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The Use of *Agaricus subrufescens* for Rehabilitation of Agricultural Soils

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Abstract: Globally, the quality of agricultural soils is in decline as a result of mismanagement and the overuse of agrichemicals, negatively impacting crop yields. *Agaricus subrufescens* Peck is widely cultivated as an edible and medicinal mushroom; however, its application in soil bioremediation and amendment remains insufficiently studied. In order to determine if *A. subrufescens* can positively impact agricultural soils, we designed two experiments: the first, a glasshouse experiment investigating the ways in which *A. subrufescens* production alters soil nutrients and soil health; the second, a laboratory experiment investigating if *A. subrufescens* can degrade beta-cypermethrin (β-CY) and glufosinate ammonium (Gla), two widely used agrichemicals. The glasshouse experiment results indicated that the use of compost and compost combined with *A. subrufescens* led to increases in soil organic matter, nitrogen, phosphorus, and potassium compared to the control treatments (sterilized soil). However, the incorporation of *A. subrufescens* with compost resulted in significantly greater levels of both available nitrogen and available phosphorus in the soils compared to all other treatments. Laboratory experiments determined that the mycelium of *A. subrufescens* were unable to grow at concentrations above 24.71 μg/mL and 63.15 μg/g for β-CY and Gla, respectively. Furthermore, results indicated that fungal mycelia were able to degrade 44.68% of β-CY within 15 days, whereas no significant changes were found in the concentration of Gla. This study highlights that cultivation of *A. subrufescens* may be a sustainable alternative for the rehabilitation of agricultural soils, whilst providing an additional source of income for farmers.

Keywords: bioremediation; compost; hyphae; mushroom cultivation; soil quality

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1. Introduction

Soil is a prerequisite for maintaining the normal operations of terrestrial ecosystems [1,2]. Soil quality directly or indirectly affects plant growth [3], food production [4,5], and human health [6]. Land degradation results from agrichemicals entering irrigation channels and irrational farming systems negatively impact soil ecosystems [7–9]. Furthermore, a number of pesticides and herbicides, which are widely used in agricultural fields, are accumulating in the soil and water systems associated with farming areas [10,11]. The two main classes of agrichemicals used are organophosphates and organochlorine compounds [12]. Both are used as prophylactic treatments (insecticide or herbicide) to maintain high agricultural production [13].

Glufosinate ammonium (Gla) is a broad-spectrum organophosphate-based herbicide used to manage glyphosate-resistant weeds [14–16]. Its toxicity towards the mammal

has been attributed to ammonia accumulation in organs [17]. Glufosinate ammonium leaching poses a risk to groundwater systems and aquatic organisms due to its high water solubility [18–21]. In soils, Gla is mainly degraded to 3-methylphosphinico-propionic acid (MPP), which may undergo further degradation into 2-methylphosphinico-acetic acid (MPA) [22]. MPP and MPA are reported to possess acute toxicity to mammals such as rats, rabbits, and dogs, but their toxic capacity remains lower compared with Gla [23]. Although Gla is mostly degraded by microorganisms in soil, the degradation of specific functional fungi on Gla has not been studied.

Pyrethroids are one of the main forms of organochlorine pesticides and are a major component of insecticides such as imiprothrin and cypermethrin. Pyrethroids have started replacing organophosphate and carbamates because they have a higher insect-to-mammal toxic ratio, thus less risk to human health [24]. Beta-cypermethrin (β-CY), a type of pyrethroid insecticide, is widely used to control pests that infest crops such as tea, orange, cotton, apple, cowpea, and brassicaceous vegetables [25]. Although β-CY is considered safe for mammals, it is increasingly viewed as an environmental risk owing to its high toxicity to aquatic organisms [26]. Application of pyrethroid pesticides began occurring much later than organophosphate-based pesticides, and the degradation of pyrethroid by microorganisms has yet to be rigorously studied [27].

Although pesticides can have acute toxic effects on natural organisms, they are mainly degraded by plants and microorganisms in the environment [28]. Microorganisms, and in particular fungi, dominate the biodegradation pathways [29]. Bioremediation with fungi, known as mycoremediation, is an innovative biotechnological approach to cleaning up pollutants through absorption or mineralization, facilitated via distinct fungal enzymes [30,31]. The screening of functional species or strains is pivotal to the sustainability of the mycoremediation process [32]. *Agaricus subrufescens* is considered a prized edible and medicinal macrofungal species which is cultivatable in the field [33,34]. Optimal conditions for the cultivation of *Agaricus subrufescens* normally include a temperature range of of 25–28 °C for mycelial growth and 22–25 °C for fruiting; humidity levels between 60–70% for the substrate and 80–85% for the ambient air, for the formation of fruitbodies [33]. In addition, *A. subrufescens* has received increased attention due to its sought-after taste and range of medicinal properties, which include: tumor growth reduction, immunomodulatory activities, immunostimulatory effects, and antimicrobial, antiviral, and antiallergy activities [33,35]. The biological efficiency (as a measure of yield) of cultivated strains of *A. subrufescens* lies between 7–29% [35]. According to previous research, *A. subrufescens* has been utilized in food production systems which recycle agricultural waste products [36] and remediate accumulated cadmium in heavy metal-contaminated soils [37]. Moreover, spent *A. subrufescens* compost was able to be used as fertilizer for crops, thus further enhancing agricultural activities [38,39]. However, no research exists regarding the use of *A. subrufescens* for rehabilitating agricultural soils nor regarding exploiting the potential of *A. subrufescens* mycelium for degrading organophosphate or organochlorine-based agrochemicals. Although the clinical toxicity of pesticides on terrestrial animals, aquatic life and humans has been well studied [40–45], the effects on fungal mycelium growth have been understudied, and moreover, studies investigating pesticide toxicity on macrofungal cultivars are almost nonexistent.

Field cultivation of mushrooms can provide food and income resources for rural communities. Previous studies focused on the domestication of novel fungal strains and increasing product diversification or developing cultivation techniques to increase crop yield. However, the use of field-based mushroom cultivation for the associated environmental benefits remains less studied, and in addition, the effects of cultivating fungi on soil improvement and degradation of pollutants are unclear. The purpose of the present study was to evaluate the extent to which *A. subrufescens* could rehabilitate agricultural soils. First, we conducted pilot glasshouse experiments investigating whether the field cultivation of *A. subrufescens* production improved the soil nutrient status of the soils it is cultivated on. Secondly, we tested the impact of two widely used agrichemicals (Gla- and

β-CY), using the "no observed effect concentration" (NOEC), on the mycelium growth of *A. subrufescens*, and whether or not *A. subrufescens* could degrade either of these chemicals.

2. Materials and Methods

2.1. Strain

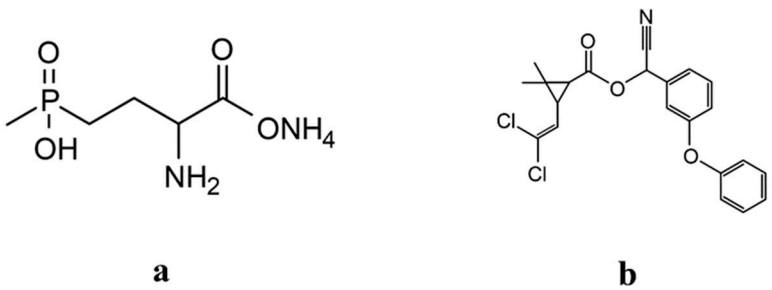
The *A. subrufescens* strain ASKIB1, from the culture collection of Kunming Institute of Botany, Chinese Academy of Sciences, was used for the purposes of our study. The fungus was sub-cultured using malt extract agar medium and then incubated at 28 °C. The sub-cultured Petri plates with fungal mycelium were prepared for identification and further use. In order to ensure we were working with the correct strain; we identified the sub-cultured specimens using internal transcribed spacer (ITS) sequence analysis. Total genomic DNA of fungal culture was extracted using DNA extraction kits (Bioer Technology Co., Ltd., Hangzhou, China). Universal primers ITS5 and ITS4 were used to amplify the complete nrITS region [46]. PCR reactions were carried out following Hu et al. [47]. The amplicons were sent to Kunming TSINGKE Biological Technology Co., Ltd. for purification and sequencing using the same primer pair in PCR reaction. The assembled ITS sequence was compared with genes available in the GeneBank Nucleotide library through a BLAST search on the National Center for Biotechnology Information (NCBI) website. BLAST results showed that the sequence yielded from this study features 100% identity with *A. subrufescens* strain JSR3, WC837-S04, and 631. The completed sequence was submitted in NCBI under accession number OM095463.

2.2. Solid Spawn and Liquid Media Preparation

Agaricus subrufescens mycelium, grown on a malt extract agar medium in sub-cultured Petri plates, was transferred to sterilized spawn bottles with 200 g of cooked wheat grain and incubated at 28 ± 1 °C. It required 28–33 days for mycelium to thoroughly colonize the wheat grain. Fully colonized solid spawn bottles were stored at 25 °C for further use. Liquid media were prepared using malt extract broth (MEB). 100 mL of dissolved MEB media were prepared and transferred to 250 mL flasks, followed by sterilization at 121 °C and 0.1 Mpa for 30 min. After sterilization, flasks were transferred to a laminar flow cabinet for cooling down and further use.

2.3. Chemicals

Beta-cypermethrin (β-CY) and glufosinate ammonium (Gla) were purchased from Jiangmen Daguangming Chemical Co., Ltd., Jiangmen, China. According to instructions, the threshold concentrations for β-CY and Gla in agricultural activities are 0.1% and 0.8%, respectively. The chemical structures of both pesticides are shown in Figure 1 [23,48].



a **b**

Figure 1. The active ingredients in (**a**) glufosinate ammonium (Gla) and (**b**) beta-cypermethrin (β-CY), representing the two pesticides used in this study.

2.4. Glasshouse Experiment

2.4.1. Cultivation

A total of 800 kg of dry raw materials were prepared for composting, and the 30-day composting process followed the methods employed by [49] (Figure 2a). The compost

formula for our mushroom production was as follows: rice straw 44%, corncobs 40%, cow manure 6%, wheat bran 6%, gypsum 1%, calcium carbonate 1%, urea 1%, and $CaP_2H_4O_8$ 1%; after composting, four samples of compost were taken to test the chemical composition. Incubation soil was transported from bulk soil taken 30–120 cm deep from an abandoned agricultural land at Panlong District, Kunming, China. Four soil samples were collected from the incubation soil and placed inside Ziploc bags (170 mm × 240 mm). Four compost samples and four soil samples were brought back to the lab for the determination of chemical properties.

Figure 2. The glasshouse experiment investigating the effects of cultivating *Agaricus subrufescens*, and subsequent basidiocarp production, for soil amendment. (**a**) composting process; (**b**) experimental layout from the four different treatments; (**c**) covering of the different treatments during the mycelial colonization of the compost; (**d**) unmatured basidiocarps; (**e**) mature basidiocarps; (**f**) soil and compost profile during the incubation period. Scare bar: (**d**,**e**), 5 cm; (**f**), 8 cm.

The glasshouse experiment was conducted on lattice steel frames in a semi-automatic-controlled glasshouse at the Kunming Institute of Botany, Chinese Academy of Sciences, from April to September 2021. A total of 36 grids, with each grid comprising 0.60 m^2 (1.71 m × 0.35 m), were constructed on the lattice steel frames. Four different treatments were designed for the glasshouse experiment, viz. soil (S); sterilized soil (SS); soil and compost (SC); and soil, compost and *Agaricus subrufescens* (SCA) (Table 1).

Table 1. Information of the four treatments in the glasshouse experiment.

Treatment Name	Basic Protocol	Abbreviation
Sterilized soil	15 cm of autoclaved (121 °C for 2 h) soil laid over grids	SS
Soil	15 cm of incubation soil laid over grids	S
Soil and compost	15 cm of incubation soil laid over grids and covered by 12 kg of compost	SC
Soil, compost and *Agaricus subrufescens*	15 cm of incubation soil laid over grids and covered by 12 kg of compost, followed by inoculating 2 containers of solid *A. subrufescens* spawn in the compost	SCA

Each treatment included nine replicates for a total of 36 grids used for the experiment. The design was conducted using a completely randomized block design [50] (Figure 2b). After the incubation soil and composts of all treatments were placed on the grids, dark garden fabrics were next used to cover the lattice steel frames for maintaining the moisture of the compost and soil; the relative humidity of the lattice steel frames was maintained at 70–80%, and the compost temperature was maintained at 20–25 °C for one month until the mycelium of *A. subrufescens* thoroughly colonized the compost in the SCA group (Figure 2c). Next, the garden fabrics were removed, and 8 cm of incubation soil was used to cover the SC and SCA groups, acting as a soil casing layer for basidiocarp production. Additionally, climate variability can impact soil fluxes, evapotranspiration and nutrient availability, ultimately affecting yield [51,52], thus, it is essential to record the temperature and humidity during the whole incubation process and ensure a stable growing environment. The temperature and humidity of the semi-automatic control glasshouse were recorded as 15–36 °C and 40–93%, respectively. Throughout the entire incubation process, each grid received 800 mL of water once per day to ensure humidity levels met conditions for fostering fungal mycelium growth. The soil temperature and humidity of all treatments were recorded as 16–28 °C and 62–96%, respectively.

2.4.2. Basidiocarp Production and Yield Record

All basidiocarps were harvested after reaching maturity (Figure 2e). The soil and compost profile of the glasshouse experiment during the fruiting stage was captured using a camera (Figure 2f). Incubation soil from the compost-soil touching layer was collected and checked under the scanning electron microscope (SEM) for characteristics of *A. subrufescens* mycelium. Fresh basidiocarps were harvested and weighed. After recording the fresh weight, basidiocarps were dehydrated at 35 °C for 48 h in an oven, dry weight was recorded, and dried basidiocarps were stored in Ziploc bags in a dark room. Dried basidiocarps of *A. subrufescens* were used to quantify the nutrient content. The biological efficiency was calculated as the fresh weight of basidiocarps per dry weight of the substrate used.

2.4.3. Soil Sampling and Chemical Analysis

Soil samples were collected three times. The first sample was taken on the day before inoculation and served as baseline soil data (day 0). The second sample was taken on the 60th day after the mycelium of *A. subrufescens* thoroughly colonized the substrate (day 60). At this stage, robust basidiocarp production was noted. The third sample was taken on the 120th day (day 120). At this stage, basidiocarp production had ceased in all grids. Soil samples were taken at a depth of 0–10 cm for each plot, after gently removing the soil casing and substrate layers. Soil samples from the four treatments were taken, consisting of nine replicates of soil samples from each treatment for a total of 36 soil samples. Sampling equipment, including mesh, scalpels, and spoons, was cleaned with 95% alcohol, followed by heating with an alcohol lamp for 30 s. Soil collections among samples were conducted after all equipment was sterilized and cooled. Each soil sample was sieved through a 2 mm mesh. Next, all soil samples were manually homogenized and divided into two parts. The first portion was stored in plastic bags for water content measurement, and the second portion was stored at 4 °C for 24 h and then dried at 25 °C for chemical analysis.

2.4.4. Mushroom Yield and Productivity

Mushroom yield included the number of basidiocarps, fresh weight, and dry weight of each grid (0.6 m^2) within the whole cropping period. Productivity was expressed in terms of biological efficiency (BE); the equation for determining BE was as follows: %BE = (fresh weight of mushrooms for each grid from the whole cropping period)/(dry weight of substrate) × 100.

2.4.5. Quantification of Nutrient Content

The dried basidiocarps of *A. subrufescens* were powdered using a blender, and powdered samples were used for the quantification of nutrient content. Ash, crude fat, crude fiber, and crude protein were analyzed based on guidelines set by the Association of Official Analytical Chemists [53]. Ash, crude fat, crude fiber, and crude protein contents were characterized based on Bandara et al. [54]. Carbohydrate content was determined using the difference method, and the calculation used to determine the carbohydrate content was as follows: Carbohydrates (%) = 100 − (ash (%) + crude fat (%) + crude fiber (%) + crude protein (%)). Energy values were calculated as follows: Energy value (kcal/100 g) = 4 × (g of protein + g of carbohydrates) + 9 × (g of fat).

2.4.6. Compost and Soil Parameters

Chemical properties of all soil samples were analyzed based on the following method: the pH value was determined in a 1:2.5 (soil to water) ratio using a pH meter with a standard combination of electrodes [55]. Soil water content (SWC) was determined using the oven-drying method [55]. Soil organic matter content (SOM) was determined through the potassium dichromate sulfuric acid oxidation method [56]. Total nitrogen (TN) was measured using Kjeldahl's method as modified by Bremner [57]. Alkali solution nitrogen (AN) was determined using the alkali-soluble diffusion method [58]. Total phosphorus content (TP) was measured using the ammonium molybdate spectrophotometric method [59]. Available phosphorus content (AP) was measured using the ammonium molybdate spectrophotometric method, following hydrochloric sulfuric acid leaching [59]. Total potassium content (TK) and available potassium (AK) were measured using cobaltinitrite method [60]. Lab analyses for each soil sample were repeated three times, and final data values were averaged.

The physio-chemical properties of the compost and soil used in the glasshouse experiment are described in Table 2. Compost and soil physio-chemical properties were used as the baseline in the soil rehabilitation experiment.

Table 2. Physio-chemical composition of the compost and soil prepared for the *Agaricus subrufescens* glasshouse experiment.

Parameter	Soil	Compost
pH value	6.77 ± 0.33	7.52 ± 0.31
Water content	20.77 ± 1.20%	53.57 ± 1.38%
Organic matter	5.44 ± 2.74 g/kg	70.45 ± 3.88%
Total nitrogen	0.54 ± 0.14 g/kg	1.34 ± 0.08%
Total phosphorus	0.71 ± 0.03 g/kg	0.39 ± 0.03%
Total potassium	4.91 ± 0.16 g/kg	1.32 ± 0.02%
Available nitrogen	25.95 ± 1.14 mg/kg	nd
Available phosphorus	3.25 ± 1.02 mg/kg	nd
Available potassium	53.45 ± 14.68 mg/kg	nd

Notes: The values were expressed as mean ± standard deviation; nd, not determined.

2.5. Liquid Culture Experiment

2.5.1. Liquid Culture Preparation

To test the toxicity of β-CY and Gla on *A. subrufescens* mycelium, liquid media was used to dissolve β-CY and Gla under different concentrations. Given the thresholds of 0.1% and 0.8% for β-CY and Gla in agricultural soils, respectively, we designed eight different concentrations, viz. 1%, 0.8%, 0.4%, 0.1%, 0.05%, 0.01%, 0.005%, and 0% (as control), for both β-CY and Gla. A total of 48 flasks (250 mL) with 100 mL liquid media, including eight concentrations, two pesticides, and three replicates. After all liquid media were dissolved with β-CY and Gla at different concentrations in different flasks, flasks were incubated at 25 °C, 150 rpm for 30 min to ensure full chemical dissolution. Next, *A. subrufescens* mycelium grown on the malt agar medium was transferred to each flask in a laminar flow

cabinet. Ten circular mycelium plugs (6 mm in diameter) were transferred to 250 mL flasks with 100 mL media and incubated in a shaker at 28 ± 1 °C, 120 rpm for growth and toxicity tests. The growth conditions of all 36 flasks were recorded during the incubation period. After the acute toxicity test, the no observed effect concentrations (NOEC) for β-CY and Gla were determined and measured separately.

After the NOEC of β-CY and Gla were obtained, 25 empty 150mL flasks were prepared for the degradation experiment. All 25 flasks with 100 mL MEB medium were sterilized at 121 °C and 0.1 Mpa for 30 min and cooled in a laminar flow cabinet. Out of 25 flasks, 10 flasks with the medium were dissolved with β-CY at NOEC (C1-C10), 10 flasks with the medium were dissolved with Gla at NOEC (G1-G10), and another 5 flasks only containing the medium were preserved as blank controls (B1-B5). All 25 flasks were incubated at 25 °C, 150 rpm for 30 min to fully dissolve the chemicals. After 25 flasks were incubated, the *A. subrufescens* mycelium grown on the malt agar medium was transferred to 15 flasks (C1-C5, G1-G5, and B1-B5) in a laminar flow cabinet. Ten circular mycelium plugs grown on malt extract agar (6 mm in diameter) were transferred to 15 flasks with 100 mL media. Ten flasks (C6-C10 and G6-G10) without mycelium inoculation were preserved as negative controls. All 25 flasks were incubated in a shaker at 28 ± 1 °C, 120 rpm for the degradation experiment.

2.5.2. Liquid Culture Sampling and Chemical Analysis

Liquid culture media was sampled twice. The first sample time refers to the first day of dissolving β-CY and Gla as well as inoculating the fungal mycelium in the flask media. Moreover, we supplemented all labels of 25 samples with -0 (C1-0 to C10-0, G1-0 to G10-0, and B1-0 to B5-0). The second sampling time refers to the last day when all mycelium had thoroughly colonized the media and white mycelium balls were produced evenly across 15 flasks. Furthermore, we supplemented all labels of 25 samples with -1 (C1-1 to C10-1, G1-1 to G10-1, and B1-1 to B5-1). A sterilized pipette was used to extract 10 mL solution from each flask before being transferred to sterilized 15 mL sample tubes in a laminar flow cabinet, followed by preserving all sample tubes in a 4 °C refrigerator. Sample storage never exceeded three days before chemical analysis. Among 50 samples, 20 samples were used to test the concentration of β-CY (C1-0 to C10-0 and C1-1 to C10-1); 20 samples were used to test the concentration of Gla (G1-0 to G10-0 and G1-1 to G10-1); and 10 samples were used to test the concentration of both β-CY and Gla (B1-0 to B5-0 and B1-1 to B5-1).

High-performance liquid chromatography (HPLC, LC-100)-grade acetonitrile used in the determination of chemicals was purchased from Shanghai Weipu Chemical Technology Service Co., Ltd. Mixed working standard solutions of β-CY and Gla in different concentrations were also prepared, respectively. HPLC and the protocols for concentration analysis followed Carazo-Rojas et al. [61] for β-CY and Yan et al. [62] for Gla.

2.5.3. Degradation Rate

The rate of pesticide degradation was calculated according to the following equation:

$$DC(\%) = (C0 - C1)/C0 \times 100\% \quad (1)$$

$$DG(\%) = (G0 - G1)/G0 \times 100\% \quad (2)$$

where DC is the degradation rate of β-CY and DG is the degradation rate of Gla; C1 is the residual concentration of β-CY in the sample solution (mg/L) and C0 is the initial concentration of β-CY at time zero; G1 is the residual concentration of Gla in the sample solution (mg/L) and G0 is the initial concentration of Gla at time zero.

2.6. Data Analysis

Raw chemical property data of soil samples were processed with Excel 2010, and results were expressed by the mean ± standard deviation. Statistical significance was determined via one-way analysis of variance (ANOVA), followed by Dunnett's T3 post

hoc test at $p < 0.05$ in SPSS version 19.0. The visualization of degradation of β-CY and Gla, and dynamic changes in soil chemical properties were calculated and plotted in GraphPad Prism version 8.0.2.

3. Results

3.1. Glasshouse Experiment

3.1.1. Yield and Nutrient Properties of Basidiocarps

During the incubation and fruiting period, fungal mycelium grew from compost to soil. SEM images from incubation soil show the characteristics of fungal hyphae of *A. subrufescens* (Figure 3a). The hyphae gathered from rod-like cells and presented as crystalline and fragile. The mycelium connected with soil particles, indicating the intimate relationship between fungi and soil in the soil system during the basidiocarp fruiting stage (Figure 3b–d).

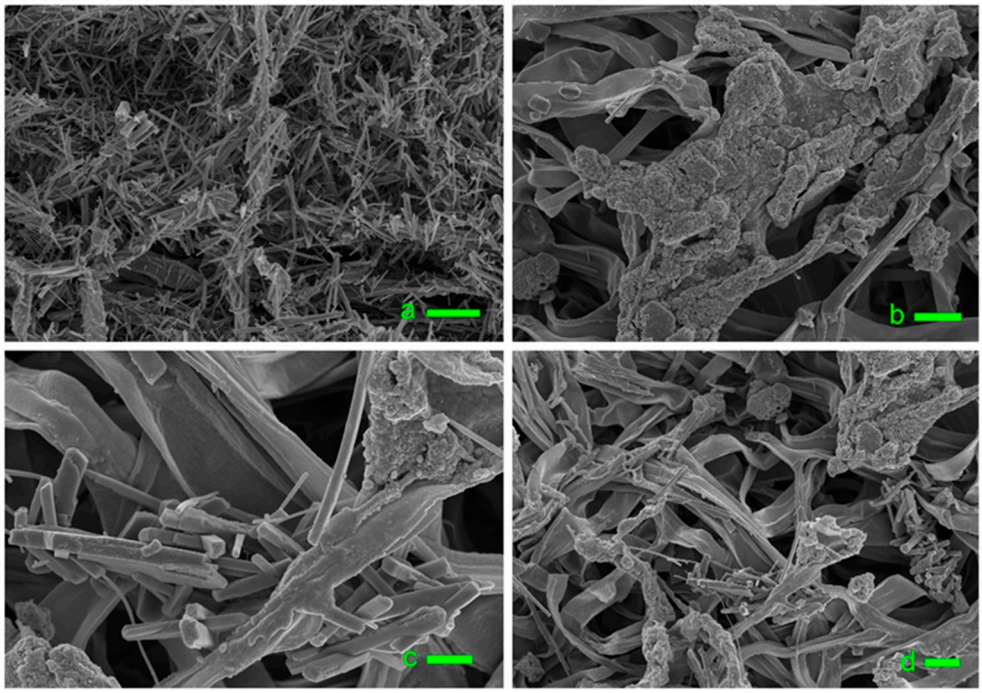


Figure 3. Cord-forming mycelial network at compost-soil touching layer during the pilot glasshouse experiment of *Agaricus subrufescens* under SEM. Photos taken from soil, compost, and *A. subrufescens* group (SCA) on day 55; (**a**) crystalline mycelium; (**b**) soil particles; (**c**,**d**) hyphae-soil connection; Scare bar: a 10 μm; (**b**,**d**) 5 μm.

The primordia formed on day 52 after spawn inoculation, and the yield was recorded from day 55 to day 146. The time duration from the first primordia emergence until the maturation of basidiocarps spanned 5–6 days, while the subsequent time duration was 3–4 days. The average number of basidiocarps harvested in each grid was 21.9; the average yield and biological efficiency of each grid were 1002.90 g and 20.10 %, respectively (Table 3). The highest content of proximate composition was carbohydrates (53.80%), followed by crude protein (31.53%). The lowest composition was crude fat (1.33%). The ash and crude fiber content were 7.73% and 5.60, respectively.

Table 3. Basidiocarps yield, biological efficiency, proximate composition, and energy of *Agaricus subrufescens*.

Parameter	*Agaricus subrufescens* Yield and Nutrition
Number of basidiocarps	21.90 ± 9.70
Fresh weight (g)	1002.90 ± 488.60
Dry weight (g)	177.40 ± 82.80
Biological efficiency (%)	20.10 ± 10.70
Ash (%)	7.73 ± 0.31
Crude fat (%)	1.33 ± 0.29
Crude fiber (%)	5.60 ± 1.82
Crude protein (%)	31.53 ± 0.91
Carbohydrates (%)	53.80 ± 2.25
Energy (kcal/100 g)	353.33 ± 9.06

Notes: proximate composition represented as % of dry weight.

3.1.2. Soil Chemical Properties during the Glasshouse Experiment

There were significant differences among the incubation soil of sterilized soil (SS), soil (S), soil and compost (SC), and soil, compost, and *A. subrufescens* (SCA) with respect to most chemical properties on day 60 and day 120 (Figure 4). The pH value of all soil samples was significantly lower on day 120 (6.66 ± 0.52 for SS; 6.46 ± 0.07 for S; 6.45 ± 0.09 for SC; 6.49 ± 0.07 for SCA) than the soil at day 0 (6.77 ± 0.33). The pH value was higher significantly in SS soils than S soils on both day 60 and day 120, while the SOM (4.10 ± 0.91 g/kg) was lower significantly in SS soils than S soils (6.36 ± 1.41 g/kg). Soil organic matter, TN, TP, TK, AN, AP, and AK significantly increased on day 60 for SC and SCA compared with day 0, and additionally, AN and AP were significantly higher in SCA soils (65.75 ± 17.87 mg/kg and 14.28 ± 2.80 mg/kg) than SC soils (43.75 ± 11.70 mg/kg and 10.85 ± 4.11 mg/kg) on day 60. For SC and SCA, SOM, TN, and TP were significantly lower on day 120 (7.78 ± 1.88 g/kg, 0.66 ± 0.08 g/kg, and 0.79 ± 0.03 g/kg) compared to day 60 (11.28 ± 1.45 g/kg, 0.90 ± 0.12 g/kg, and 0.89 ± 0.07 g/kg). On day 120, The AN and the AK were significantly higher in SCA soils (81.92 ± 25.24 mg/kg and 515.42 ± 214.46 mg/kg) than in SC soils (52.38 ± 7.52 mg/kg and 362.88 ± 108.81 mg/kg) (Figure 4).

3.2. Agaricus Subrufescens Liquid Culture Experiment

3.2.1. Toxicity of β-CY and Gla on Agaricus Subrufescens Mycelial Growth

The growth of *A. subrufescens* mycelium responds to both β-CY and Gla, which shows an inhibition effect in high concentrations. The mycelium in MEB media ceased growth when the concentration of β-CY exceeded 0.1%, while the mycelium grew normally and produced uniform mycelial pellets when the concentration of β-CY was less than 0.1% (Table 4). The mycelium in the MEB media ceased to grow when the concentration of Gla exceeded 0.01%, while the mycelium grew normally and produced uniform mycelial pellets when the concentration of Gla was less than 0.01% (Table 4). The NOEC of β-CY for *A. subrufescens* mycelium was determined at a concentration of 24.71 μg/mL (with a concentration of 0.1% in MEB media). The NOEC of Gla for *A. subrufescens* mycelium was determined at a concentration of 63.15 μg/g (with a concentration of 0.01% in MEB media) (Figure 5). The NOEC of β-CY for *A. subrufescens* mycelium is the same as the threshold concentration in agricultural use. However, the threshold concentration for the agricultural use of Gla is higher than the NOEC for *A. subrufescens* mycelium.

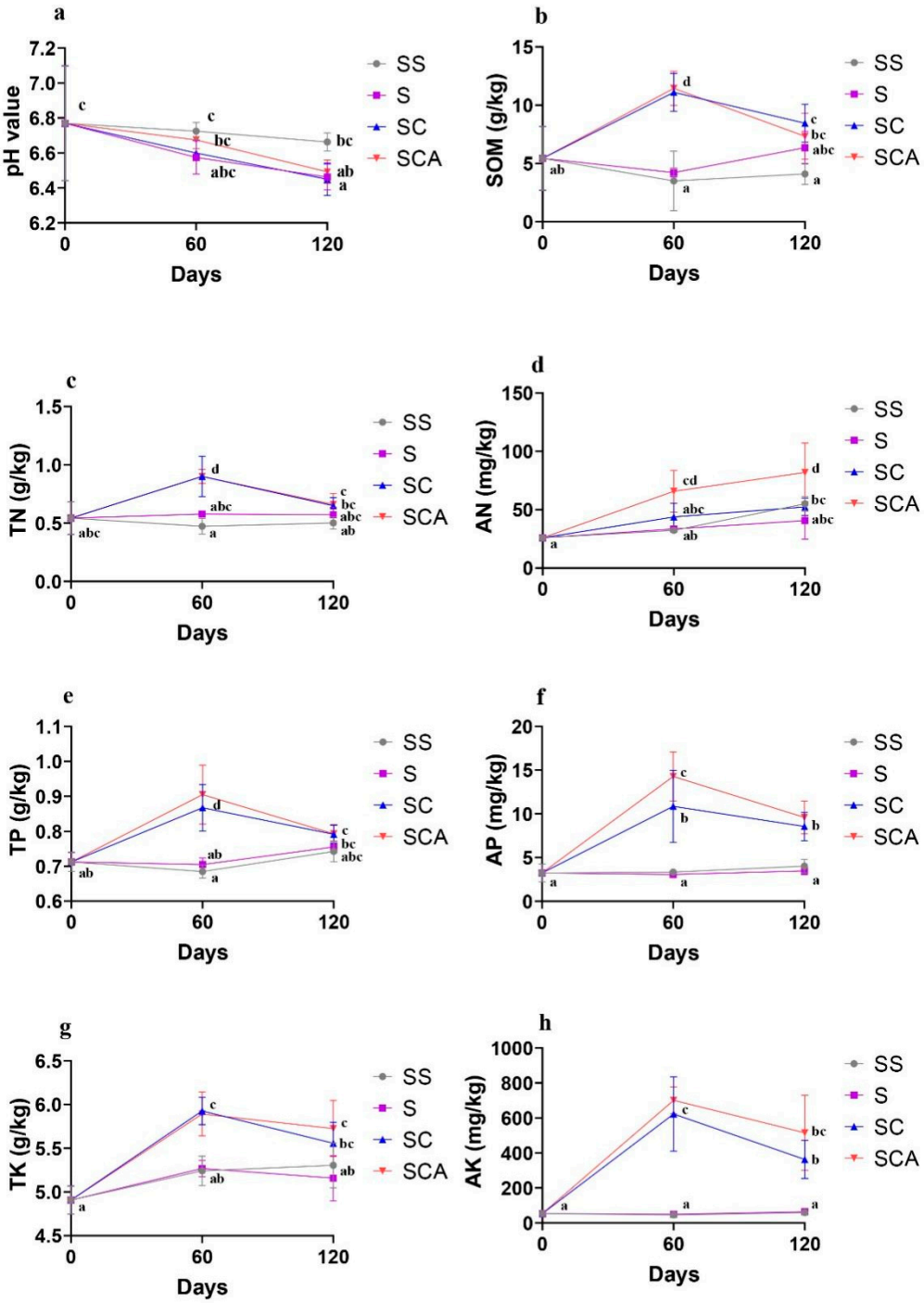


Figure 4. Chemical properties of soil samples on day 0, day 60, and day 120 during the soil amendment experiment. SS, sterilized soil; S, soil; SC, soil and compost; SCA, soil, compost, and *Agaricus subrufescens*; (**a**), soil pH value; (**b**), soil organic matter; (**c**), soil total nitrogen; (**d**), soil alkali solution nitrogen; (**e**), soil total phosphorus; (**f**), soil available phosphorus; (**g**), soil total potassium; (**h**), soil available potassium; SOM, soil organic matter; TN, total nitrogen; AN, alkali solution nitrogen; TP, total phosphorus; AP, available phosphorus; TK, total potassium; AK, available potassium; plots with different letters across different treatments are significantly different under Dunnett's T3 post hoc test at $p < 0.05$.

Table 4. Mycelial growth conditions of *Agaricus subrufescens* in MEB medium dissolved with different concentrations of Beta-cypermethrin and Glufosinate ammonium.

Mycelial Growth in MEB Media with Different Concentrations of Pesticides *v*/*v*	Beta-Cypermethrin (C22H19Cl2NO3)	Glufosinate Ammonium (C5H15N2O4P)
0%	**** (nd)	**** (nd)
0.005%	*** (nd)	*** (nd)
0.01%	*** (nd)	** (63.15 μg/g)
0.05%	*** (nd)	– (nd)
0.1%	** (24.71 μg/mL)	– (nd)
0.4%	– (nd)	– (nd)
0.8%	– (nd)	– (nd)
1%	– (nd)	– (nd)

Notes: –, mycelium growth ceased growth; *, mycelium grew and produced uniform mycelial pellets; the number of asterisks indicates the relative number of uniform mycelial pellets; nd, not determined.

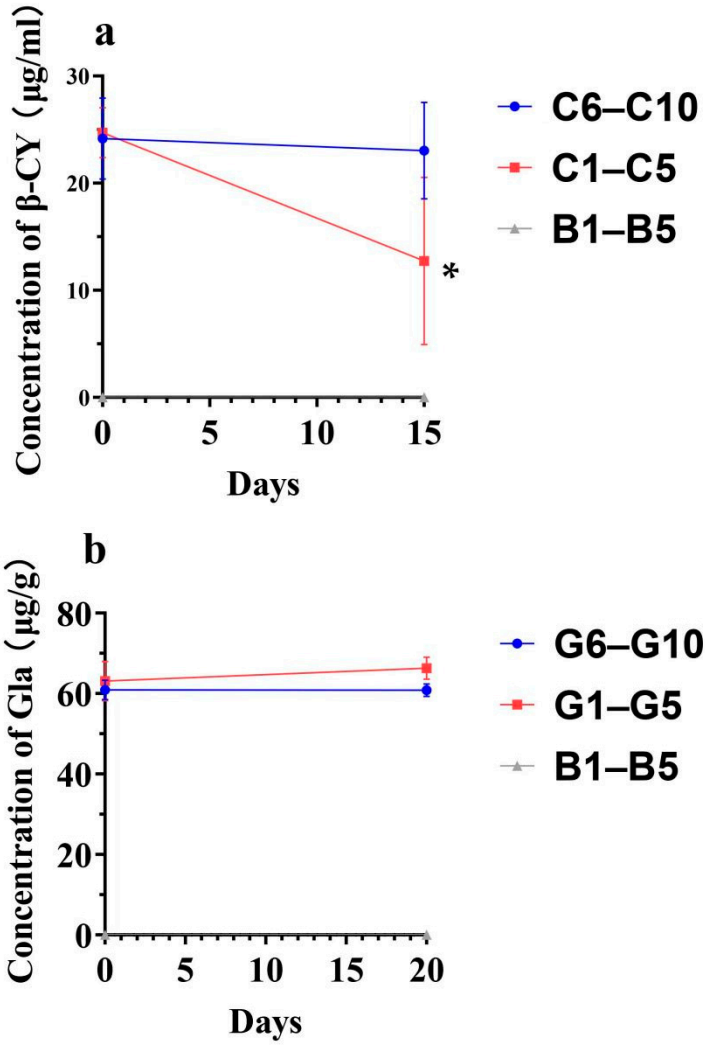


Figure 5. Beta-cypermethrin and glufosinate ammonium fates in MEB media in 250 mL flasks inoculated with *Agaricus subrufescens* fungal plugs. (**a**), concentration of beta-cypermethrin during the incubation period; (**b**), concentration of glufosinate ammonium during the incubation period; β-CY, beta-cypermethrin; Gla, glufosinate ammonium. B1–B5, 5 repeated flasks with MEB; C1–C5, 5 repeated flasks with MEB, β-CY and *A. subrufescens* plugs, C6–C10, 5 repeated flasks with MEB and β-CY; G1–G5, 5 repeated flasks with MEB, Gla and *A. subrufescens* plugs; G6–G10, 5 repeated flasks with MEB and Gla. *, significant difference among different groups.

3.2.2. Degradation of β-CY and Gla by Fungal Mycelium under NOEC

It required 15 days for *A. subrufescens* mycelium to fully colonize the MEB media dissolved with β-CY under NOEC and 20 days for mycelium to fully colonize the MEB media dissolved with Gla under NOEC. The concentrations of β-CY and Gla during the incubation period are shown in Figure 5. Results indicate that the concentration of β-CY significantly decreased after 15 days of incubation inoculated with *A. subrufescens* fungal mycelium (C1–C5) (Figure 5a), while there were no significant differences between day 0 and day 15 in the control group (C6–C10) (Figure 5a). The average concentration of Gla increased on day 20 in media inoculated with *A. subrufescens* fungal mycelium (G1–G5) when compared to the control group (G6–G10), though the difference was not significant (Figure 5b). For the control group, β-CY and Gla were not detected during the incubation period (<0.01 μg/mL for β-CY and <0.01 μg/g for Gla) (B1–B5) (Figure 5a,b).

4. Discussion

Composting is a critical process for accelerating the degradation of organic matter, recycling organic matter for use as a soil amendment [63]. Composting for basidiocarp production has been applied for a few species such as *A. bisporus* (J.E. Lange) Imbach, *A. subrufescens*, *Lepista sordida* (Schumach) Singer and *Stropharia rugosoannulata* Farl. ex Murrill [31,49,64–66]. However, few studies have investigated the benefits of combining composting with macrofungal species for soil rehabilitation. Results of our study showed that SOM, TN, AN, TP, AP, TK, and AK significantly increased in SC and SCA groups when compared to control treatments. This is likely owing to compost nutrients transferring to the incubation soil over the runtime of the experiment. Compared to compost amendments, composting with *A. subrufescens* mycelium improves soil AN and AP significantly, with the added benefit of producing approximately 1 kg of basidiocarps per grid (0.6 m^2). These results are consistent with a recent soil amendment project conducted in shady forested areas using another macrofungal species, *S. rugosoannulata* [67]. The observed increases in soil N and P from the SCA group could be a consequence of the large mycelium network. Based on the SEM images of cord-forming mycelial networks at the compost-soil-touching layer (Figure 3), *A. subrufescens* mycelium surrounded soil particles, a necessary process to facilitate nutrient acquisition and transport, working as the critical connective pathway between the compost and soil layers. Moreover, soil pH values decreased across the entire cultivation period, which has been shown to increase acid phosphatase activity and AP concentrations in soils [68]. In addition, for the SCA treatment, there was a decline in soil TN, SOM, TP, and AK between days 60 (robust fruiting) and 120 (end of fruiting period), which is likely because these nutrients were absorbed by the fungal mycelium for basidiocarps production [69], lowering nutrient content by day 120. Overall, soil nutrients on day 60 and day 120 were significantly higher than day 0. A limitation of this study is that we were not able to trace the movement of nutrients from the soil to the basidiocarps and future studies should make use of radio-labelled isotopes to track the movement of soil nutrients. Additionally, further studies should monitor the changes not only in soil nutrients, but also soil aggregates along with the broader soil microbial communities to determine if the cultivation of *A. subrufescens* has any further impacts on the soils systems in which they are growing. Lastly, field-based studies would enable researchers to determine if the noted changes occur at larger scales, in an agricultural setting.

With regards to the effect of pesticides on mycelium growth, our results demonstrated that mycelium growth was inhibited when exposed to high concentrations of β-CY and Gla (Table 4). The inhibitory effect of exposure to agrichemicals at high concentrations is consistent with the results of past studies investigating the toxicity of β-CY and Gla on other organisms (frog embryos, newborn mice, soybean aphid, and zebrafish embryos) [20,70–72]. A previous study reported that agrichemical use decreases soil microbial biomass, diversity, and function [73]. However, the mechanism by which pesticides inhibit the proliferation of fungal hyphae and formation of fungal fruitbodies remains unclear and further research is required. In this study, we found that β-CY and Gla inhibited the propagation of hyphae

and formation of mycelium for *A. subrufescens* with concentrations at or above 24.71 μg/mL (0.1%) for β-CY and 63.15 μg/g (0.8%) for Gla, representing the respective NOECs for these pesticides. Thus, before the field cultivation of *A. subrufescens* baseline testing of soils should be performed to ensure pesticide concentrations are below these values. This is especially notable with Gla, whose recommended concentration for general use is 80 times that of the NOEC for *A. subrufescens* mycelium. Therefore, the long-term application of Gla in agricultural soils is likely to inhibit soil fungal development, and potentially damage related soil ecosystems.

Of particular value are the results of our study which indicate that *A. subrufescens* can degrade β-CY, suggesting the need for future studies on the rehabilitation of soils contaminated with this pesticide. We found that *A. subrufescens* degraded 44.68% of β-CY, provided the exposure levels remained below the NOEC, within 15 days. These results add to the growing list of fungi which are able to degrade pesticides, for example *Eurotium cristatum* (Raper & Fennell) Malloch & Cain was found to degrade 57.93% [74] and *Aspergillus niger* Tiegh 54.83%, of β-CY [75]. Moreover, some bacterium strains have also been reported to possess β-CY degradation capacities, such as 90% for *Ochrobactrum lupini* DG-S-01 [76], 76% for *Brevibacillus parabrevis* BCP-09 [77], 81% for *Streptomyces aureus* HP-S-01 [78], and 80% for *Bacillus thuringiensis* SG4 [79]. However, we did not observe any significant degradation of Gla by *A. subrufescens*, and even though the mycelium could form when the concentration of Gla did not exceed the NOEC, it has been suggested that, even if mycelium is able to form, Gla could still inhibit the production of certain enzymes within the mycelium [80].

5. Conclusions

This study not only shows how the field cultivation of *Agaricus subrufescens* can improve soil nutrient concentrations in one cultivation cycle, but also the potential for this mushroom-producing fungus to degrade β-CY. Although there were fluctuations within the soil nutrient concentrations during the course of the study, overall quality of the soil was improved by the end of the experiment. However, we recommend trials be conducted over a longer duration of time in the future to allow us to understand the long-term effects that the cultivation of *Agaricus subrufescens* has on soil nutrient status. Furthermore, this is the first study to provide evidence that a multipurpose species such as *Agaricus subrufescens* can be used to not only improve soil health, but also function as a cash crop of mushrooms that are safe for consumption. Moreover, the inhibition of fungal mycelial growth by agrichemicals provides useful insights to be incorporated into future field-based experiments. However, more research is required to determine if the results obtained from the liquid culture experiments in our study can be replicated under field conditions, and if other species of mushroom-producing fungi are able to provide the same type of benefits when cultivated in agricultural fields, thus improving the income and productivity per unit of land.

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