impact of soil carbon release on atmospheric CO_2 concentration [39].

Our results showed that during the 35-day incubation period, all SMS-amended treatments exhibited an initial increase in CO_2 release rate followed by a decrease and stabilization, similar to patterns observed with straw addition in previous studies [23,35,40-42]. This pattern occurs because SMS is rich in proteins, NPK, medium and trace elements, ash, crude fat, crude protein, crude fiber, and various amino acids. As an external organic material, SMS provides readily available nutrients and energy sources for microorganisms, thereby stimulating soil respiration. During the early stage, readily decomposable components in SMS and soil were rapidly mineralized, leading to rapid increases in soil organic carbon mineralization rate and amount. As incubation progressed, these readily decomposable components were depleted, and microorganisms began utilizing more recalcitrant components, causing mineralization rates to slow and organic carbon decomposition to decrease accordingly.

After 35 days of incubation with different SMS ratios, soil organic carbon mineralization intensity was minimal, with most organic carbon being sequestered in the soil, particularly in the TM treatment which showed the lowest mineralization intensity. This indicates that higher SMS application rates result in greater carbon sequestration, similar to effects observed with straw return [23].

It should be noted that this study was conducted under controlled laboratory conditions with small samples and constant temperature. Under field conditions, factors affecting SMS decomposition and soil CO_2 release are more complex and require further validation.

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