Industrial Crops & Products 240 (2026) 122656

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop

Sawdust-cow manure compost and non-sterile soil casing synergistically enhance *Stropharia rugosoannulata* yield: Carbon-use dynamics, enzyme activities and microbial drivers

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ARTICLE INFO

Keywords:
Stropharia rugosoannulata
Agroforestry
Mushroom cultivation
Sustainable agriculture
Microbial community structure

ABSTRACT

This study explores the utilization of agroforestry biowastes as compost substrates for edible mushroom cultivation, a practice that mitigates environmental burdens in agroecosystems. Here we performed a fully replicated, factorial experiment to quantify how (i) substrate type (sawdust–cow-manure vs. straw–cow-manure compost), (ii) casing material (non-sterile soil, sterile soil, or perlite + vermiculite) and (iii) fungal inoculation duration (0, 30, 90, 210 d) control *S. rugosoannulata* productivity. By integrating yield data with high-resolution enzyme assays and 16S amplicon sequencing, we aimed to identify the carbon-compound and microbial drivers that underpin high biological efficiency and to benchmark the protein conversion efficiency of the system against insect- and livestock-based platforms. Our findings identify primary determinants for improving mushroom yield and compost quality: i) Application of unsterilized soil as a casing material induced substantial shifts in substrate nutrient composition, particularly in total carbon and nitrogen fractions; ii) Selection of sawdust-cow manure blends as initial composting substrates enhanced cellulase activity, facilitating decomposition of hemicellulose, cellulose, and lignin—key C-compounds critical for fungal nutrition. *Cellvibrio* and *Stenotrophomonas* emerged as dominant bacterial genera, with bacterial Shannon diversity increasing progressively with inoculation duration. Throughout cultivation, substrate moisture and cellulose content were identified as primary drivers of bacterial diversity, while total sugar, cellulose, and lignin levels significantly shaped community structure. These results provide actionable guidelines for optimizing substrate formulations, casing material selection, and environmental parameters in *S. rugosoannulata* cultivation. Their implementation would enable efficient conversion of agroforestry biowaste composts into edible mushroom production systems, thereby advancing sustainable agricultural practices and circular bioeconomy objectives.

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https://doi.org/10.1016/j.indcrop.2026.122656
Received 1 September 2025; Received in revised form 6 January 2026; Accepted 6 January 2026
Available online 14 January 2026

1. Introduction

The escalating challenges of global food security and environmental sustainability have intensified the search for circular agricultural systems that integrate waste valorization with high-efficiency biomass production. Among these, fungal-based conversion systems—particularly those utilizing edible mushrooms—have emerged as promising biotechnological platforms for transforming agroforestry biowaste into nutrient-rich food products. The cultivation of saprophytic edible mushrooms on composted agroforestry residues represents a paradigmatic shift toward closed-loop bioeconomies, wherein organic waste streams are upcycled into high-value biomass while simultaneously reducing environmental externalities (Jorge et al., 2020; Hamza et al., 2024; Timm et al., 2024).

Stropharia rugosoannulata, commonly known as the wine cap mushroom, exemplifies this potential (Allen et al., 2023; He et al., 2024). As a lignocellulolytic saprotroph, it possesses the enzymatic machinery necessary to degrade complex plant polymers, thereby converting lignocellulosic waste into edible biomass while contributing to soil organic matter cycling (Yang et al., 2022; Liu et al., 2021). However, the scalability and commercial viability of such systems are contingent upon a mechanistic understanding of the substrate-microbe-mushroom continuum, particularly in non-sterile, open-environment cultivation systems typical of circular agriculture.

From a material flow analysis (MFA) perspective, the conversion of agroforestry biowaste into mushroom biomass can be conceptualized as a three-phase biotransformation process: (i) the enzymatic solubilization of recalcitrant carbon polymers into bioavailable nutrients; (ii) the assimilation of these nutrients into fungal mycelial biomass and subsequent fruiting body formation; and (iii) the reintegration of residual substrate into the soil as organic amendment. Here, we test whether targeted manipulation of the substrate-casing-microbiome continuum can improve carbon-use efficiency and yield stability under low-input conditions. Substrate composition is a critical determinant of system performance. Our comparative studies have demonstrated that sawdust-cow manure substrates yield 25.8 % higher fresh mushroom biomass (3.36 kg vs. 2.67 kg per cultivation unit) and 43 % greater biological efficiency (42.9 % vs. 30.0 %) compared to straw-cow manure formulations when paired with non-sterile soil casing. Despite marginally higher input costs, sawdust-based substrates exhibit superior moisture retention and nutrient stability, resulting in a 33 % reduction in energy consumption per kilogram of fresh mushroom produced (0.12 kWh/kg vs. 0.18 kWh/kg), thereby improving the overall cost-benefit ratio under scaled conditions.

At the biochemical level, carbohydrate heterogeneity—encompassing total sugars, reducing sugars, cellulose, and hemicellulose—modulates fungal growth kinetics and enzymatic activity profiles (Lu et al., 2020; Zhang et al., 2025). Readily metabolizable sugars (e.g., glucose, fructose) serve as primary carbon sources during early colonization (Andlar et al., 2018), while structural polysaccharides (cellulose and hemicellulose) are progressively hydrolyzed by fungal oxidative and hydrolytic enzymes, including laccases, cellulases, and xylanases (Zeng et al., 2018; Wang et al., 2024). The temporal dynamics of these enzymes are tightly coupled with fungal developmental stages, and any perturbation in their activity can compromise substrate utilization efficiency and yield (Jorge et al., 2020; Wang et al., 2024).

In addition to carbon compounds and enzymes, the microbial communities associated with the compost and the fungal growth also play a vital role in the cultivation of the edible mushroom (Liu et al., 2021). These microbial communities are involved in a variety of ecological processes, including nutrient cycling, organic matter decomposition, humus accumulation, and the production of growth-promoting substances (Hicks et al., 2021; Zhou et al., 2025). The composition and dynamics of these microbial communities can influence the availability of nutrients, the activity of enzymes, and the overall health and productivity of the fungal culture (Li et al., 2019). Understanding the key microbial communities and their interactions with fungi is crucial for maintaining a stable, efficient, and productive system. Previous studies have provided some insights into the cultivation of edible mushrooms and the role of various factors in their growth. For example, research on the effects of different substrates and casing materials on mushroom fruiting has shown the importance of these factors in determining yield and quality (Ayyub et al., 2014; Vieira et al., 2023; Otieno et al., 2022). Additionally, studies on the impact of mushroom cultivation on soil nutrients and microbial communities have highlighted the potential ecological benefits of this practice (Gong et al., 2018; Tang et al., 2022). Meanwhile, the system constructed by *S. rugosoannulata* for converting agricultural waste offers advantages in agricultural waste reduction, high-quality protein production. Comparative analyses of protein conversion efficiency, reveal that the *S. rugosoannulata*-based system exhibits a high biological efficiency comparable to insect protein production systems (Schneider et al., 2025; Sideris et al., 2021), with a balanced amino acid composition that equates its nutritional value to insect protein (Schneider et al., 2025; Wang et al., 2025), further supporting its potential as a high-quality protein source to replace traditional animal-derived proteins (Flachowsky, 2002).

Although *S. rugosoannulata* is increasingly promoted as a saprophytic platform that simultaneously valorises lignocellulosic wastes and generates protein-rich biomass, its adoption in circular-agriculture pipelines remains empirical. Three critical knowledge gaps currently constrain rational process design: (i) how substrate-derived carbon fractions (soluble sugars, hemicellulose, cellulose, lignin) are sequentially remodelled during crop progression; (ii) which microbial taxa and enzyme activities govern this temporal carbon flux; and (iii) how casing physicochemistry interacts with the substrate microbiome to set the theoretical upper limit for protein conversion efficiency relative to insect-based or conventional livestock systems.

In an attempt to narrow these knowledge gaps, we carried out a replicated, full-factorial experiment in which the cultivation substrate (sawdust- or straw-based compost) and the casing material (non-sterile soil, sterile soil, or perlite + vermiculite) were varied independently. Substrate chemistry, enzyme activities (cellulase and laccase) and bacterial community composition were monitored at successive intervals. The data were then used to examine the relationships between substrate composition, microbial succession, enzyme dynamics and mushroom yield.

On this basis, we compare the protein yield and apparent resource-use efficiency of the *Stropharia* system with published values for black-soldier-fly larvae and conventional livestock, recognising that such a comparison is preliminary and subject to considerable uncertainty. We hope these provisional metrics will offer a starting point for further efforts to model and optimise fungal-based waste-to-protein conversion.

2. Materials and methods

2.1. Agroforestry biowaste composting for mushroom cultivation

2.1.1. Raw material preparation and pretreatment

Straw substrate: Corn straw and rice straw were air-dried to a moisture content of 12 ± 1 %, then crushed into 2–3 cm segments using a straw crusher. A total of 150 kg (dry weight) of mixed straw (corn straw:rice straw = 1:1, w/w) was prepared.

Sawdust substrate: *Quercus variabilis* and *Alnus japonica* wood were processed into sawdust with a particle size of 0.8–1.2 mm using a wood crushe, air-dried to 11 ± 1 % moisture content. A total of 150 kg (dry weight) of mixed sawdust (*Q. variabilis*:*A. japonica* = 1:1, w/w) was prepared. Sawdust and straw were selected due to their wide availability as agroforestry biowastes and differing lignocellulose contents (key for fungal nutrition). Their comparison helps identify optimal substrate properties.

Cow manure: Fresh cow manure was sun-dried for 72 h to reduce moisture content to 15 ± 2 %, then sieved through a 5-mm mesh to

remove impurities (e.g., stones, plastic debris). A total of 100 kg (dry weight) of processed cow manure was prepared for each substrate type.

Additives: Corn cobs (crushed to 1–2 cm), gypsum (analytical grade), and urea (agricultural grade, N content ≥46 %) were purchased from local suppliers and used without further purification. The basic physicochemical properties of the original compost substrate are detailed in Supplementary Table 1.

2.1.2. Substrate compounding and mixing

For each substrate type (straw or sawdust), the base materials (150 kg straw/sawdust + 100 kg cow manure) were first spread evenly on a concrete platform (10 m × 3 m) and manually mixed for 5 min. Additives were incorporated sequentially: 21 kg corn cobs, 9 kg gypsum, and 9 kg urea were added to the base mixture, followed by mixing to ensure homogeneous distribution. Deionized water was sprayed into the mixture during mixing to adjust the initial moisture content to 65 ± 2 % (determined by the oven-drying method: 105°C for 48 h). The moisture content was verified by randomly sampling 5 points (each 500 g) from the mixed substrate and measuring the mass loss after drying.

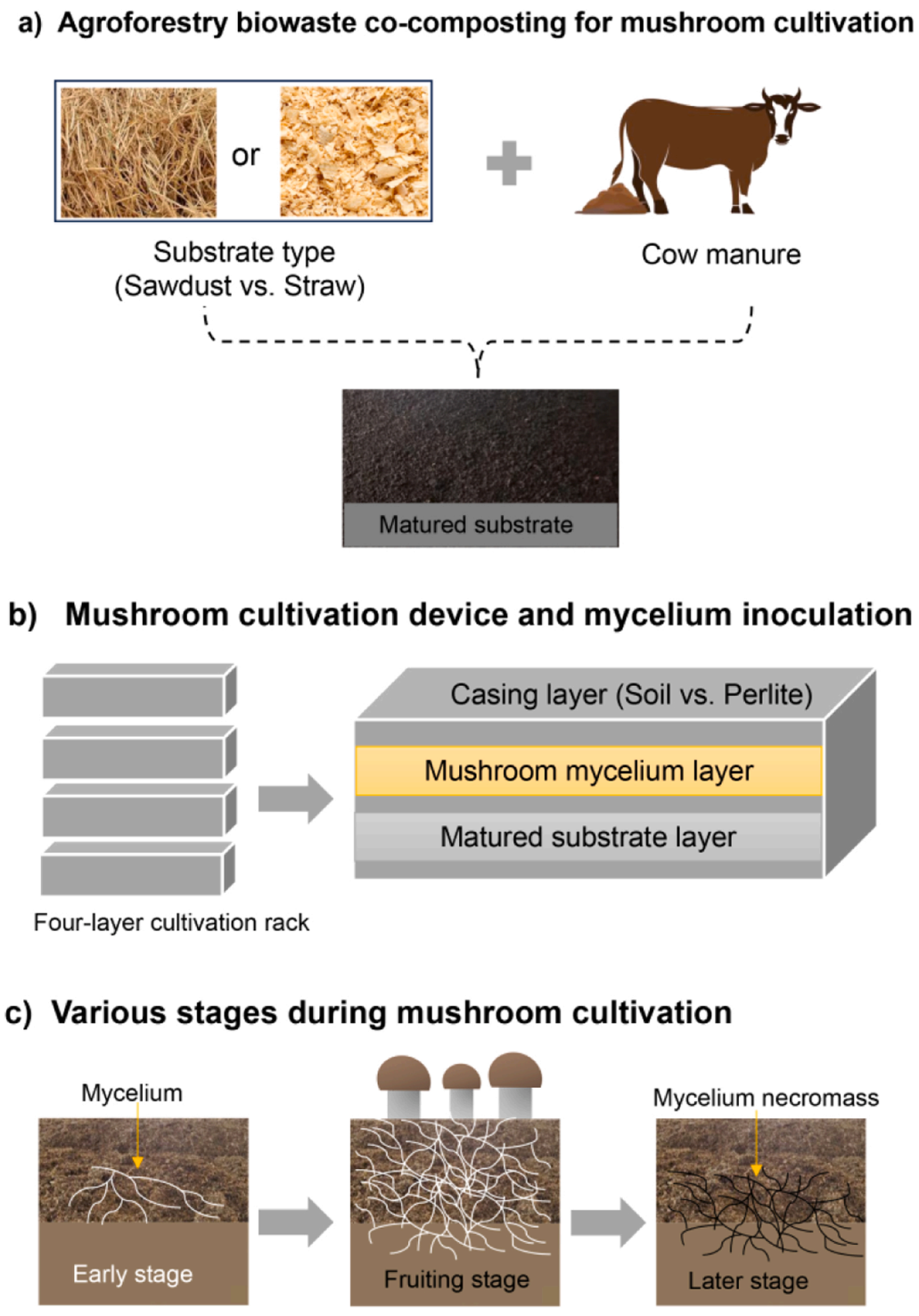

Fig. 1. Experimental procedure from composting preparation to mushroom cultivation.

2.1.3. Aerobic composting process

The mixed substrate was piled into trapezoidal heaps (length × width × height = 5 m × 1.5 m × 1 m) with 5 cm-diameter ventilation holes drilled every 30 cm along the heap to ensure oxygen supply. Composting was conducted in a greenhouse with ambient temperature ranged between 20–30°C. Turning and mixing were performed on the 10th, 20th, and 30th days of composting (Fig. 1). During each turning, the moisture content was rechecked and adjusted to 60–65 % by spraying deionized water if necessary. Composting was terminated after 40 days when the substrate temperature stabilized at 25 ± 2°C (monitored by a soil thermometer inserted 30 cm deep into the heap) and the C/N ratio reached 25 ± 2 (measured by an Elementar Vario MICRO Cube analyzer).

2.2. Mushroom cultivation set-up, mycelium inoculation and casing materials

The prepared cultivation substrates were then positioned onto multi-layer racks (Fig. 1b). This approach, commonly adopted in the mushroom industry, improves space utilization and management. Casing materials were also used to maintain optimal moisture for enhanced mushroom quality. Experimental cultivation of the edible mushroom (hereafter referred to as *Stropharia rugosoannulata*) was conducted within a semi-open four-layer cultivation rack (Fig. 1b). The dimensions of each cultivation layer were specified as length × width × height, equaling 120 × 16 × 20 cm. Each cultivation layer was composed of a substrate bottom layer and a top casing layer. Two casing materials were utilized and compared: sterilized/non-sterilized soil, and a mixture of perlite and vermiculite in a 1:1 (v/v) ratio.

To avoid clogging of drainage outlets, a single layer of non-woven fabric was positioned at the bottom of the cultivation rack. slot. A 6-cm-thick layer of substrate was then laid down. The solid mushroom mycelium, whose diameter ranged from 3-to-5 cm, was evenly distributed across the surface of the substrate at a density of 0.75 kg per slot (the detailed preparation process of the solid mushroom mycelium, can be found in Supplementary file 1). Subsequently, a 7 cm-thick layer of substrate was added, accompanied by an additional 0.75 kg of mushroom spawn per slot. Finally, a layer of approximately 3 cm of substrate was laid down, followed by a casing with a 2-to-3 cm thick casing material (Fig. 1b). In the control group, all the operations were identical to those described above, with the exception of the mushroom mycelium inoculation process.

2.3. Mushroom fruiting management, sample collection and pre-processing

2.3.1. Cultivation environment setup

The semi-open four-layer cultivation rack was placed at the Edible Fungi Cultivation Center of Kunming Institute of Botany, Chinese Academy of Sciences. The entire cultivation center is located under the forest, and such an environment is mainly to maintain appropriate temperature (~22 ± 1°C) and relative humidity (RH) 70 ± 5 %. Meanwhile, temperature and humidity data loggers were placed at the center of each cultivation layer to record temperature and substrate moisture content at 30-min intervals throughout the experiment.

2.3.2. Layered substrate loading and inoculation

A single layer of non-woven fabric was laid at the bottom of each cultivation slot (120 cm × 16 cm × 20 cm) to prevent substrate leakage while allowing drainage. The matured compost was loaded into the slot in two layers: the first layer (6 cm thick) was compacted gently, followed by uniform placement of solid mushroom mycelium (diameter 3–5 cm) at a density of 0.75 kg per slot (equivalent to 3 mycelium pieces per 100 cm^2). A second layer of compost (7 cm thick) was added to cover the mycelium, and an additional 0.75 kg of mushroom spawn was sprinkled on the surface. The final substrate layer was compacted to the same density as the first layer. Casing material (2–3 cm thick) was evenly spread on top of the substrate. For non-sterilized/sterilized soil casing: the soil was passed through a 2 mm sieve, and sterilized soil was autoclaved at 121°C, 0.1 MPa for 2 h. For perlite+vermiculite casing: the mixture (1:1, v/v) was soaked in deionized water for 24 h and drained to a moisture content of 70 ± 5 % before use.

2.3.3. Stage-specific environmental control

Mycelial colonization stage (0–20 days post-inoculation): Temperature was maintained at 25 ± 1°C, RH at 60 ± 3 %, to promote mycelial growth. Daily manual watering (100 mL per slot) with deionized water was performed using a spray bottle to maintain the casing layer moisture content at 60 ± 2 %.

Mycelial maturation stage (21–89 days post-inoculation): Temperature was adjusted to 22 ± 1°C, RH to 55–65 %. Watering frequency was reduced to once every 3 days, with the amount adjusted to keep the substrate moisture content at 55–65 % (monitored by the data loggers: OnSet Computer Corporation, HoBo, USA).

Fruiting stage (90–210 days post-inoculation): Temperature was maintained at 18–20°C, RH increased to 85 ± 5 %. Watering was performed twice daily (morning and evening) with 150 mL per slot each time to ensure the substrate moisture content remained at 70–80 %.

2.3.4. Fruiting body harvesting

Harvesting was conducted when the mushroom caps were fully expanded (diameter 8–12 cm) and the annulus was intact. Fruiting bodies were carefully cut at the base using sterile scissors to avoid damaging the mycelium. Fresh weights were measured immediately after harvesting with a precision balance. After harvesting, one-third of the collected substrate was processed for the determination of physicochemical properties, enzyme activity, and sugar content. The remaining substrates were stored at −20°C prior to DNA extraction. Yield data were recorded for each cultivation slot, and three biological replicates were maintained for each treatment.

2.4. Physicochemical, enzymatic and nutritional properties

The physicochemical properties, enzyme activity, and sugar content of the substrate, were determined by Nanjing Convinced-test Technology Co., Ltd. Total carbon (TC) and total nitrogen (TN) were determined on 10 mg freeze-dried, ball-milled aliquots combusted at 950 °C in an Elementar Vario MICRO cube (Elementar, Hanau, Germany); quantification was by thermal-conductivity detection against acetanilide standards (n = 3 per biological replicate). For inorganic N, 5 g fresh substrate was extracted with 50 mL 1 M KCl (1:10 w/v, 60 min, 180 rpm), filtered (0.45 μm cellulose acetate)(Wang et al., 2018), and NH_4^+-N and NO_3^--N were measured concurrently on a SEAL AA3 continuous-flow analyser (salicylate–hypochlorite and cadmium-reduction cartridges, respectively; LOD 0.05 mg N L^{-1}). pH was recorded in a 1:5 (w/v) slurry after 30 min equilibration (Mettler Toledo InLab® Expert Pro) (Yang et al., 2012), while gravimetric moisture was obtained by drying 10 g fresh material at 105 °C to constant mass (48 h).

Structural carbohydrates and lignin were sequentially analysed on the same 0.5 g sample: hemicellulose was solubilised by refluxing in 2 M HCl (2 h), neutralised, and the liberated pentoses quantified at 620 nm with anthrone (xylose calibration) (Xiong et al., 2005). The remaining residue was digested in 67 % H_2SO_4 (v/v, 25 °C, 24 h) to release glucose for cellulose determination (anthrone, glucose standard) (Wang, 2006). Klason lignin was calculated as mass loss-on-ignition (550 °C, 4 h) of the acid-insoluble residue, and all values are expressed as mg g^{-1} dry substrate (Xiong et al., 2005). Soluble sugars were extracted with 80 % ethanol (80 °C, 30 min); total sugars were assayed in the ethanolic supernatant with anthrone (620 nm), and reducing sugars with 3,5-dinitrosalicylic acid (540 nm)(Wen et al., 2005). Enzyme activities were measured under substrate-saturated conditions: cellulase (EC 3.2.1.4)

activity was estimated by incubating 1 g substrate with 1 % carboxymethyl-cellulose in 50 mM citrate buffer (pH 5.0, 40 °C, 1 h) and quantifying the released reducing sugars by DNS (μmol glucose g^{-1} DM h^{-1}) (Xu, 1986); laccase (EC 1.10.3.2) activity was determined with 1 mM ABTS in 50 mM tartrate buffer (pH 5.0) and monitoring oxidation at 420 nm ($\varepsilon = 36\ mM^{-1}\ cm^{-1}$, μmol ABTS oxidised g^{-1} DM h^{-1}) (Zhang et al., 2019). All spectrophotometric readings were performed in triplicate on a Shimadzu UV-1800 with concurrent blanks and internal standards; calibration curves ($R^2 \geq 0.998$) were verified every 20 samples to ensure linearity.

2.5. DNA extraction, sequencing for soil microbial communities and bioinformatics

Total DNA was extracted from 0.5 g of fresh substrate sample using the FastDNA Spin Kit for Soil (Qbiogene, Irvine, CA, USA), following manufacturer. Extracted DNA quality and quantity were determined using a NanoDrop spectrophotometer (NanoDrop C2000, Thermo Fisher Scientific, Waltham, MA, USA) (Strychalski et al., 2022). Substrate sample DNA fragments were subjected to paired-end sequencing using the Illumina NovaSeq PE250 platform. The V4-V5 region of the 16S rRNA gene was amplified using the primer set (F: GTGCCAGCMGCCGCGGTAA, R: CCGTCAATTCMTTTRAGTTT), which provides accurate classification with minimal bias in bacterial taxonomy. The ITS1 gene was amplified using the primer set (F: GGAAGTAAAAGTCGTAACAAGG, R: GCTGCGTTCTTCATCGATGC), which accurately assigned fungal taxonomy (He et al., 2020).

Sequences were de-noised or clustered using the DADA2 and Vsearch with Vsearch software (v2.13.4_linux_x86_64) and cutadapt (v2.3). Subsequently, QIIME2 (2019.4) was utilized to align the sequences against reference sequence databases (Bokulich et al., 2018) for species annotation. Raw sequence data were deposited in the NCBI Sequence Read Archive (PRJNA1164471) under accession numbers SUB14728682 (Bacterial sequences) and SUB14746724 (Fungal sequences).

2.6. Statistical analysis

Prior to analysis, the Shapiro-Wilk test was performed to examine data normality. Five-way analyses of variance (ANOVAs) were conducted using SPSS (Chicago, USA, Version 20) to evaluate the impacts of casing materials (soil/perlite), cover soil microbial activities (sterilized/non-sterilized), cultivation substrates (straw/sawdust), mycelium inoculation (inoculated/non-inoculated), and temporal variations (early stage “E”, fruiting stage “F”, and late stage “L”) on nutrients, physicochemical properties, carbon components (cellulose, hemicellulose, total sugar, and reducing sugar), carbon-degrading enzymes (lignin, laccase, and cellulase), and microbial communities (diversity and community structure).

Significant differences (means ± standard deviation, n = 3) between treatments were compared using the Least Significant Difference (LSD) test at $P < 0.05$. The Simpson Diversity Index was employed to estimate the alpha-diversity of soil microbial communities. Nonmetric Multidimensional Scaling (NMDS) ordination plots were utilized to visualize the differences in microbial community structures). The Mantel test (in R 4.1.0 with the packages of ape and linkET) was adopted to assess relationships between microbial communities and environmental parameters.

3. Results and discussion

3.1. Yield, fruiting date and biological efficiency

Mushroom yield: For the same casing materials, mushroom production was significantly higher under sawdust treatment than under straw treatment ($P < 0.05$; Table 1). Keeping substrate constant, mushroom yields were highest in non-sterilized soil > perlite and vermiculite > sterilized soil (Table 1). The observed higher yield in non-sterilized soil compared to sterilized soil and other materials like perlite and vermiculite can be attributed to the persistence of a diverse microbial communities in the non-sterilized soil. Yield benefits of enhanced substrate quality have been linked with increased microbial community diversity in previous studies (Suwannarach et al., 2022; He et al., 2024). This may be driven by the fungus’s symbiotic relationships with other soil microorganisms, which aid nutrient mobilization and bioavailability. These symbioses appear crucial for the growth and fruiting of mushrooms (Carrasco and Preston, 2020). Full soil sterilization eliminates the beneficial microbial community almost completely, aside from endosymbionts from the original inoculum, leading to reduced yields. Yield differences between sawdust-treated and straw-treated groups with the same casing material could be due to the physical and chemical properties of these two substrates. Sawdust may have a more favorable structure for mycelial colonization and nutrient absorption (Jung et al., 2010), with superior water-holding capacity and air porosity compared to straw (Shaheen et al., 2022). Additionally, the chemical composition of sawdust, including higher lignin content, positively correlates with mushroom yield and biological efficiency. High lignin content and a low cellulose/lignin ratio in sawdust substrates tend to benefit mushroom growth (Akter et al., 2022; Atila, 2019).

Initial Fruiting Time: With the same casing materials, sawdust substrates promoted significantly earlier ($P < 0.05$) mushroom emergence than straw substrates (Table 1). This might be due to faster mycelial growth rates in sawdust, especially at the fruiting stage, afforded by a more conducive environment for mycelium establishment and colonization. Mycelium utilization of sawdust components for nutrients and energy conversation may be more efficient for fruiting body initiation compared to straw (Abdullah et al., 2013) Differences in substrate density and accessibility of nutrients between the two substrates may affect mycelium penetration and nutrient mining.

Biological Efficiency: When the casing materials are the same, the biological efficiency of sawdust- treated samples for mushroom production is higher ($P < 0.05$) than that of straw-treated ones. Notably, the combination of non-sterilized soil and sawdust treatment exhibits the highest biological efficiency (Table 1). For the biological efficiency, the

Table 1
Yield, fruiting date and biological efficiency of *Stropharia rugosoannulata* under the different experimental treatments.

Casing material	Substrate type	Mushroom Yield[a] (kg)	Fruiting date[b] (Days)	Biological efficiency[c] (%)
Unsterilized soil	Sawdust+Manure	3.36 ± 0.25 a	38 days	42.9 ± 0.6 a
Unsterilized soil	Straw+Manure	2.67 ± 0.59 b	45 days	30.0 ± 4.5 b
Sterilized soil	Sawdust+Manure	1.66 ± 0.09c	40 days	22.1 ± 1.1 c
Sterilized soil	Straw+Manure	1.32 ± 0.22 d	51 days	14.6 ± 2.3 d
Perlite+Vermiculite	Sawdust+Manure	2.10 ± 0.24 b	42 days	27.9 ± 1.1 b
Perlite+Vermiculite	Straw+Manure	1.63 ± 0.21 c	68 days	15.8 ± 1.8 d

Notes: [a]Yield was evaluated based on fresh weight; [b]Fruiting date was estimated from days after initial inoculation; [c]Biological efficiency is a measure of the productivity of a mushroom-growing substrate. It is expressed as a percentage and represents the amount of fresh mushroom biomass obtained relative to the dry weight of the substrate used for cultivation. Letters within the biological efficiency and yield columns signify significant differences at $P < 0.05$ (ANOVA) between means (Tukey’s HSD pairwise comparisons, $n = 3$).

higher biological efficiency in sawdust-treated groups with the same casing material is likely a result of better utilization of resources. Sawdust may offer a more balanced supply of carbon, nitrogen, and other essential elements required for mushroom growth (Jung et al., 2010; Shaheen et al., 2022). The mycelium can convert these nutrients into mushroom biomass more effectively. The highest biological efficiency in the 'non-sterilized soil + sawdust' treatment combination can be explained by the combined positive effects of the beneficial soil microorganisms and the suitable substrate properties of sawdust. The microorganisms in the non-sterilized soil can enhance the overall nutrient cycling and availability, while sawdust provides an ideal physical structure and nutrient source for the mycelium to grow and develop into mature mushrooms. This synergy between the substrate and the soil environment leads to optimal biological efficiency.

These results highlight the importance of both the substrate type and the soil condition in mushroom cultivation. A further in-depth understanding these mechanisms can help to optimize cultivation practices resulting in higher yields and better biological efficiencies.

3.2. Nutrient and physicochemical variations during mushroom cultivation

Significant differences ($P < 0.05$) in pH, nitrate-nitrogen, ammonium-nitrogen, total nitrogen, and total carbon during the fruiting period are observed among the treatments with different casing materials (Table 2). In the early fruiting stage, the nitrate-nitrogen and ammonium-nitrogen in the treatments with inoculating of *Stropharia rugosoannulata* mycelium are significantly higher ($P < 0.05$; Table S2) than those in the treatments without inoculating (Table 2).

During the early fruiting stage, significant differences ($P < 0.05$; Table 2) in pH, water content, and ammonium-nitrogen are presented among the treatments with different cultivation substrates. Furthermore, the sterilization treatment of the casing soil layer can lead to a significant difference ($P < 0.05$) in the carbon-to-nitrogen ratio of mushroom cultivation substrate during fruiting period (Table 2).

The cultivation of *S. rugosoannulata* has been studied for its effects on soil nutrients and microbial communities.

Our aim was to understand how different factors such as casing materials, microbial activity of casing soil, substrates, and mycelium inoculation influence key nutrient and physicochemical variations during the fruiting period. For the impact of casing materials, it was observed that different casing materials significantly affect the cultivating substrate's pH, nitrate-nitrogen, ammonium-nitrogen, total nitrogen, and total carbon during the fruiting period (Table 2), these results illustrate the significant impact of casing materials on mushroom fruiting, which has been widely reported in the casing cultivation of edible mushrooms (Ayyub et al., 2014; He et al., 2024). Moreover, the beneficial roles of *Stropharia* on nutrients were also reported either in forestlands (Gong et al., 2018) or in croplands (Tang et al., 2022).

In the early fruiting stage, *S. rugosoannulata* inoculation significantly enhances nitrogen metabolism and utilization in cultivating substrates, leading to increased nitrogen contents. This phenomenon can be attributed to the increased activities of nitrogen metabolic enzymes and gene expression after inoculating *S. rugosoannulata* (Zhang et al., 2023).

The early fruiting stage of mushroom cultivation is significantly influenced by the properties of the cultivation substrates (Table 2). Key factors such as pH, water content, and ammonium-nitrogen levels are affected by the type of substrate used, which in turn impacts the overall growth and productivity of mushrooms. It is demonstrated that variations in mushroom cultivation substrates can exert a significant impact on water content, pH, and oxygen levels (Suwannarach et al., 2022; He). Additionally, both the cultivation substrates and fungal (mushroom) inoculation have a significant effect on ammonium- nitrogen (with remarkable changes prior to the fruiting stage), suggesting that the process of organic nitrogen mineralization to ammonium-nitrogen is highly active during the early fruiting period (Lou et al., 2017). Simultaneously, the significant increases in both nitrate-nitrogen and ammonium-nitrogen during the fruiting stage also reflect the high cycling of nitrogen due to increased nitrification process during this time (Majchrowska-Safaryan et al., 2020). In the same study, it was also observed that the sterilization treatment of the casing soil layer can result in a significant difference in the substrate C/N ratio during the fruiting period. This might be because the sterilization of the casing soil layer has a significant impact on the bacterial community composition,

Table 2
Main-factor effects and significant interactions derived from four-way ANOVA analyses of the key nutrient and physicochemical variations investigated in the *Stropharia rugosoannulata* cultivation experiment.

Key parameter	Cultivation stage	Casing material		Soil sterilization		Cultivation substrate		Mycelium inoculation	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
pH	Early	10.727	0.003***	1.51	0.247	14.492	0.001***	4.255	0.066
	Fruiting	22.947	0.000***	6.389	0.030*	2.44	0.133	9.743	0.011*
	Later	0.661	0.425	0.832	0.383	0.824	0.374	21.655	0.001***
Moisture	Early	1.66	0.211	1.027	0.335	13.979	0.001***	1.665	0.226
	Fruiting	1.042	0.318	0.433	0.526	6.622	0.017*	1.821	0.207
	Later	25.758	0.000***	9.962	0.010**	0.855	0.365	0.14	0.909
NH_4^+-N	Early	8.554	0.008**	0.872	0.372	7.808	0.011*	146.199	0.000***
	Fruiting	8.361	0.008**	23.839	0.001***	4.269	0.051	3.732	0.082
	Later	7.771	0.011*	0.154	0.703	7.381	0.013*	27.961	0.000***
NO_3^--N	Early	0.783	0.386	0.007	0.935	0.176	0.679	36.125	0.000***
	Fruiting	9.247	0.006**	17.959	0.002**	4.924	0.037*	0.545	0.477
	Later	1.952	0.176	0.78	0.398	46.491	0.000***	0.056	0.818
Total N	Early	0.004	0.95	0.877	0.371	3.428	0.078	0.059	0.813
	Fruiting	8.013	0.010**	0.32	0.584	0.824	0.374	0.599	0.456
	Later	0.095	0.761	1.192	0.3	0.885	0.357	5.437	0.042*
Total C	Early	4.262	0.051	5.3	0.044*	0.267	0.611	0.019	0.894
	Fruiting	6.183	0.021*	11.138	0.008**	0.109	0.745	9.673	0.011*
	Later	1.865	0.186	0.186	0.676	2.867	0.105	7.978	0.018*
C/N ratio	Early	1.473	0.238	3.515	0.09	1.156	0.294	0.341	0.572
	Fruiting	0.855	0.365	5.133	0.047*	0.388	0.54	3.838	0.079
	Later	1.739	0.201	0.459	0.513	5.066	0.035*	0.397	0.543

Notes: The four-factor effects under examination are as follows: Casing materials (soil or perlite + vermiculite; Microbial activity of casing soil (unsterilized or sterilized); Substrates (sawdust/straw combined with cow manure); Mycelium inoculation (plus/minus inoculation). *, ** and *** indicate the 0.05, 0.01, and 0.001 significance levels.

which subsequently affects the mushroom yield and the C/N ratio of the substrate. For instance, non-sterilized casing layers with higher populations of beneficial *Pseudomonas fluorescens* can boost mushroom production (Cho et al., 2008), thereby leading to an increased C/N ratio.

3.3. Variations in carbon components and C-degrading enzymes

During the cultivation process of *S. rugosoannulata*, the content of reducing sugar in each treatment exhibits a pattern of decreasing initially and then increasing (Fig. 2). It is highly probable that significant alterations occur within the microbial community during the development of *S. rugosoannulata*. These modifications subsequently give rise to changes in the decomposition of organic matter and the efficiency of reducing-sugar utilization.

The shifts in the microbial community resulting from the cultivation of *S. rugosoannulata* can exert an impact on the decomposition of organic matter. This could potentially explain the observed fluctuations in reducing-sugar content during the mushroom's development, as documented by Zhou et al. in 2023. Specific enzyme activities, such as the elevated activities of xylanase and laccase, are associated with the changes in the microbial community. These enzymatic actions contribute to the decomposition of lignocellulose, thereby potentially influencing the availability of reducing sugars (Zhou et al., 2023).

In each treatment, the content of total sugar gradually decreases as the cultivation time progresses (Fig. 2). This may be strongly correlated with the enhanced efficiency of carbon metabolism during the cultivation process. Likewise, the up-regulation of carbon metabolism in *S. rugosoannulata* has an impact on starch and sucrose metabolism (Zhang et al., 2023). During the cultivation of the mushroom, the contents of hemicellulose, cellulose, and lignin in each treatment generally exhibit a gradual decreasing trend, as presented in Fig. 1. This implies that in the process of mushroom cultivation, the total carbon compounds within the substrate are gradually utilized and reduced. This can be attributed to the genome structure of this mushroom which encompasses over 400 Carbohydrate-Active Enzyme-encoding genes (Li et al., 2022).

As a litter-decomposing fungus, *S. rugosoannulata* primarily decomposes cellulose and hemicellulose (Yang et al., 2022), and has a limited capacity to decompose and utilize lignin. Our results also

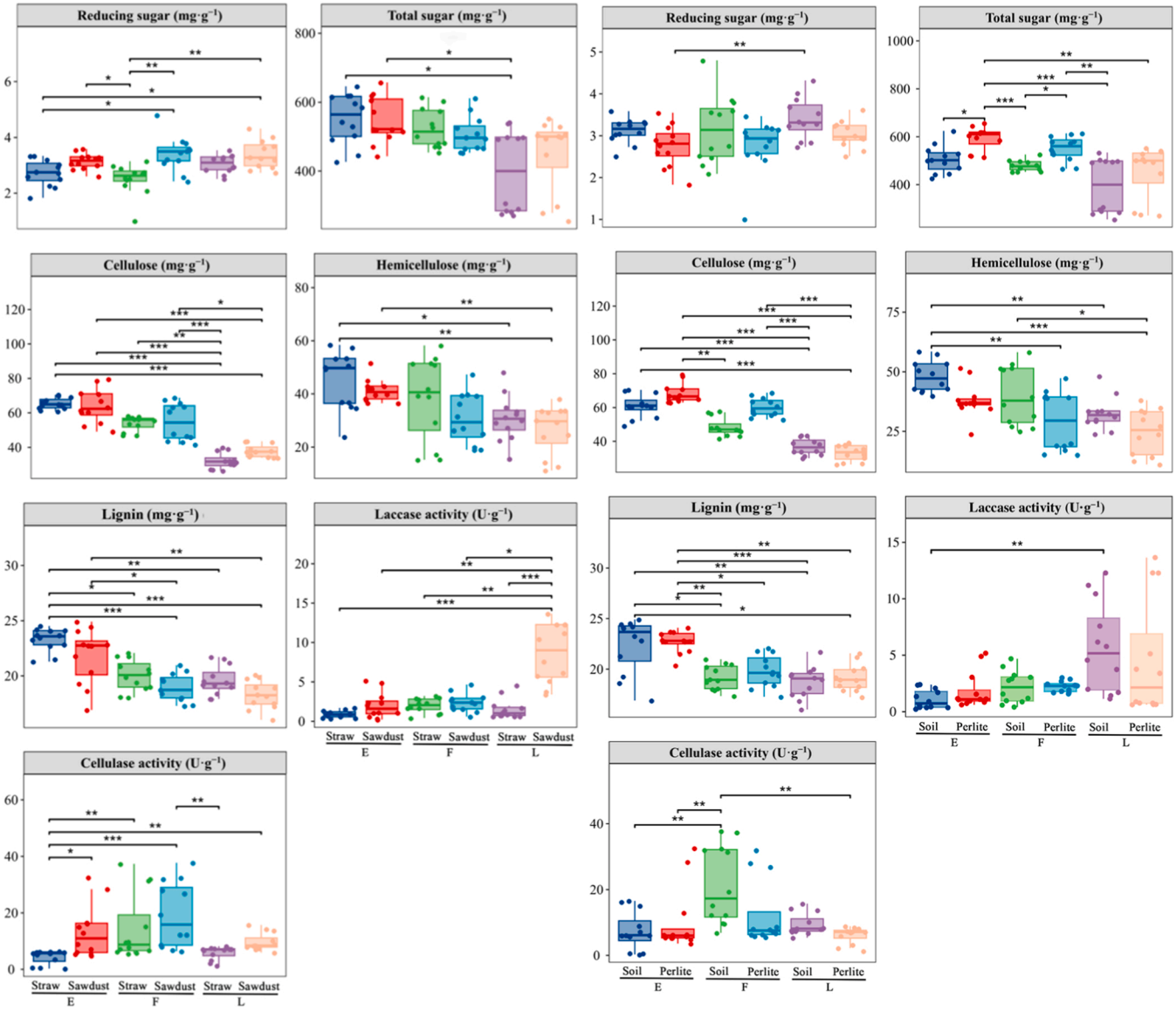

Fig. 2. Changes in total sugar, reducing sugar, carbon-containing components (hemicellulose, cellulose, lignin) and activities of C-degrading enzymes (laccase and cellulase) from different casing materials (A) and mushroom cultivation substrates (B) during *Stropharia rugosoannulata* cultivation, namely the early stage (E), the fruiting stage (F), and the later stage (L). The symbol * represents a significant difference between different treatments. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

demonstrated that the degradation amounts of cellulose and hemicellulose in each treatment are greater than that of lignin (Fig. 1). This might be related to the limited production of ligninolytic enzymes, such as laccase, by *S. rugosoannulata* (Baldrian and Šnajdr, 2006). In our experiment, it was found that the laccase activity of each treatment during the later stage of fruiting is higher than that in the other two periods (Fig. 2). During the later stage of fruiting, the lignin content might remain relatively high, and the substrate contains abundant organic nitrogen derived from fungal hyphae residues, thereby providing a favorable ecological niche for other laccase-producing microorganisms. Consequently, the laccase activity increased while the lignin content decreased in our study.

The cellulase activity during the fruiting period of the treatment with *S. rugosoannulata* inoculation was significantly higher than that in other periods. In contrast, the cellulase activity of the treatment without *S. rugosoannulata* inoculation gradually decreased with the cultivation time (Fig. 2). This demonstrates rapid mushroom fruiting process requiring a high-expression of cellulase. This can be ascribed to the fact

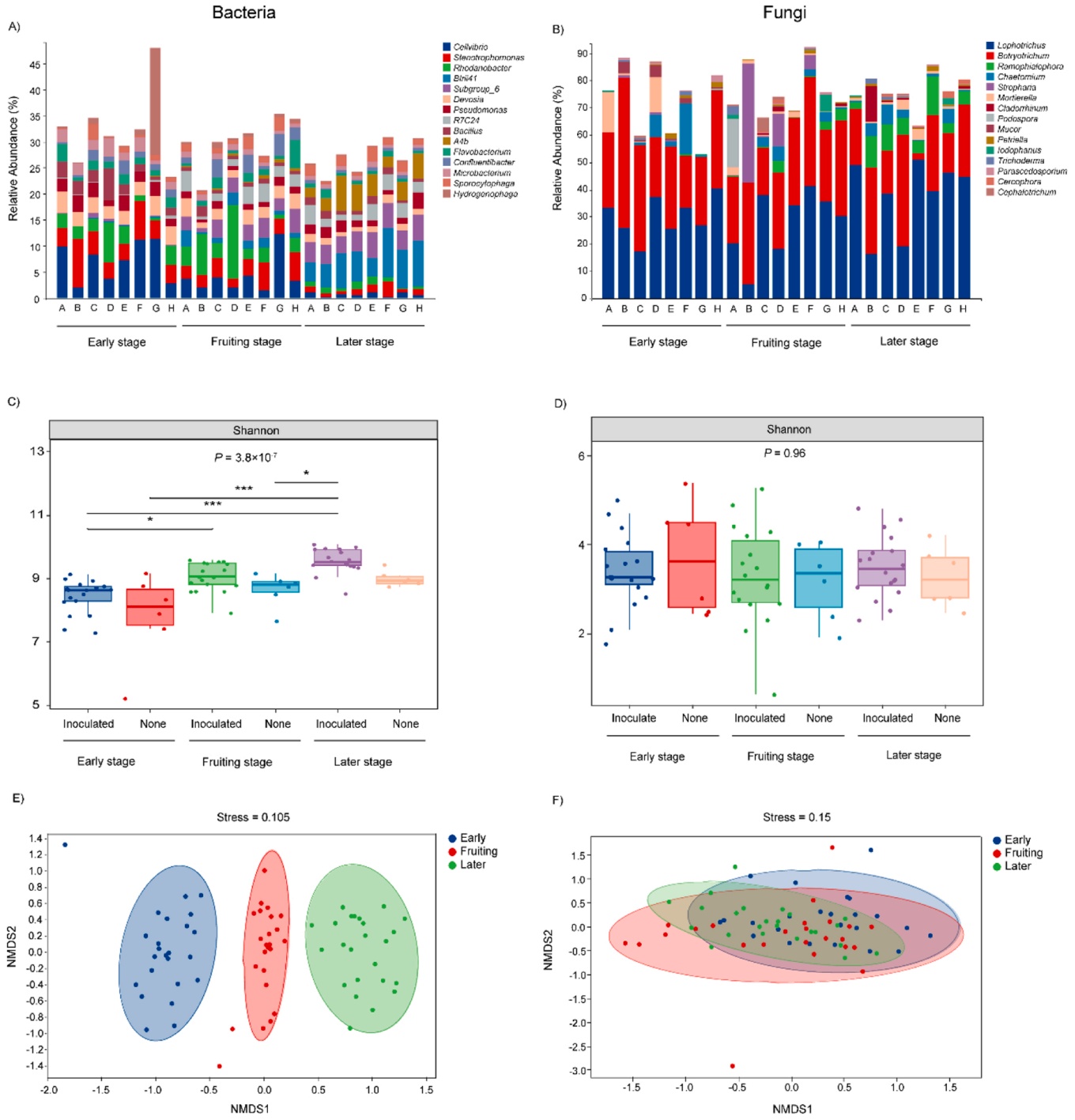

Fig. 3. Variations in microbial genus composition (A, bacteria; B, fungi); Shannon diversity index (C, bacteria; D, fungi) and structure using Nonmetric Multidimensional Scaling (NMDS) ordination (E, bacteria; F, fungi) during cultivation phases (early, fruiting, and late) across a range of substrates. Treatments are as follows: A, Sterilized Soil + Straw; B, Sterilized Soil + Sawdust; C, Soil + Straw; D, Soil + Sawdust; E, Perlite + Straw; F, Perlite + Sawdust; G, Perlite + Straw (inoculated); H, Perlite + Sawdust (not inoculated).

that the higher expression of carbohydrate-enzyme genes during the hyphal stage is associated with substrate degradation (Hao et al., 2022).

3.4. Changes of microbial community structure and diversity during cultivation

During the cultivation process of *S. rugosoannulata*, the dominant bacterial genera were *Cellvibrio*, *Stenotrophomonas*, *Rhodanobacter*, *Devosia*, *Pseudomonas*, and *Bacillus*; the dominant fungal genera are *Lophotrichus*, *Botryotrichum*, *Ramophialophora*, *Chaetomium*, *Stropharia*, *and Mortierella* (Fig. 2a, b). In general, as the inoculation time of *S. rugosoannulata* increased, the Shannon diversity index of bacteria in the cultivation substrate rose gradually and significantly. Moreover, during different periods, the Shannon diversity index of the treatment with *S. rugosoannulata* inoculation was higher than that of the non-inoculation treatment (Fig. 3c). The inoculation of *S. rugosoannulata* enhanced the bacterial diversity within the substrate. It may have generated a nutrient-rich hot zone as demonstrated by the increased organic matter after its cultivation (Gong et al., 2018), thereby recruiting and attracting some eutrophic bacteria.

In this study, the NMDS analysis revealed bacterial communities organization under different treatments (variations in the casing layer and cultivation substrate) during the same period (e.g., before, during, and after fruiting) with high similarities (the ellipse represents the 95 % confidence ellipse of the samples). This may suggest that inoculating mushrooms may have had a positive effect on enhancing the overall bacterial diversity albeit with a limited turnover in the community structure (Fig. 3e). Many previous studies demonstrated the cultivation period of *S. rugosoannulata* significantly affected the structures of microbial communities during composting period (Gong et al., 2018; Hao et al., 2022). The potential reasons for these changes could be that the inoculated *S. rugosoannulata* quickly develops into a dominant community due to favorable micro-environmental conditions, including the substrate, oxygen, water, and pH value. As a result, it substantially impacts the growth and turnover of on other co-existing microbial communities (Carrasco and Preston, 2020).

We observed differences in the dominant bacterial genera during mushroom cultivation periods. Vertically, the heatmap represents the variations in mushroom cultivation period. Horizontally, it depicts the branching of microorganisms based on phylogenetic relationships, where the groups within each branch indicate closer genetic ties with microbes having similar functions (Fig. 4).

The dominant bacterial groups are mainly divided into four branches (Fig. 4). Specifically, in the early fruiting stage, the dominant groups within Branch 1 include *Hydrogenophaga, Bacillus, Microbacterium, Pseudomonas, Devosia, Cellvibrio, Stenotrophonomas*, and *Flavobacterium*. These dominant bacteria focus on carbon-compounds degradations as they have robust abilities to degrade various polysaccharides including cellulose, xylan, and pectin through a diverse array of carbohydrate-active enzymes (Arfi et al., 2014; Gardner et al., 2021).

During the fruiting period, the dominant bacteria was *Rhodanobacter*. Usually, this bacterium demonstrates strong survival capacities by adapting to various environmental conditions that vary with have a wide range of pH and temperature levels (Prakash et al. in 2020). Simultaneously, *Rhodanobacter* strains possess most of the genes necessary for denitrification processes (Kostka et al., 2012). And these denitrifying bacteria may interact with saprophytic fungi and in conjunction enhance the decomposition and transformation of organic matter and a resultant increase in nutrients in their growth zone (Fan et al., 2014). In the later stage of fruiting, the dominant bacteria were *Blrii41, A4b, R7C24, Subgroup 6*, and *Sporocytophaga*. Limited knowledge exists on the succession of these dominant bacterial genus except that *Sporocytophaga* genus consists of highly efficient aerobic bacteria that can decompose various forms of cellulose into simpler substances, making a significant contribution to the carbon cycle in cellulose-rich environments (Taillefer et al., 2018; Yang et al., 2021).

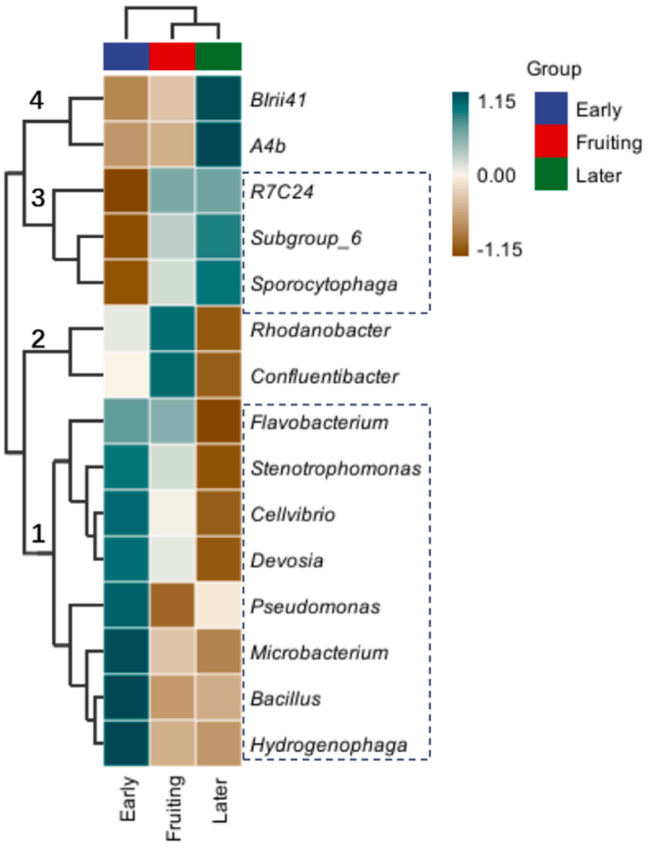

Fig. 4. Hierarchically clustered heatmaps showing variations in bacterial community composition of the top 15 genera across *Stropharia rugosoannulata* cultivation stages. The relative abundances of the genera are the average of triplicated MiSeq sequencing data.

3.5. Relationships between microorganisms and key physicochemical properties, carbohydrates, carbon structural components, and carbon-decomposing enzyme activity

Prior to the fruiting stage of *S. rugosoannulata*, a robust correlation was identified between the bacterial diversity and the carbohydrates present in the compost, with particular emphasis on hemicellulose and reducing sugar (Fig. 5a). This implied a complex ecological association, wherein the carbohydrates were presumably crucial to support the survival of bacterial populations. Hemicellulose, a significant component of the compost, can be enzymatically degraded by specific bacteria, liberating sugars that influence the bacteria's metabolic processes and overall diversity (López-Mondéjar et al., 2016). Reducing sugars, due to their chemically reactive properties, engage in numerous biochemical reactions and pathways, thereby modulating the growth and survival patterns of the bacteria and contributing to the observed correlation (Yoneyama et al., 2007).

Subsequently, as the mycelium proliferates extensively and the fruiting process assumes a predominant role within the substrate. This transformation in the fungal life cycle leads to a weakening of the connections among bacterial communities (Fig. 5b). The underlying causes likely involve alterations in the substrate's physicochemical characteristics, such as fluctuations in nutrient availability, pH levels, and oxygen concentrations. These changes affect the ecological niches and competitive dynamics of the bacterial species.

Additionally, the fungal mycelium may secrete substances that directly or indirectly disrupt the structure and function of the bacterial community, thereby disrupting the previously established correlations (Abeysinghe et al., 2020).

Following the completion of the fruiting stage, the residual

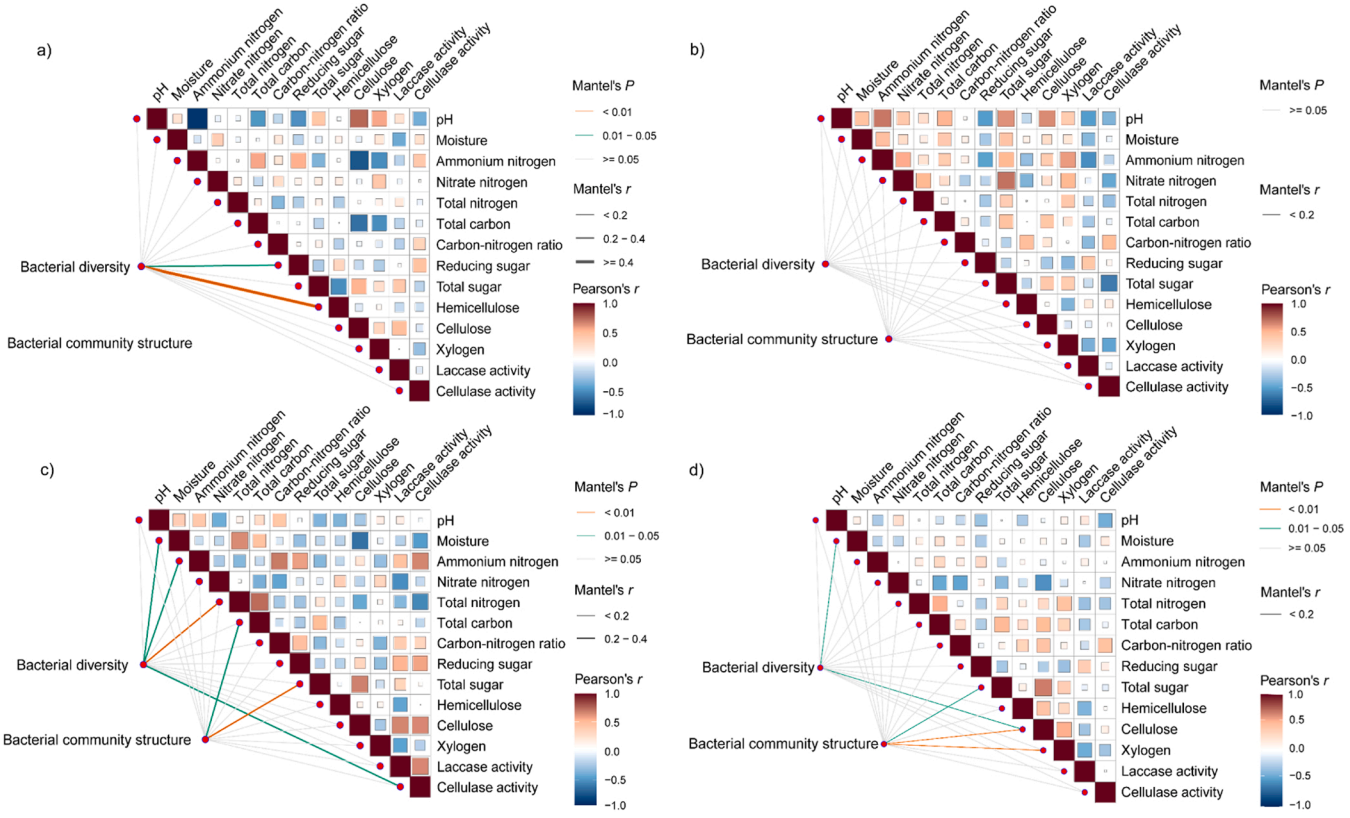

Fig. 5. Mantel test of the correlations between microbial communities and environmental factors during the early stage (A), fruiting stage (B), later stage (C), and the entire process (D) of *Stropharia rugosoannulata* cultivation. Bacterial diversity was assessed using the Shannon diversity index. Changes in community structure are signified by the first representative component derived from the nonmetric multidimensional scaling of the pairwise Bray-Curtis distance. The width of the line is directly proportional to the partial Mantel's *r* statistic, and the color of the line indicates the statistical significance based on 999 permutations. The heatmap presents pairwise comparisons of environmental factors, in which the color gradient and the size of the square denote Pearson's correlation coefficient.

necromass of the mushroom and the degraded substrate components remain. During this post-fruiting period, the bacteria resume their function as active agents of decomposition and nutrient cycling. The significant correlations between the bacterial diversity and elements like moisture, ammonium nitrogen, total nitrogen, and cellulase activity demonstrate the bacteria's crucial role in mediating the transformation of the residual organic matter (Fig. 5c). For instance, moisture affects the transport of nutrients and the occurrence of chemical reactions, as well as the growth and activity of the bacteria. Ammonium nitrogen and total nitrogen are essential for bacterial biosynthesis, and the bacteria regulate their availability and cycling (Kuypers et al., 2018). Cellulase activity reflects the ability of the bacterial community to hydrolyze cellulose-rich substrates to obtain carbon and energy.

Moreover, the levels of total carbon and total sugar were generally the primary determinants of the structure of the bacterial communities (Fig. 5c). The total carbon content provides an indication of the available organic matter, and the total sugar content affected the trophic interactions and metabolic adaptability of the bacterial community (Błońska and Lasota, 2017; Jeckelmann and Erni, 2020). Furthermore, the changes in the composting substrate were closely related to the inherent microbes in the composting units. Nevertheless, the findings of this study demonstrated that during the cultivation of *S. rugosoannulata*, the moisture and cellulose in the compost substrate happen to be the key factors governing the co-existence of bacterial diversity and community structure. Furthermore, moisture modified the physical and chemical microenvironment, and cellulose served as a major carbon source and a constituent of the substrate. Concurrently, the levels of total sugar, cellulose, and lignin had significant impacts on the structure of the bacterial community within the composts.

These components not only defined the resource availability and energetic context but also regulated the competitive and cooperative interactions among the bacterial species, thereby determining the overall community composition and its functional characteristics. A detailed understanding these relationships shall not only enhance our knowledge of the microbial ecology within the context of saprophytic mushroom cultivation but may also give indicators for potential optimization of composting processes at industrial scale while also retaining sustained sustainable agriculture management practices.

3.6. Comparative analysis of protein conversion efficiency among fungal conversion system (Stropharia rugosoannulata), insect system, and conventional livestock system

This work highlights the fungal conversion system of agricultural wastes (specifically that constructed by *Stropharia rugosoannulata*) as a critical component of sustainable agriculture and waste resource utilization. Its core contribution lies in achieving synergistic waste reduction, high-quality protein production, and improved farmers' income through fungal degradation and protein synthesis.

A comparative analysis of protein conversion efficiency—benchmarked against 1 ton of dry substrate/feed—reveals that the *S. rugosoannulata*-based system and insect protein production systems exhibit similar biological efficiency (~60 %) (Schneider et al., 2025; Sideris et al., 2021), significantly exceeding that of traditional animal husbandry (e.g., pig and chicken farming) (Table 3). Both systems also achieve an average protein yield of ~1.5, approximately three times higher than the 0.5 observed in traditional animal husbandry (Table 3).

Notably, detailed analysis of *S. rugosoannulata* confirms it contains 18 essential amino acids (Table S2). Comparative assessments of self-

Table 3
Comparative analysis of protein conversion efficiency: fungal proteins versus insect proteins and conventional livestock protein production (based on 1 ton of dry substrate/feed).

Indicator	Fungal conversion system[a] (Stropharia rugosoannulata)	Insect conversion system[b] (Hermetia illucens)	Conventional livestock husbandry[c]
Biological efficiency	40 %-85 % (62 % on average)	50 %-70 % (60 % on average)	10 %-25 % (15 % on average)
Dry protein yield	9.6–20.4 kg	12.5–17.5 kg	3.0–7.5 kg
Protein productivity	0.96 %-2.04 % (1.3 %-1.5 % on average)	1.25 %-1.75 % (1.5 % on average)	0.3 %-0.75 % (0.5 % on average)
Resource conversion traits	Organic matter utilization rate: 70 %-90 % Energy consumption: ≤ 0.5 kWh/kg fresh fungal product (mushrooms)	Organic matter utilization rate: 50 %-70 % Energy consumption: 1.2–2.0 kWh/kg fresh insect	Organic matter utilization rate: 20 %-40 % Energy consumption: 5–8 kWh/kg product
Environmental friendliness	Carbon emissions reduced by 60 %-70 % and water consumption reduced by 40 %-50 % compared to conventional livestock	Carbon emissions reduced by 40 %-50 % and water consumption reduced by 30 %-40 % compared to conventional livestock	Set as a reference benchmark for comparing carbon emissions and water consumption.

Notes: Fungal conversion system[a], this refers to a system involving saprophytic macrofungi, which act as decomposers to convert and utilize agricultural, forestry, and pastoral wastes (e.g., crop straws, livestock manure) for the production of fruiting bodies. A representative example is *Stropharia rugosoannulata*, a globally recognized rare edible saprophytic macrofungus highlighted in this experiment; Insect conversion system[b], this denotes a self-reproductive conversion system centered on insects. *Hermetia illucens* (black soldier fly) is selected as the representative, given its status as an efficient protein-converting insect that has garnered widespread attention in sustainable protein production and organic waste utilization in recent years. Its larvae can efficiently transform various low-value agricultural and forestry organics into protein-rich (and fat-containing) biomass, serving as a critical source of animal feed and industrial raw materials; Conventional livestock husbandry[c], this system, included for comparison with the first two, refers to traditional animal farming (e.g., sheep, pig, and chicken rearing). Its protein output—calculated based on 1 ton of dry feed—is compared with that of the fungal and insect systems. As the most commonly adopted protein production model in agricultural, forestry, and pastoral ecosystems, it serves as a baseline for evaluating the efficiency of alternative systems.

cultivated and commercially sourced samples show an average total amino acid content of ~1.5 g per 100 g of *S. rugosoannulata* mushrooms (Table S2). From a nutritional perspective, *S. rugosoannulata* has a balanced composition of essential amino acids (Wang et al., 2025), and its nutritional value is comparable to that of insect protein (Schneider et al., 2025).

Beyond comparable biological efficiency and protein yield to insect systems, the fungal conversion system demonstrates superior resource conversion and environmental performance: it achieves 70–90 % organic matter utilization (Heines et al., 2022), consumes nearly 50 % less energy, and reduces carbon emissions and water use by ~10 % relative to insect-based production. These attributes collectively highlight the multifaceted advantages of *S. rugosoannulata*. While this study demonstrates the species' high biological efficiency and protein productivity under optimized cultivation conditions, it is important to note that the reported environmental performance metrics—specifically, reductions in carbon emissions and water use—are based on comparative estimates derived from existing literature (Flachowsky, 2002; Schneider et al., 2025; Sideris et al., 2021), rather than direct experimental data from the present work. Nevertheless, the cultivation process of *S. rugosoannulata* offers significant ecological benefits, including the reduction of agricultural waste—particularly straw, which can be decreased by over 50 %—and the enhancement of soil fertility (Gong et al., 2018). Furthermore, its fruiting bodies possess functional properties that render them suitable for use as functional foods or feed additives. These combined advantages underscore the species' potential value in promoting sustainable ecological agriculture (Huang et al., 2023; Wang et al., 2025; Zhang et al., 2023).

4. Conclusion

In this study we explored one possible route for adding value to agroforestry waste by examining whether combined adjustment of the substrate, casing microbiome and enzyme environment could improve the yield of *Stropharia rugosoannulata*. Under the specific conditions tested, a sawdust–cow-manure substrate overlain with non-sterile loam gave a mean biological efficiency of 42.9 %, compared with ~14 % observed for a conventional straw-based mix. This modest increment coincided with a higher relative abundance of cellulolytic bacteria (*Cellvibrio*, *Stenotrophomonas*) and with somewhat more persistent cellulase activity, suggesting that faster hemicellulose/cellulose turnover may have contributed additional soluble carbon. Mass-balance calculations indicated that 70–90 % of the initial organic matter was converted, while estimated greenhouse-gas and water requirements appeared lower than published coefficients for livestock protein. The protocol can be implemented on small, low-input farms, although further validation under contrasting climates and management regimes will be needed before any wider recommendation can be made.

CRediT authorship contribution statement

Chengmo Yang: Project administration, Investigation, Conceptualization. **Kaixuan Zhang:** Methodology, Investigation, Conceptualization. **Fuqiang Yu:** Writing – review & editing, Supervision, Project administration, Funding acquisition. **Tingting Dou:** Methodology, Data curation. **Xiaofei Shi:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Ziyan Zhou:** Validation, Methodology, Data curation. **Fengming Zhang:** Methodology, Formal analysis. **Yan He:** Resources, Formal analysis, Conceptualization. **Shimei Yang:** Project administration, Investigation, Data curation, Conceptualization. **Jishao Jiang:** Resources, Methodology, Data curation. **Parag Bhople:** Writing – review & editing, Resources, Conceptualization. **Jianou Gao:** Methodology, Investigation, Conceptualization. **Dong Liu:** Writing – review & editing, Writing – original draft, Supervision. **Zhenyan Yang:** Resources, Project administration, Investigation. **Chater Caspar C. C.:** Writing – review & editing, Supervision.

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.

Acknowledgments

The authors acknowledge the support received from the Germplasm Bank of Wild Species, Kunming Institute of Botany, Chinese Academy of Sciences. This work received funding from the 'Strategic Priority Research Program' of the Chinese Academy of Sciences (XDA26050302), the Yunnan Revitalization Talent Support Program 'Young Talent' Project (YNQR-QNRC-2019–025), the Yunnan Technology Innovation Program (202205AD160036), the Clean Conversion of Agricultural Waste and Efficient Recycling through Edible Mushroom Cultivation

(2023520000240179), and the Yunnan Revitalization Talent Support Program awarded to Jesus Pérez Moreno and Xinhua He.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.indcrop.2026.122656.

Data availability

Data will be made available on request.

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