RESEARCH ARTICLE

Influence of *Pleurotus* spent mushroom substrate on root-knot nematode infection and soil microbial dynamics

Jenjira Chobyen[1], Pornsuk Chaisuk[1], Yupa Chromkaew[2, 3], Nakarin Jeeatid[2], Krit Sujarittam[4], Terd Disayathanoowat[3, 4, 6], Milan Chameera Samarakoon[1*], and Nilita Mukjang[1, 3, 4*]

[1]Chiang Mai University, Faculty of Agriculture, Department of Entomology and Plant Pathology, Chiang Mai 50200, Thailand.
[2]Chiang Mai University, Faculty of Agriculture, Department of Plant and Soil Sciences, Chiang Mai 50200, Thailand.
[3]Chiang Mai University, Faculty of Science, Center of Excellence in Microbial Diversity and Sustainable Utilization, Chiang Mai 50200, Thailand.
[4]Chiang Mai University, Faculty of Engineering, Department of Mechanical Engineering, Chiang Mai 50200, Thailand.
[5]Chiang Mai University, Faculty of Science, Department of Biology, Chiang Mai 50200, Thailand.
[6]Chiang Mai University, Research Center of Deep Technology in Beekeeping and Bee Products for Sustainable Development Goals (SMART BEE SDGs), Chiang Mai 50200, Thailand.
[*]Corresponding authors (nilita.m@cmu.ac.th; milanchameerasamar.s@cmu.ac.th)
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ABSTRACT

Spent mushroom substrate (SMS) offers a sustainable approach to managing plant-parasitic nematodes, particularly root-knot nematodes (RKNs; *Meloidogyne* spp.), which cause severe root damage and yield loss in crops including *Capsicum* spp. This research investigated the effect of *Pleurotus* spp. The SMS on chili root-knot disease and the dynamic of the soil bacterial community of six treatments which are sterile soil with *Meloidogyne* spp. inoculation (SN), sterile soil control (SC), live soil with nematode inoculation (LN), live soil control (LC), live soil mixed with 15% SMS and nematode inoculation (MN), and live soil mixed with 15% SMS control (MC). Before treatment application, the antagonistic activity of *Pleurotus* spp. was evaluated, revealing RKN-inhibitory effects, with about 40% immobilization of second-stage juveniles (J2) by mycelium after 24 h and up to 67.5% paralysis caused by the fungal culture filtrate. *Pleurotus* SMS-amended soil treatments reduced root gall formation by approximately 85%-90%. Regarding changes in the bacterial community, it was found that bacterial communities profiling revealed that the application of SMS had no negative impact on soil bacterial diversity. Bacterial community composition differed markedly between sterilized and live soils, while SMS-amended soil treatments closely resembled live soils, with higher relative abundances of Actinobacteriota (41%-48%) and detectable Acidobacteriota (3%-4%), groups associated with soil health and plant growth promotion. In contrast, sterilized soil treatments were dominated by Bacilli (49%-56%). These findings indicated that SMS play a crucial role in promoting plant growth and assisting in RKN control without affecting the indigenous soil bacterial community.

Key words: Biological control, *Capsicum*, *Meloidogyne*, microbiome, next-generation sequencing.

INTRODUCTION

When the mushroom harvest is complete, the by-product is known as spent mushroom substrate (SMS). The SMS is rich in nutrients and organic substances, and contains metabolites such as cellulose, lignin, vitamins, and various enzymes that have a direct effect in inhibiting the growth of bacteria, fungi, and nematodes (Yang et al., 2023; Cruz-Arévalo et al., 2024). One edible fungal mushroom popularly commercially cultivated in Thailand is *Pleurotus* spp. (oyster mushroom) (Rungjindamai et al., 2024). *Pleurotus* spp. can be used to trap nematodes

by using loop-like structure and produce secondary metabolites that are toxic to nematodes. Generally, 5 kg growing material can yield 1 kg mushrooms (Chandana et al., 2023). Root-knot nematodes (RKNs: *Meloidogyne* spp.) are one of the pests that can cause damage and yield loss in economic crops, especially in chili (*Capsicum* spp.) In Thailand, the yield loss of chili caused by RKNs has been reported as high as 50%-100% in Ubon Ratchathani and Sisaket Provinces, Thailand (Boonrin et al., 2024). Biocontrol is a widely recognized approach as it does not pose risks to human health or the environment. Biocontrol is living organisms such as bacteria, fungi, and viruses or extracts from living organisms such as antibiotic or attractant activities (e.g., pheromones), that can inhibit diseases or the growth of pathogens, either directly or indirectly (Collinge et al., 2022).

There are many studies using SMS to control RKNs at many ratios, it was found that a 15% SMS ratio could reduce J2 population growth by up to 98.68% (Lopes et al., 2023). Additionally, SMS can improve soil structure and microbial composition, as it contains a variety of microorganisms (Pintarič et al., 2024). The SMS can help control pathogenic microorganisms while promoting beneficial microbes in the rhizosphere when mixed with soil for cultivation (Lopes et al., 2023). This promotion may lead to changes in soil microbial populations, particularly in bacterial communities, which are the most abundant group per unit area compared to other microorganisms. Bacterial communities can suppress RKNs infection through various mechanisms that help manage nematode population density. Previous studies have showed that the relationship between RKNs and bacterial communities affects plant development and yield (Wu et al., 2023; Nimnoi et al., 2024). However, there is limited research on the effects of soil amendment with SMS, which has RKNs-controlling properties and may be associated with changes in the structure and diversity of bacterial communities. These changes may promote certain bacterial groups that suppress RKNs. This research aims to study the effect of using SMS on chili root-knot disease caused by nematodes and to study the effect of using SMS on soil bacterial community for enhanced plant protection.

MATERIALS AND METHODS

Evaluation of *Pleurotus* spp. against J2 in vitro

Pleurotus spp. were isolated from mature fruiting bodies on spent mushroom substrate (SMS). The mushroom cap was wiped with 70% alcohol on the outside. A sterile scalpel was used to cut through the middle of the cap, and a piece of internal tissue that had not been exposed to alcohol, measuring 0.5 × 0.5 mm, was removed. The tissue was transferred onto potato dextrose agar (PDA), and the sample was incubated at room temperature for 3-5 d. After incubation, the growing mycelium was transferred onto a new PDA plate to obtain a pure culture.

Plant samples showing root-knot disease from *Meloidogyne* spp. were provided by Lom Ruk Farm, June District, Phayao Province. Plant samples were thoroughly washed and cut into small pieces approximately 0.5-1.0 cm in size. The root pieces were shaken at 0.5% sodium hypochlorite (NaOCl) for 3-5 min, then filtered using sieves N° 60, 100, and 325. The samples were washed using distilled water 2-3 times, or until it was ensured that the nematode eggs were free of NaOCl (Wu et al., 2021). The suspension collected on sieve N° 325 was adjusted to a final volume of 10 mL. Egg counts were performed under a stereo microscope, and the total number of nematode eggs was calculated. The nematode eggs were separated into two groups: The first part was used in vitro to produce second-stage juveniles (J2) in sterilized distilled water for 7-14 d, while the second part was used in the greenhouse experiments.

The effect of *Pleurotus* spp. mycelium on the mobilization of J2 was tested. The 0.5 mm agar disc of *Pleurotus* spp. mycelium with PDA was placed on a 2% water agar (WA) and incubated at room temperature for 10 d. Then, 20 J2 in 50 µL sterile distilled water were added. This procedure was repeated four times. The samples were incubated at room temperature in the dark (da Silva et al., 2024). Observations were recorded at 1, 6, 12, 18, and 24 h.

The effect of *Pleurotus* spp. culture filtrate on the mobilization of J2 was evaluated using a method adapted from Chandana et al. (2023). *Pleurotus* spp. mycelium cultured on PDA was taken, and a 0.5 mm agar disc was cut. The disc was placed into potato dextrose broth (PDB) and shaken at 180 rpm at room temperature for 7 d. After incubation, the culture was filtered through a 0.22 µm filter paper to obtain *Pleurotus* spp. culture filtrate. Then, 3 mL culture filtrate of *Pleurotus* spp. was pipetted into a sterile 5 cm plate, followed by the addition of 20 J2. This procedure was repeated four times, and the samples were incubated at room temperature in the dark. Samples were observed and recorded at 1, 6, 12, 18, and 24 h.

Evaluation of SMS on root-knot nematodes and plant growth in the greenhouse

Chili plants (*Capsicum frutescens* L. var. *frutescens*) 25-30 d old were planted in pots filled with soil for six treatments with three replicates each, as follows: Sterilized soil with 2000 root-knot nematodes (RKN) eggs (SN), sterilized soil (control; SC), live soil with 2000 RKN eggs (LN), live soil (control; LC), live soil with 15% SMS and 2000 RKN eggs (MN), and live soil with 15% SMS (control; MC). Sterilized coconut coir was placed at the bottom of each pot. After 7 d, a suspension containing 2000 RKN eggs mL^{-1} in 50 mL sterile distilled water was applied to the soil around the chili plants according to each treatment. After 90 d, plant samples, particularly around the root area, were carefully collected, and various growth parameters of the chili plants were recorded. These parameters included height, weight, and dry weight of the chili plants. The severity of the disease was also assessed by counting the number of galls on the root system and by determining the gall index, which classifies galling into six levels: Level 0 = no galls on the root system, Level 1 = 1-2 galls, Level 2 = 3-10 galls, Level 3 = 11-30 galls, Level 4 = 31-100 galls, and Level 5 = more than 100 galls on the root system (Keshtvarz et al., 2025). For the analysis of nutrient uptake, including N, P, K, Ca, and Mg in the plants, tissue samples of 0.5 g (dry weight) from each plant were weighed and digested with nitric-perchloric acid to determine the total nutrient content from the nutrient uptake of the chili plants. The data were analyzed for variance (ANOVA), and the means of each treatment group were compared using Tukey's multiple range test.

Analysis of soil physiological and chemical property from six treatments

Soil samples were randomly collected from three points around the rhizosphere of the chili roots to determine the nematode population using Cobb's sieving and the Baermann funnel technique (Oka, 2019). For soil nutrient analysis, soil samples were weighed at 0.10 g for N, P, K, Ca, and Mg. Tissue samples of 0.5 g (dry weight) from each plant were also digested with nitric-perchloric acid to determine the total nutrient content in the soil. Additionally, pH, electrical conductivity (EC), and organic matter (OM) were analyzed. The data were ANOVA, and the means of each treatment group were compared using Tukey's multiple range test.

Extraction DNA of soil samples and soil bacterial community analysis

Soil samples from each treatment were extracted for DNA using the Quick-DNA Fecal/Soil Microbe Miniprep Kit (Zymo Research, Tustin, California, USA). The DNA was then measured for concentration and purity and sent for amplicon metagenomic sequencing targeting bacterial 16S rRNA genes using 515F and 806R primers. The amplicon reads obtained from MiSeq sequencing were processed using the DADA2 pipeline in R program (R Core Team, Vienna, Austria). The phyloseq package in R program was then used to generate operational taxonomic units (OTU) tables for further analysis. Permutational analysis of variance (adonis function in vegan) was performed to determine if compositional differences were associated with the use of SMS in soil. Diversity (OTU richness) for each community was calculated, and ANOVA was used to examine the impact of plant growth substrate and root-knot nematode inoculation on diversity.

RESULTS

Evaluation of *Pleurotus* spp. against J2 in vitro

The nematicidal activity of *Pleurotus* spp. mycelium against second-stage juveniles (J2) was examined. The fungus grew on an agar medium and developed specialized structures, including loop-like structure (Figure 1A) and toxin sacs (Figure 1B). After adding J2 nematodes to the culture medium, they moved across the surface and passed through the small loop-like structure, which trapped the nematodes. After 24 h, J2 nematodes were immobilized (Figure 1C), accounting for 40.00 ± 23.80% (Table 1) and the RKNs began to be digested (Figure 1D). The fungus penetrated the nematode cells, using nutrients from the digestion of RKNs for growth until the nematodes were completely digested (Figure 1E). The *Pleurotus* spp. culture filtrate exhibited strong nematocidal activity, with the highest level of J2 paralysis observed after 24 h (Figures 1F and 1G), reaching approximately 67.50 ± 18.48% (Table 1).

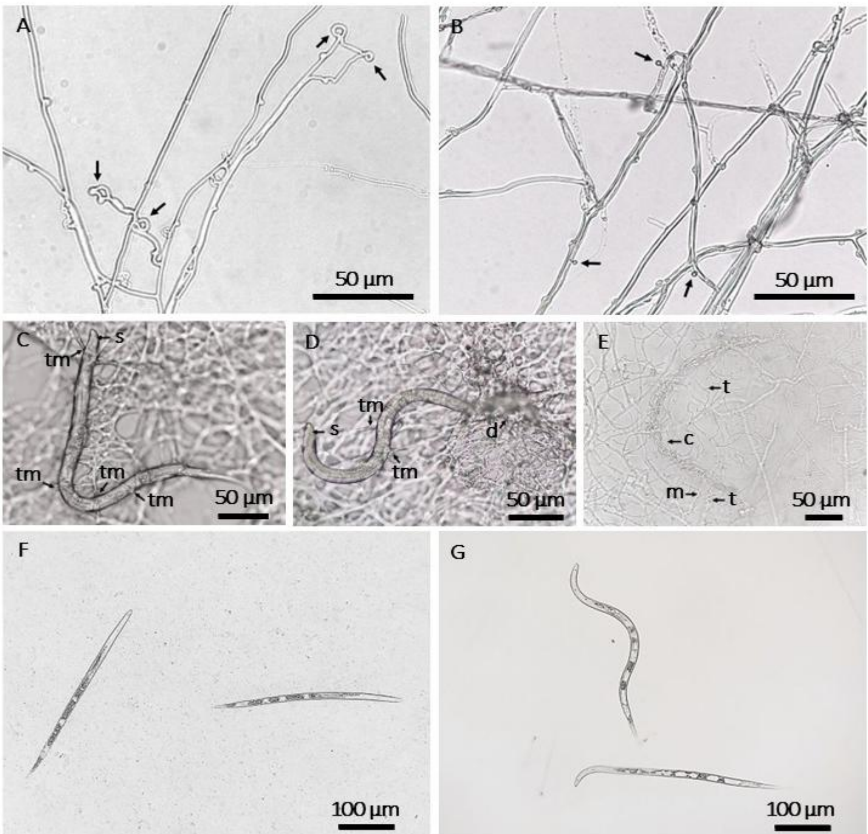

Figure 1. Effect of culture media and cultural filtrate of *Pleurotus* with second-stage juveniles *Meloidogyne*; loop-like structure (A), stalked toxin product (B), nematode immobilized and trapped by *Pleurotus* (C), lysis of the colonized nematode (D), extensive colonization of nematode (E), *Pleurotus* spp. culture filtrate treats (F) and control (G). s: Stylet; tm: trapping mycelium; d: degradation; t: toxin product; c: cuticle of root-knot nematodes; m: mycelium.

Table 1. Effect of the percentage of trapping and immobilization of *Pleurotus* spp. on second-stage juveniles (J2). Distinct letters in the row indicate significant differences according to Tukey's test ($p \leq 0.05$).

	Culture media (trapping)		Culture filtrate (immobilization)	
Time (h)	Inoculation	Control	Inoculation	Control
1	1.25 ± 2.50^a	0.00 ± 0.00^b	2.50 ± 2.89^a	0.00 ± 0.00^b
6	1.25 ± 2.50^a	0.00 ± 0.00^b	10.00 ± 10.80^a	0.00 ± 0.00^b
12	21.25 ± 16.01^a	0.00 ± 0.00^b	25.00 ± 19.58^a	0.00 ± 0.00^b
18	30.00 ± 19.58^a	0.00 ± 0.00^b	35.00 ± 14.14^a	0.00 ± 0.00^b
24	40.00 ± 23.80^a	0.00 ± 0.00^b	67.50 ± 18.48^a	0.00 ± 0.00^b

Evaluation of SMS with root-knot disease and plant growth in greenhouse

A study evaluating the effect of spent mushroom substrate (SMS) on the control of root-knot nematodes (RKNs) found significant differences in the number of galls in the root system and gall index compared to the control group (ANOVA, df = 5, $p < 0.05$). In the SN treatment, the highest number of galls was recorded at 146.5 ± 15.50 (Level 5), followed by LN at 12.00 ± 8.50 (level 2) and MN at 6.00 ± 4.36 (level 1), respectively (Figures 2A and 2B). These results indicated that SMS-amended soil could reduce root gall formation by approximately 85%-90%, with gall severity decreasing from level 5 to level 1 of gall index. Regarding the number of J2 RKNs in the

soil, MN had the highest count, followed by SN and LN, with values of 10.00 ± 4.00, 4.00 ± 2.00, and 0.67 ± 1.15, respectively. These values showed significant differences compared to the control group (ANOVA, df = 5, $p < 0.05$; Figure 2C).

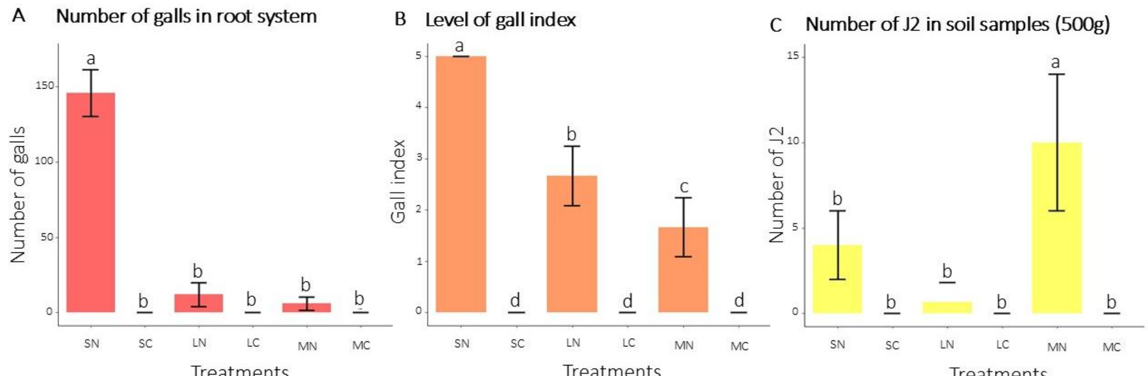

Figure 2. Effect of six treatments on the number of galls in root system (A), gall index (B) and number of second-stage juveniles (J2) in soil (500 g) (C). Sterile soil with 2000 eggs root-knot nematodes (RKN) (SN), sterile soil (SC), live soil with 2000 eggs RKN (LN), live soil (LC), live soil mixed with 15% spent mushroom substrate (SMS) with 2000 eggs RKN (MN) and live soil mixed with 15% SMS (MC). ANOVA for number of galls in root system: df = 5, $p \leq 0.05$. ANOVA for gall index: df = 5, $p \leq 0.05$. ANOVA for number of J2 in soil (500 g): df = 5, $p = 0.000136$. Distinct letters in the row indicate significant differences according to Tukey's test ($p \leq 0.05$).

In this experiment, there were nonsignificant differences in chili plant growth parameters, including height, fresh weight, and dry weight, across all treatments. However, there were trends that can be observed as MC treatment resulted in the best parameters, including height, weight, and dry weight, with values of 80.67 ± 8.13, 56.89 ± 16.91, and 7.85 ± 2.34, respectively. However, when compared to the test group exposed to RKN infestation, MN treatment showed plant growth was reduced, with values of 72.33 ± 9.61, 51.37 ± 3.86, and 8.09 ± 0.91, respectively. Although the differences were nonsignificant, there was a positive trend in plant growth when SMS was incorporated into the soil compared to untreated and sterilized soil (Figures 3A (A1-A3), 3B, and 3C; ANOVA: df = 5, $p > 0.05$) for all analyzed plant growth parameters.

In this experiment, SMS incorporation in the control group (MC) enhanced the uptake of both macronutrients and micronutrients, including N, P, K, Ca, and Mg with average values of 7.76 ± 0.06, 0.23 ± 0.02, 2.17 ± 0.07, 3.94 ± 0.15, and 0.32 ± 0.02, respectively (Figure 4). However, in the test group with RKNs, despite the addition of SMS (MN), nutrient uptake was lower, with values of 7.36 ± 0.08, 0.18 ± 0.05, 2.22 ± 0.08, 4.18 ± 0.12, and 0.32 ± 0.05, respectively (Figure 4). Tukey's HSD analysis indicated that the uptake of P, K, and Mg in SMS-amended soils did not differ significantly among treatments (ANOVA: df = 5, $p > 0.05$), whereas the uptake of N and Ca showed significant differences (Figure 4).

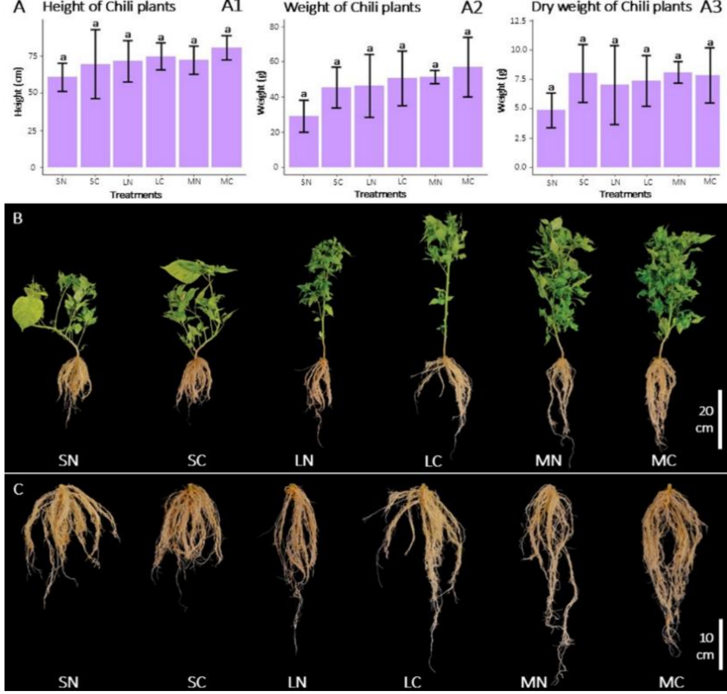

Figure 3. Effect of six treatments on the number of galls in root system (A), gall index (B) and number of second-stage juveniles (J2) in soil (500 g) (C). Sterile soil with 2000 eggs root-knot nematodes (RKN) (SN), sterile soil (SC), live soil with 2000 eggs RKN (LN), live soil (LC), live soil mixed with 15% spent mushroom substrate (SMS) and 2000 eggs RKN (MN) and live soil mixed with 15% SMS (MC). ANOVA for number of galls in root system: df = 5, $p \leq 0.05$. ANOVA for gall index: df = 5, $p \leq 0.05$. ANOVA for number of J2 in soil (500 g): df = 5, $p = 0.000136$. Distinct letters in the row indicate significant differences according to Tukey's test ($p \leq 0.05$).

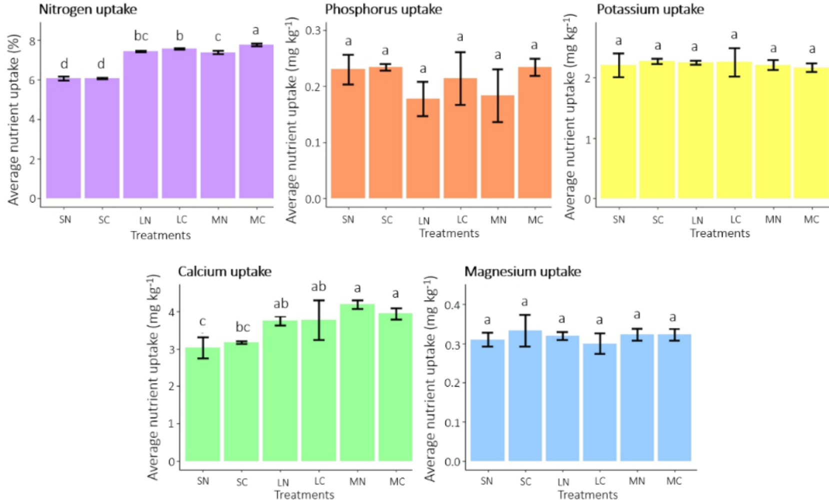

Figure 4. Average nutrient uptake analysis for N, P, K, Ca and Mg. SN: Sterile soil with 2000 eggs root-knot nematodes (RKN); SC: sterile soil; LN: live soil with 2000 eggs RKN; LC: live soil; MN: live soil mixed with 15% SMS and 2000 eggs RKN; MC: live soil mixed with 15% spent mushroom substrate (SMS). ANOVA for average N: df = 5, $p \leq 0.05$ for all analyzed nutrients. ANOVA for average Ca: df = 5, $p = 0.00111$. ANOVA for average another nutrient: df = 5, $p > 0.05$. Distinct letters in the row indicate significant differences according to Tukey's test ($p \leq 0.05$).

Soil physicochemical properties from six treatments

After the greenhouse experiment was completed, the soil was analyzed for the remaining levels of macro- and micronutrients, as well as pH, electrical conductivity (EC), and organic matter (OM) values. It was found that the nutrient levels, particularly N, were relatively higher in the MN and MC treatments, which contained SMS. These treatments showed significantly higher residual N values of 0.61 ± 0.01% and 0.64 ± 0.01%, respectively. In comparison, the other treatments, including LN, SC, SN, and LC, exhibited lower residual N values of 0.51 ± 0.01%, 0.54 ± 0.01%, 0.55 ± 0.02%, and 0.57 ± 0.01%, respectively ($p \leq 0.05$).

Additionally, the pH values showed nonsignificant differences, but soils treated with sterilization in the SN and SC treatments had higher pH compared to the other treatments. The OM content differed significantly among treatments, with MN and MC treatments (with SMS) showing higher OM content compared with other treatments with 12.23 ± 0.17 and 12.87 ± 0.20 dS m^{-1}, respectively ($P \leq 0.05$). The other treatments which were LN, SC, SN, and LC exhibited lower OM values of 10.21 ± 0.14, 10.85 ± 0.19, 11.04 ± 0.26, and 11.34 ± 0.20 dS m^{-1}, respectively. Tukey's HSD analysis indicated that P, K, Ca, Mg, pH, and EC did not differ significantly among treatments (ANOVA: df = 5, $p > 0.05$), whereas significant differences were observed for N and OM.

Bacterial communities

The initial bacterial count was examined using the plate count method. Before the greenhouse experiment, the soil was categorized into three types: Sterilized soil (SN and SC), live soil (LN and LC), and live soil mixed with 15% SMS (MN and MC). After the experiment, the soil was divided into six treatments: SN, SC, LN, LC, MN, and MC. The bacterial count in the soil before the experiment showed significant differences. The live soil mixed with 15% SMS had a bacterial count of 1.25×10^5 CFU mL^{-1}, while live soil had 7.47×10^4 CFU mL^{-1}. No bacteria were detected in the sterilized soil. The bacterial count after the experiment showed nonsignificant differences, with an average bacterial count ranging from 1.09×10^7 to 2.59×10^7 CFU mL^{-1}.

After extracting DNA and analyzing the bacterial community diversity at the phylum level, bacteria from the phyla Acidobacteriota, Actinobacteriota, Chloroflexi, Firmicutes and Gemmatimonadota were detected in all treatments. The phylum Actinobacteriota (LN: 4%, LC: 3%, MN: 3%, and MC: 3%) was the most abundant except in sterilized soil samples (SN and SC), where Acidobacteriota was not detected. Instead, Firmicutes (SN: 49% and SC: 56%) was the most dominant compared to other bacterial phyla in the same treatment. Additionally, when soil samples were categorized into three types including sterilized soil (SN and SC), live soil (LN and LC) and live soil mix 15% SMS (MN and MC), it was observed that the percentage of bacteria in the phyla Acidobacteriota of LC (4%) more than LN, MC and MN (3%). In sterilized soil treatment, the phylum Firmicutes was significantly more abundant in the SC (56%) (Figure 5A).

When analyzing diversity at the class level, the five most abundant classes in all treatments were Actinobacteria, Thermoleophilia, Alphaproteobacteria, Gammaproteobacteria and Chloroflexia, respectively, across all experiments. However, the most abundant classes were Bacilli (SC: 56% and SN: 49%), Actinobacteria (SC: 25 and SN: 30%), Gemmatimonadetes (SC: 5% and SN: 7%) and Alphaproteobacteria (SC: 3% and SN: 3%), respectively in sterilized soil. When comparing the differences between soil types, sterilized soil (SN and SC) had a significantly higher difference ($p = 0.001$) of the Bacilli class, which differed from the other soil types. In live soil, Actinobacteria increased after the test, while Thermoleophilia decreased. Additionally, after tests in greenhouse live soil mixed with 15% SMS (MC and MN) showed Actinobacteria decreased, but Thermoleophilia increased in treatments MC (Figure 5B).

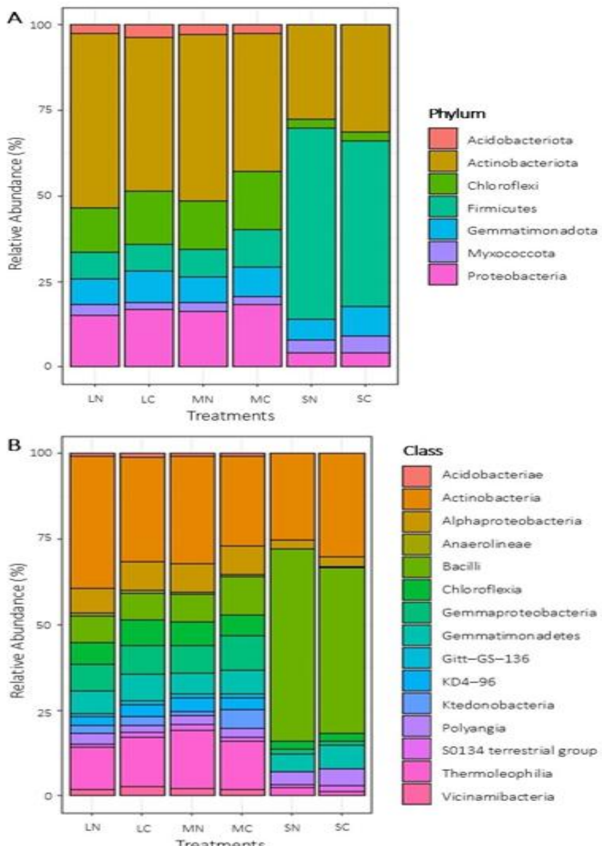

Figure 5. Bacterial communities at the phylum level (a) and the class level (b) in the soil of different treatments. LN: Live soil with 2000 eggs RKN; LC: live soil; MN: live soil mixed with 15% SMS and 2000 eggs RKN; MC: live soil mixed with 15% spent mushroom substrate (SMS); SN: sterile soil with 2000 eggs root-knot nematodes (RKN); SC: sterile soil. Permutational multivariate analysis of variance (PERMANOVA: df =5, p = 0.001 for all analyzed soil bacterial communities at the phylum and class level).

Diversity of bacterial communities in the soil of different treatments by Shannon and Simpson indices showed as consistent values, and when considering analysis of Shannon index of the orange point meaning live soil (LC and LN) and green points of live soil mixed with 15% SMS (MC and MN) were distributed in a nearby area, indicating a nearly equal level of diversity of bacterial community. As for the sterilized soil (SC and SN) observed from the blue points that are distributed in a nearby area. Shannon index showed that it had the average diversity of the orange point meaning live soil (LC and LN) and green points meaning live soil mixed with 15% SMS (MC and MN) that were distributed in a nearby area, indicating a nearly equal level of diversity of bacterial community. But when considering the diversity of bacterial community before and after, it showed that bacterial community before test have slightly lower diversity than after test. As for the sterilized soil (SC and SN) observed from the blue points that were distributed in a nearby area. So, the use of the Shannon and Simpson indices in this experiment showed consistent values, indicating that soil sterilization before testing affected the diversity of the bacterial community, whereas the use of SMS did not influence bacterial community diversity (Figure 6).

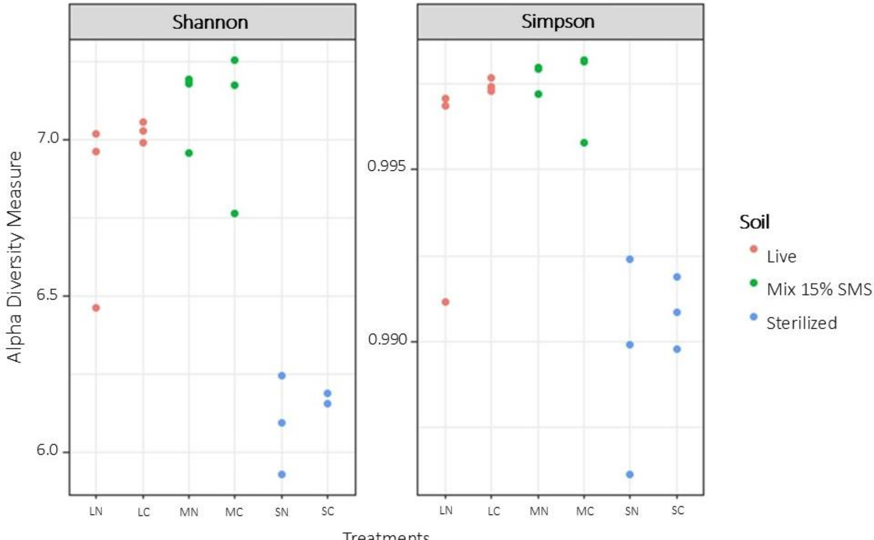

Figure 6. Shannon and Simpson indices for alpha diversity of bacterial communities in the soil of different treatments. LN: Live soil with 2000 eggs RKN; LC: live soil; MN: live soil mixed with 15% SMS and 2000 eggs RKN; MC: live soil mixed with 15% spent mushroom substrate (SMS); SN: sterile soil with 2000 eggs root-knot nematodes (RKN); SC: sterile soil.

DISCUSSION

Laboratory tests revealed that mushroom mycelium and *Pleurotus* spp. culture filtrate have the potential to inhibit second-stage juveniles (J2), as the loop-like structure formed by the mushroom was able to trap the nematodes. Additionally, toxin sacs were produced to help degrade the cuticles when trapped by the mycelium. Previous studies have shown that *Pleurotus* spp. act as antagonistic fungi through both direct trapping structures and the production of nematicidal metabolites (Chandana et al., 2023), both directly and indirectly. Direct inhibition occurs through the formation of loop-like structure, while indirect inhibition involves the production of toxin sacs containing secondary metabolites and enzymes such as cellulase, protease, and chitinase (Yang et al., 2023; Cruz-Arévalo et al., 2024). These enzymes degrade nematode cuticles, which are the main composed of chitin, making them susceptible to chitinase activity, ultimately leading to cuticle destruction (Lopes et al., 2023). Various components, including intracellular nutrients, serve as a food source for mushroom mycelium, leading to the replacement of root-knot nematodes (RKNs) by mushroom mycelium (Chandana et al., 2023). Additionally, a crude extract of the culture medium containing secondary metabolites was tested directly on RKNs, resulting in J2 paralysis (Cruz-Arévalo et al., 2024).

The use of spent mushroom substrate (SMS) from *Pleurotus* spp. can control RKNs because the number of root galls were found to be the least compared to other treatments. This was consistent with previous reports showing that mushroom mycelia secrete various hydrolytic enzymes during cultivation, which remain in the SMS and can degrade the cuticle of residual RKNs (Lopes et al., 2023). In addition, using 15% SMS, nonsignificant differences were observed in plant growth parameters, including plant height, fresh, and dry weight, compared to other treatments. When applied at low rates, SMS did not affect plant growth, resulting in nonsignificant differences. However, it tended to promote better plant development. Previous studies stated that application rates upper 30% tend to adversely affect plant growth, especially in terms of plant height, fresh, and dry weight (Lopes et al., 2023). Since SMS contains lignocellulosic compounds, which are sources of C and protein derived from rice bran, it may influence N availability. Moreover, additives like lime and gypsum commonly used in mushroom production can increase soil salinity, thereby affecting plant development and growth (Lopes et al., 2023).

Analysis of plant uptake showed macronutrient analysis, particularly N, showed a significant difference when compared to sterilized soil treatments, while P and K levels did not differ significantly which was consistent with research reported using SMS with an appropriate N fertilizer rate enhanced yield and nutritional value

especially plant protein indicating improved N uptake (Wiśniewska-Kadżajan and Malinowska, 2022). As for micronutrients, Ca levels showed a significant difference, whereas Mg did not when compared to other treatments. This aligns with research found that using SMS at a 15% ratio improved Ca uptake more effectively than standard substrates (Jesus et al., 2023). Therefore, SMS showed potential in enhancing the absorption of certain plant nutrients. The post-experiment analysis of soil showed SMS treatment revealed that N levels showed significant differences, while P and K levels showed nonsignificant differences when compared to other treatments. Several studies have reported similar results (Yang et al., 2023), explaining that N in SMS is present in a form that plants can readily absorb. When mixed into the soil for cultivation, it becomes easily accessible for plant used. Consequently, the amount of N detected in the soil after the experiment was higher in SMS treatment compared to other treatments. In addition, a significant different increase in organic matter (OM), research has shown that there was an increase in the amount of OM received from SMS (Lopes et al., 2023). This may be because the spent mushroom substrate (SMS) was produced from sawdust (Rungjindamai et al., 2024), which contained lignocellulosic compounds with a high C content and was not fully fermented. Fermentation can reduce both OM content and salinity (Lopes et al., 2023).

After extracting DNA and analyzing the bacterial community diversity at the phylum level and class, bacteria from the phyla Acidobacteriota, Actinobacteriota, Chloroflexi, Firmicutes, Gemmatimonadota, Myxococcota and Proteobacteria were detected in all treatments. When considering the class level, the five most abundant classes were Actinobacteria, Thermoleophilia, Alphaproteobacteria, Gammaproteobacteria and Chloroflexia, respectively. In sterilized soil where only class Acidobacteria in phylum Acidobacteriota was not detected. In addition, the five most abundant classes in this treatment were Bacilli, Actinobacteria, Gemmatimonadetes, Polyangia and Alphaproteobacteria, respectively. All detected bacterial taxa were commonly found in soil (Hua et al., 2024). When considering only the live soil and live soil mixed with SMS, the treatment showed similar percentage structure of the bacterial community. However, when sterilized soil was compared with live soil, with or without SMS, the class Acidobacteria within the phylum Acidobacteriota was not detected in the sterilized soil. It was possible that the live soil used prior to the experiment contained a low proportion of the phylum Acidobacteriota. Therefore, when the soil was used for plant cultivation under different treatments, especially those sterilized soil treatment, this bacterial group was not detected at the end of the experiment. In addition, Acidobacteriota exhibited slow growth (Dedysh and Damsté, 2018) and they were unable to compete with other bacterial groups (López et al., 2023). Therefore, bacteria from this group could not be detected in the sterilized soil at the end of the experiment. The phylum Actinobacteriota in class Actinobacteria was detected to be the highest percentage of bacterial community in all treatments. These bacteria possessed diverse metabolic capabilities that enabled them to utilize a wide range of C sources, including complex organic compounds, allowing survival in nutrient-limited soils (Barka et al., 2016). The class Bacilli of the phylum Firmicutes was found in higher abundance in the sterilized soil compared to the live soil, with or without SMS, because plant cultivation may have supported re-colonization after the microbial community was destroyed by sterilization. There were many reports indicating that the use of SMS helps increase the abundance of Firmicutes and Actinobacteriota (Yang et al., 2024). Firmicutes are capable of decomposing C in the soil, thereby promoting plant growth, while Actinobacteriota thrives well and possesses diverse metabolic pathways that enable C sequestration in the soil, contributing to the stability of soil C composition (Mitra et al., 2022).

The class Acidobacteria, a member of the phylum Acidobacteriota, has been reported to act as plant growth-promoting bacteria by producing the plant hormone indole-3-acetic acid (IAA). In addition, there was reported that Acidobacteriota can secrete exopolysaccharide (EPS), which were key components of biofilms. The EPS contributes to the formation of the soil matrix, prevents water loss, and supports bacterial adhesion (Kielak et al., 2016). The EPS production also plays a role in mineral and water absorption by plant roots (Kalam et al., 2020). The class Actinobacteria, which belongs to the phylum Actinobacteriota, was found to be more abundant in the treatments inoculated with RKN than in the control. This result was consistent with previous studies showing that infection by RKN alters soil bacterial community structure, leading to an increased abundance of Actinomycetales within the class Actinobacteria (phylum Actinobacteriota) in the rhizosphere of infected plants (Lamelas et al., 2020). Some genera within this class, such as *Streptomyces* spp., may function as biocontrol agents (BCAs) against RKNs by producing nematicidal compounds, such as actinomycin (Sharma et al., 2019), which exhibit cytotoxicity and inhibit egg hatching (Sharma et al., 2019). They can stimulate plants by induced systemic resistance (ISR) and systemic acquired resistance (SAR) (Saeed et al., 2021) activating plant defense

mechanisms (Mogollón-Ortiz et al., 2024). In addition, bacteria in this group could produce IAA and siderophores to promote plant growth (Ling et al., 2021). Interactions between RKNs and rhizobacteria can alter the structure of the bacterial community, leading to an initial increase in the abundance of the class Bacilli (Song et al., 2023). This observation was consistent with the findings of the present study. *Bacillus* spp., in class Bacilli of phylum Firmicutes, were widely tested and commercially formulated BCA against RKN can producing enzymes and toxin such as protease and chitinase that inhibit reproduction, egg hatch and J2 of RKN (Das et al., 2021). Additionally, bacteria this group is one of plant growth-promoting rhizobacteria (PGPR) and induce ISR and SAR.

This study demonstrated that live soil for planting, whether supplemented with SMS or not, tended to develop a similar soil bacterial community after a period of plant cultivation, both in terms of structure and diversity. However, the use of SMS-mixed soil slightly increased soil fertility and diversity (Shannon and Simson indices) (Nimnoi et al., 2024), which was consistent with previous reports showing that SMS application enhanced bacterial abundance and diversity (Wang et al., 2025). This effect was attributed to the residual nutrients in SMS, such as N, P, and K, which serve as important nutrient sources for bacterial growth. Sterilization of soil before test can affect diversity, it could be observed from analysis of soil bacterial community by Shannon and Simpson indices lower than live soil and live soil mixed with SMS. Because soil sterilization destroys all living organisms, including bacteria and fungi, even plant cultivation cannot restore the structure and diversity of the bacterial community to the same extent as in live soil or live soil mixed with SMS within a short period of time (Luo et al., 2022). Moreover, the use of SMS has been shown to be more effective than live soil alone to enhance microbial diversity and overall soil fertility.

The study results showed that the use of SMS could help reduce the population of nematodes in the soil that cause RKN disease. It also improved soil structure and increased essential nutrients, which positively affect plant growth. Additionally, SMS did not negatively impact the soil bacterial community and appeared to promote the growth of PGPR. Moreover, the use of SMS could help reduce agricultural waste.

CONCLUSIONS

The *Pleurotus* spp. from spent mushroom substrate (SMS) showed the potential in inhibiting *Meloidogyne* spp. with 40.0% of second-stage juveniles (J2) immobilization using their mycelium after 24 h of incubation and 67.5% paralysis when using the fungal culture filtrates. The use of the *Pleurotus* SMS has also shown positive effects in inhibiting the development of root-knot nematodes (RKN), as evidenced by the reduction in gall formation on chili roots by 85%-90% and decreased the gall index from level 5 to level 1 compared with the untreated soil treatments. The SMS not only enhanced the availability of essential soil nutrients, particularly N, which showed higher residual values in SMS-amended treatments, where residual N reached 0.61% in live soil with 15% SMS and 2000 RKN eggs (MN) and 0.64% in live soil with 15% SMS (MC), but also improved organic matter (OM) content. The SMS treatments (MN and MC) showed significantly higher OM content than the other treatments, with values of 12.23 and 12.87 $dS.m^{-1}$, respectively. Regarding the bacterial community, it was found that bacterial communities profiling revealed that the application of SMS had no negative impact on soil bacterial diversity. Clear differences in bacterial community composition were observed between sterilized soils (sterilized soil with 2000 RKN eggs; SN and sterilized soil; SC) and live soils, including live soils (live soil with 2000 RKN eggs; LN and live soil; LC) and live soils mixed with SMS (MN and MC), particularly in the distribution of bacterial phyla and classes. Sterilized soils (SN and SC) were dominated by the class Bacilli (49%-56%) within the phylum Firmicutes and lacked the class Acidobacteria (phylum Acidobacteriota), indicating that soil sterilization strongly affected this bacterial group. In contrast, SMS-amended soils (MN and MC) exhibited higher relative abundances of Actinobacteriota (41%-48%) and detectable levels of Acidobacteriota (3%-4%), resulting in bacterial community structures like those of live soil (LN and LC). These bacterial groups were commonly associated with improved soil health and potential plant growth-promoting rhizobacteria activity, enhancing plant growth and disease resistance. Therefore, the use of SMS in chili cultivation did not negatively affect the soil bacterial community. This study demonstrated that SMS could be used as a sustainable soil amendment that effectively suppresses RKNs and enhanced nutrient availability without disrupting native soil bacterial communities.

Author contribution

Conceptualization: J.C., P.C., M.S., N.M. Methodology: J.C., P.C., Y.C., N.J., K.S., M.S., N.M. Validation: P.C., Y.C., N.J., T.D., M.S. Formal analysis: J.C., M.S., N.M. Investigation: M.S., N.M. Resources: P.C., Y.C., N.J., K.S., T.D., M.S., N.M. Data curation: J.C., M.S., N.M. Writing-original draft: J.C., M.S., N.M. Writing-review & editing: J.C., P.C., Y.C., N.J., K.S., T.D., M.S., N.M. Visualization: J.C., N.M. Supervision: P.C., Y.C., N.J., T.D., M.S., N.M. Project administration: T.D., M.S., N.M. Funding acquisition: Y.C., N.J., T.D., M.S., N.M. All co-authors reviewed the final version and approved the manuscript before submission.

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