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Research article

Microbial potential of spent mushroom compost and oyster substrate in horticulture: Diversity, function, and sustainable plant growth solutions

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1. Introduction

Horticulture is a vital global sector dedicated to producing nutritious and high-quality crops. However, its capacity to sustain high yields depends on effective methods of fertilization and disease control, which gives rise to environmental challenges such as greenhouse gas emissions, eutrophication, and the extensive use of synthetic fertilizers and pesticides. European Union (EU) legislation strongly advocates a decrease in synthetic input and promotes alternative strategies (Farm to Fork strategy, 2022). Another concern in horticulture is the reliance on peat as the primary growing medium. While *Sphagnum* peat is widely preferred by European horticulturalists, because of its affordability and favorable properties such as water retention and nutrient exchange (Owen, 2007), the environmental ramifications of its use and its non-renewable nature present significant disadvantages. The quest for alternative growing media in order to reduce peat dependency is not only an environmental imperative but also aligns with EU legislation (Owen, 2007).

In the pursuit of a circular economy, agro-food industries harbor valuable resources with the potential to address significant challenges related to sustainability. Biomass production within these sectors can be valorized and repurposed into essential products. The cultivation of white mushrooms (*Agaricus bisporus*) and oyster mushrooms (*Pleurotus ostreatus*), for example, produces significant quantities of post-harvest byproducts, namely the spent white mushroom compost (SMC) and spent oyster mushroom substrate (SOS). For every kilogram of white mushroom cultivated, approximately 2.5–5 kg of SMC is generated (Sample et al., 2001). Europe produces more than three million tons of SMC annually (García-Delgado et al., 2013), presenting an escalating environmental concern for the mushroom industry, and underscoring the urgency for sustainable solutions for this organic waste. European production of oyster mushrooms is considered to be lower than for white mushrooms, but has been increasing over the past few years (Jongman et al., 2018).

Both SMC and SOS can enhance soil health, by acting as biofertilizers and helping with bioremediation (García-Delgado et al., 2013; Carrasco et al., 2018). Their inclusion in horticultural systems could probably alleviate issues associated with the use of peat, chemical fertilizers, and pesticides. The intrinsic value of SMC and SOS stems from their rich content of fungal mycelium, biologically active compounds, organic substances, and essential mineral elements such as nitrogen, phosphate, and potassium (Paula et al., 2017). As highlighted (Paula et al., 2017), the application of SMC in agriculture is challenged by the stability and maturity of the compost, especially regarding the microbial activities that drive biodegradation. The microbial communities play an important role as predictors of plant health because they improve plant fertilization and protection (Banerjee and van der Heijden, 2023).

To understand fully the potential applications of SMC and SOS in horticulture, particularly for plant protection, growth, and fertilization, it is vital we gain a deeper understanding of their stability and properties during composting after the harvesting of the edible mushrooms. This includes examining their microbial activity and nutrient profiles. Such insights are critical for their sustainable integration into horticultural practices. The rich organic matter and mushroom mycelia present in the substrates require additional composting to mitigate any adverse effects on plant growth (Paula et al., 2017). Microbial stabilization encompasses aspects such as the suppressive effect on plant pathogens and the diversity, functionality, and composition of the microbiome. This specifically pertains to the presence of microbial groups with beneficial traits. For example, studies by Wang et al. (2020) have hinted at the presence of microbial communities within the residues with antagonistic potential against plant pathogens. Moreover, the cultivation conditions used in the production of white or oyster mushrooms, with emphases on the substrate used, might have an impact on the composition and

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function of the microbial communities during the cultivation process, as well as in the composting process after cultivation. White mushrooms are secondary decomposers and heterotrophic organisms that require external nutrients to grow vegetative mycelium (Suwannarach et al., 2022). They grow on decomposed substrate prepared through aerobic solid-state fermentation. Oyster mushrooms, in contrast, can grow on non-degraded sterile substrates, where fruiting bodies can be produced directly from lignocellulotic substrates (Suwannarach et al., 2022).

Although there has been significant research on the suppressive potential of SMC and SOS against root pathogens, their effectiveness against pre- and post-harvest pathogens that cause economic losses in high-value crops, such as *Phytophthora infestans* in potatoes (Akino et al., 2014) and *Botrytis cinerea* in strawberry fruits (Petrasch et al., 2019), remains underexplored. Additionally, the bacterial-to-fungal ratio is a crucial microbial parameter indicating compost stability and maturity. Studies utilizing phospholipid fatty acids (PLFA) have shown a higher bacterial biomass in SMC compared with fungal biomass (Wei et al., 2020). The prevalence of actinomycetes in relation to oyster mushrooms has also been noted (Ko et al., 2005). However, these findings are based on SMC-amended soil or the substrates used for oyster mushroom cultivation, not on the composted material remaining after mushroom harvest, which is pertinent when considering disease suppression (Hernández et al., 2021). While existing studies have focused on the microbial diversity and composition in SMC, there is a need for research on microbial functions such as enzyme production, nutrient breakdown and consumption, and plant growth promotion.

Regarding nutrient stabilization, SMC and SOS are characterized by a high nutrient content, along with elevated pH and electrical conductivity (EC). These properties pose challenges for nutrient uptake. Further research is required to understand nutrient breakdown post-harvest and to monitor pH and EC levels. This knowledge is essential to enhance the use of these residues as biofertilizers or as peat substitutes in growing media.

This study therefore explored the microbial diversity and functionality of composted SMC and SOS, assessing their potential for plant pathogen suppression and plant growth enhancement. PLFA analyses were used to evaluate how the type of cultivated fungus influences microbial dynamics, and their functionality was determined based on nutrient source utilization and extracellular enzyme production. We hypothesized that (i) both SMC and SOS composted after the harvest of the white or oyster mushrooms host microbial communities advantageous for plant growth and antagonistic to plant pathogens; (ii) the composted SMC and SOS microbial communities display structures and functions specific to the type of cultivated fungus; and (iii) plant growth and protection attributes are more pronounced in SMC than SOS microbial communities because of the respective substrates used in fungus cultivation.

2. Materials and methods

2.1. Source of SMC and SOS

SMC and SOS were sourced from a commercial mushroom producer in southern Sweden. As described in Table (1), SMC contained aerobic fermented compost and comprised 80% Phase-3 compost (60% organic straw, 39.5% organic chicken manure, and 0.5% spawn), 18.5% casing soil, and 1.5% organic supplement. SOS contained straw material augmented with wheat bran and gypsum, which provided sources of nitrogen and calcium. After the harvest of white or oyster mushrooms, a mixed amount of 50 L of either SMC or SOS substrate were taken from the cultivation trays and put in 100 L containers. The containers were placed in a climate chamber at room temperature for further analyses. Three replicates (containers) of either SMC or SOS were used in the subsequent analyses. The samples were collected from four different sites in the container and mixed to obtain one representative sample.

2.2. Chemical analyses

Chemical analyses of macro- and micronutrients in the SMC and SOS were carried out by a commercial agriculture laboratory (LMI AB, Helsingborg, Sweden) using the Spurway method (Asp et al., 2022). Peat (SWHorto, Hammenhög, Sweden), used as a control material, again with three replicates, was also subjected to chemical analyses.

2.3. Microbial analyses

2.3.1. Viable count and microbial enumeration

Viable counting by means of serial dilutions and enumeration on selective media is a commonly used technique in microbial assessment studies (Rosberg et al., 2021). In the current study, the viable count was determined using agar media: (i) 0.1% tryptic soya agar (TSA; DIFCO 0369-17-6) supplemented with cycloheximide (100 μL/mL) for enumeration of general bacteria; (ii) 0.5% malt extract agar (MA; DIFCO 0186-17-7) for general fungi; and (iii) King Agar B (KB; 1,5 g K_2HPO_4, 1, 5 g $MgSO_4$*$7H_2O$, 10 mL glycerol, 20 g proteose peptone #3 (Difco) and 15 g agar dissolved in 1000 mL H_2O) with cycloheximide (100 μg/mL) for fluorescent *Pseudomonas* (Khalil et al., 2021).

2.3.2. Microbial diversity

Microbial diversity analyses were performed as described by Darlison et al. (2019) and Khalil et al. (2021). DNA extraction was performed using a ZymoBIOMICS DNA kit (D 4300; Sigma Aldrich), following the manufacturer's recommendations. Microbiome communities were sequenced with an Illu-mina MiSeq (2 × 300 bp) at LGC Genomic GmbH (Berlin, Germany).

The bacterial 16S ribosomal gene was targeted using the forward primer B341F (CCTACGGGAGGCAGCAG) and reverse primer B806R (GGACTACHVGGGTWTCTAAT). The forward primer ITS1F (CTTGGTCATTTAGAGGAAGTAA) and the reverse primer ITS2 (GCTGCGTTCTTCATCGATGC) were used to target the ITS region for fungal assessment. The oomycete genes were targeted using the forward primer ITS1-O (CGGAAGGATCATT-ACCAC) and the reverse primer 5.8s-O-Rev (AGCCTAGACATCCACTGCTG). Data pre-processing and operational taxonomic unit (OTU) picking from amplicons were performed using MOTHUR pipelines (version 1.35.1). The sequenced data were submitted to the GenBank database under submission number SUB14031714.

2.3.3. Microbial composition

PLFA analyses were used to study the composition of the microbial communities. The extraction method followed that described in Frostegård and Bååth (1996). Briefly, 1 g of each replicate was extracted with a one-phase mixture of chloroform, methanol, and citrate buffer (0.15 M, pH 4.0) in the proportions 1:2:0.8 v/v/v. After centrifugation, the pellet was washed once with the one-phase mixture, and the supernatants combined and split into two phases by adding chloroform and citrate buffer. An aliquot of the lower, organic, phase was dried under a gentle stream of nitrogen. The polar lipids were then isolated using a silicic acid column (Bond elute 1CC LRC-SI; Scantec Lab, Sweden). The fatty acid 19:0 was added as an internal standard. The phospholipid fraction was subjected to a mild alkaline methanolysis, and the resulting fatty acid methyl esters were separated using a Hewlett Packard 5890 gas chromatograph (GC; HewlettPackard Co., Palo Alto, CA) equipped with a flame ionization detector. A 50-m HP5 capillary column (phenylmethyl silicone; HewlettPackard Co.) was used. Hydrogen was used as a carrier gas, and injections were made in the splitless mode. The injector temperature was 230 °C and the detector temperature 270 °C. The temperature was initially held at 80 °C (1 min), then increased by 20 °C/min to 160 °C and then by 5 °C/min to a final temperature of 270 °C.

2.3.4. Microbial activity

A phenotypic microarray was performed using Biolog plates (Biolog, Haywood, CA, USA) as described by Mackie et al. (2014). Ten grams of SMC or SOS were added to 90 mL of sterile 0.85 % w/v NaCl and shaken at 200 rpm overnight. The suspension was then centrifuged at 2500 rpm for 15 min and the pellets resuspended in 0.85 % w/v NaCl and adjusted to an optical density $(OD)_{590}$ of 0.1 (Khalil and Alsanius, 2009). Biolog 96-well plates (Biolog Inc.) were used for each of three different nutrient sources, carbon (PM01), nitrogen (PM03), and phosphorus (PM04). An aliquot of 150 μL of each replicate was inoculated into each well of the plates, and the plates incubated at 25 °C for 96 h. The reduction of tetrazolium, a redox indicator dye, caused a shift from colorless to purple, indicating utilization of the nutrient compounds. The color development was measured as absorbance at a wavelength of 590 nm using Omnilog software (Biolog Inc.).

2.3.5. Pure bacterial cultures and enzyme activity

Isolation of bacterial and enzyme activities was performed according to Morales and Holben (2009) and Khalil et al. (2021). In total, 10 colonies were selected from each TSA and KB plate. Colonies were transferred to broth media comprising tryptic soy broth (TSB) and King B broth (KBB), respectively, and inoculated at 25 °C with shaking (140 rpm) for 24 h. The 16s rRNA region of all the bacterial isolates was amplified individually by polymerase chain reaction (PCR) with the universal primer pairs 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 907R (5′-CCGTCAATTCMTTTRAGTTT-3′), using an initial denaturation step at 94 °C for 3 min, followed by 35 cycles at 94 °C for 45 s, 50 °C for 30 s, and 72 °C for 30 s, then a final extension step at 72 °C for 5 min. The isolated bacterial colonies were screened for production of enzymes protease, phosphatase, cellulase, and xylanase on functional media using a plate assay as described by Choudhary et al. (2009).

2.3.6. In vitro antagonistic assay

To assess any antagonistic activity, both the dual-culture method and the detached leaf assay (DLA) were employed, alongside the detached fruit assay (DFA). For the dual-culture approach, the ten isolates were tested for antagonism against various plant pathogens. Specifically, *Phytophthora infestans* was grown on rye agar for 14 days; *Phytophthora colocasiae* and *Phytophthora cactorum* were grown on corn meal agar (CMA; Difco 213,400) for 10 days; *Rhizoctonia solani* and *Botrytis cinerea* were grown on CMA for 7 days; and *Alternaria alternata* was cultured on potato dextrose agar (PDA; Difco 218,630) for 14 days. All the plates were incubated at 20 °C. Radial inhibition was determined following the protocols outlined by Landa et al. (1997) and Balouiri et al. (2016). Sterile water was used as a control, and the radial inhibition percentage was defined as: radial inhibition (*RI*) (%) = $(RC - RT)/RC \times 100$, where RT = the minimal distance between the center and fungal margin of the experimental plate, and RC = the distance between the center and fungal margin on the control plate (Schaad et al., 2001). For each experiment, three replicates were used.

Based on the cumulative results, isolates 4 and 8 (SK4 and SK8, respectively) were selected for further testing using DLA and DFA, to determine their efficacy in stalling pathogen spread on both leaves and fruits. This was tested with *P. infestans* on potato (*Solanum tuberosum*) leaves, *P. colocasiae* on taro (*Colocasia esculenta*) leaves, and *B. cinerea* on tomato (*Solanum lycopersicum*) and grape (*Vitis vinifera*) fruits. For DFA, 22 tomatoes and 18 grapes were treated with SK4, SK8, or water as a neutral control. For DLA, six mature leaves from two-month-old plants were cleaned with sterile water before inoculation.

The bacterial inoculum was prepared meticulously by introducing a single bacterial colony (either isolate 4 or 8) into Erlenmeyer flasks filled with 300 mL of Lysogeny broth (LB). After incubation at 28 °C with constant agitation at 220 rpm for 16 h, bacterial cells were collected via centrifugation at 3000 rpm for 5 min. The resulting cell pellet was then combined with 200 mL of sterile 1 X phosphate-buffered saline (PBS) to achieve a concentration of 2×10^7 colony-forming units (CFU)/mL.

Spore suspensions of the pathogens *P. colocasiae*, *P. infestans*, and *B. cinerea* were made using distilled water and filtering out the mycelium, and standardized to 25,000 spores/mL using a hemocytometer. For the treatments, 20 μL of the bacterial suspensions were applied to the leaves or fruits, with an additional 20 μL of pathogen suspension introduced after 24 h. The samples were incubated in a growth chamber maintained at 20 °C at a humidity level of 75% over 5 days. Distilled water and bacterial suspensions served as controls. The DLA and DFA analyses were repeated four times.

To monitor disease progression, trypan blue staining was administered. Both potato and taro leaves were submerged in trypan blue solution for 30 min, then rinsed with 100% ethanol, and left to soak overnight in the same ethanol concentration at room temperature. These samples were then transferred to a 20% glycerol solution and scanned using an Epson V850Pro.

2.3.7. Seedling colonization assay

Tomato seeds (*S. lycopersicum*) of the variety "Money maker" underwent surface sterilization in a 3% sodium hypochlorite solution for 5 min, after which they were rinsed with distilled water twice. The seeds were then sown in plastic pots filled with commercial potting soil (SWHorto-Sweden) with no fertilizers. Three treatments were applied: (i) treatment with bacterial isolate 4, (ii) treatment with bacterial isolate 8, and (iii) a control that was not exposed to any bacterial inoculation. Each treatment was replicated six times.

To prepare the bacterial suspensions, isolates 4 and 8, grown in LB medium, were centrifuged at 3000 rpm for 10 min. The resultant pellet was resuspended in 1 X PBS to reach a viable concentration of 2×10^7 CFU/mL. Five weeks post-germination, the roots of the young tomato plants were submerged in this bacterial suspension, or in 1 X PBS as a control, for approximately 30 min. Subsequently, these seedlings were transplanted to 30 × 30-cm plastic pots filled with cultivation soil fertilized with inorganic fertilizers containing nitrogen, phosphorus, and potassium (SWHorto). The growth conditions in the climate chamber were maintained at 20 °C, with 80% relative humidity, a light intensity of 200 μmol $m^{-2}s^{-1}$, and a 16-h photoperiod.

Eight weeks after germination, a second inoculation with the bacteria at a viable concentration of 2×10^7 CFU/mL was administered. At week 12, the plant biomass was analyzed, focusing on plant height, fresh and dry weight of both root and shoot systems, chlorophyll content (as measured with a chlorophyll concentration meter; MC-100), and leaf count, in comparison with the control group. To determine the dry weight, the roots and shoots were placed separately in paper bags and subjected to a constant 70 °C heat in an oven for 48 h, and then weighed.

2.4. Statistical analyses

Statistical analyses of the Chao and Shannon indices were carried out using Microbiome Analyst v1.0, following the methods outlined by Dhariwal et al. (2017) and Khalil et al. (2021). Microbial enumeration data underwent a log transformation to ensure conformity with the assumptions of homogeneity and normality. The impact of treatments on microbial enumeration, as well as on the *in vitro* and seed germination assays, was determined using analysis of variance (ANOVA). The analysis was conducted using Tukey's multiple comparison test at a significance level of $p < 0.05$, in Minitab version 18. For the phenotypic microarray data, the analysis was performed in R-studio with the Opm package as described by Karlsson et al. (2023). This analysis centered on the area under the curve (AUC) values and was followed with ANOVA and Tukey tests, all executed within R-studio. Lastly, principal component analyses (PCA) of the PLFA data were undertaken using Minitab version 18.

3. Results and discussion

3.1. Nutrient content

Overall, SMC exhibited superior macronutrient content compared with SOS and the control peat (Table 1). Notably, elements such as nitrogen, calcium, magnesium, phosphorus, sulfur, chloride, and sodium were more abundant in SMC. Additionally, SMC displayed elevated levels of micronutrients such as iron, boron, aluminum, manganese, and silica. Exceptions were zinc and copper, their concentrations being higher in SOS and peat. Compared with peat, SOS also showed a significance increase in elements such as nitrogen, calcium, sodium, chloride, magnesium, iron, silica, and zinc. Both pH and electrical conductivity measurements were significantly higher in SMC and SOS compared with peat.

These results highlight the enriched micro- and macro nutritional content in SMC and SOS compared with peat, emphasizing their potential utility from a plant nutrition perspective. Previous studies (Martín et al., 2023) have alluded to the promise of SMC in this context, attributing the nutrient enhancement to the supplementation that occurs during pre-mushroom cultivation stages (Carrasco et al., 2018). However, the amount of copper in SOS is of concern if it occurs in higher concentrations, as highlighted by Góngora-Gómez et al. (2017).

Furthermore, the elevated salt content, particularly sodium and chloride in both SMC and SOS, warrants further attention, given its potential to impede plant growth (Kaiwen et al., 2020). Additionally, the pH and electrical conductivity levels observed in SMC and SOS were notably higher than in peat. Elevated pH levels might pose challenges for optimal nutrient absorption by plants. Generally, for effective nutrient absorption by plants, the recommended pH range is 5–6, and EC should be between 1 and 3 mS/cm (Kaiwen et al., 2020). The values observed in our samples were higher than these recommendations, highlighting the need for effective management strategies to optimize the use of SMC and SOS for plant growth. As indicated by Hernández et al. (2021), however, the proportional amendment of SMC or SOS with peat can enhance plant growth and production. This might be a viable strategy to manage the high pH and salt content.

Table 1
Chemical characters of spent mushroom compost (SMC), spent oyster substrate (SOS) and the growing media, peat, based on nutrient content in mg.L^{-1}, pH and electrical conductivity (mS. cm^{-1}). The values indicate mean ± SE (n = 3) and the different letters on the values indicate significant differences between treatments ($p < 0.001$) according to one-way ANOVA.

Chemical characters	SMC	SOS	Peat
pH	8,2^{a}	7,4^{a}	4,9^{b}
Electrical conductivity	10^{a}	7^{a}	0,7^{b}
Nitrogen	3,8^{a}	2,3^{b}	1,2^{c}
Potassium	47^{a}	26^{b}	47^{a}
Calcium	1500^{a}	927^{b}	990^{b}
Magnesium	961^{a}	187^{b}	140^{b}
Phosphor	85^{a}	30^{b}	40^{b}
Sulfur	31^{a}	20^{b}	21^{b}
Sodium	120^{a}	77^{b}	60^{c}
Chloride	540^{a}	420^{b}	51^{c}
Aluminum	3^{a}	3^{a}	0,77^{b}
Boron	1,7^{a}	0,25^{b}	0,21^{b}
Copper	0,56^{b}	1,1^{a}	1,3^{a}
Iron	20^{a}	15^{b}	0,81^{c}
Molybdenum	0,12^{b}	0,12^{b}	0,54^{a}
Silica	7,8^{a}	6,0^{b}	1,2^{c}
Manganese	4,3^{a}	1,0^{c}	2,1^{b}
Zinc	2,1^{c}	6,9^{a}	4,9^{b}
Organic straw (%)	60	NA	NA
Organic chicken manure (%)	39,5	NA	NA
Spawn (%)	0,5	NA	NA
Casing soil (%)	18,5	NA	NA
Organic supplement (%)	1,5	NA	NA
Straw material (%)	NA	50	NA
Wheat bran (%)	NA	45	NA
Gypsum(%)	NA	5	NA

NA = Not applicable.

3.2. Microbial enumeration

Microbial analyses using selective media revealed a greater presence of bacteria and fluorescent *Pseudomonas* in SMC than in SOS (Fig. 1). Conversely, SOS exhibited a more pronounced general fungal count compared with SMC. This suggests that, while SMC microbial communities were predominantly bacterial, SOS communities displayed a higher fungal dominance. The elevated bacterial content in SMC may be associated with its richer nutrient and organic matter composition, conditions known to promote bacterial growth in compost (Qian et al., 2022). As highlighted by Braat et al. (2022), a high bacterial abundance could be present in the compost material used for *Agaricus* cultivation, and therefore levels could be higher in SMC samples than SOS.

3.3. Microbial diversity

The alpha diversity of the bacterial communities identified in SMC was significantly higher than in SOS (ANOVA, Tukey test $p < 0.05$; Fig. 2). This was confirmed by both Chao1 (Fig. 2A, $p = 0.015$) and Shannon (Fig. 2B, $p = 0.022$) indices. Fungal communities displayed a similar trend (Fig. 2), with Chao1 (Fig. 2C) and Shannon (Fig. 2D) indices emphasizing a greater alpha diversity in SMC. However, for oomycete communities, there was no significant difference based on Chao1 indices (Fig. 2E), although Shannon indices (Fig. 2F) indicated a higher diversity in SOS compared with SMC. Beta diversity clearly distinguished between bacterial, fungal, and oomycete communities in SMC versus SOS (supplement SFig. 1).

SMC bacterial communities primarily comprised the phyla Proteobacteria (23.0%) and Bacteroidetes (62.0%), followed by Firmicutes (13.0%) (Fig. 3A). Less dominant phyla included Actinobacteria (2.0%). The genera *Pedobacter* (48%), *Deyobacter* (12%), and *Sphigomonas* were dominant in SMC samples, while less dominant genera included *Lysinibacillus* (4%), *Pseudomonas* (4%), and *Bacillus* (2%) (Fig. 3B). SOS presented bacterial communities dominated by the phyla Proteobacteria (43.0%) and Bacteroidetes (33.0%), followed by Firmicutes (7.0%) and Actinobacteria (5%) (Fig. 3C), and the genera *Geofilum, Pseudomonas*, *Treponema*, *Alkaliflexus*, and *Fibrobacter* (Fig. 3D).

Concerning fungal communities, Basidiomycetes dominated in SOS, while Ascomycetes was dominant in SMC (Fig. 4A). The genus *Pleurotus* was particularly dominant in SOS (Fig. 4B), whereas SMC included

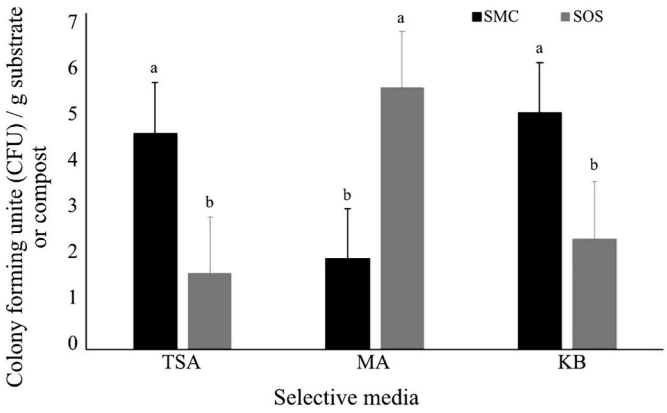

Fig. 1. Microbial content as colony forming units (CFU)/gram of spent mushroom compost (SMC) or spent oyster substrate (SOS) and enumerated on 0.1% tryptic soya agar (TSA) for general bacterial flora, 0.5% malt extract agar (MA) for general fungal flora, and King Agar B (KB) for fluorescent pseudomonads. Letters above the bars indicate significant differences between treatments with $p < 0.05$. Mean ± standard deviation are shown, n = 3.

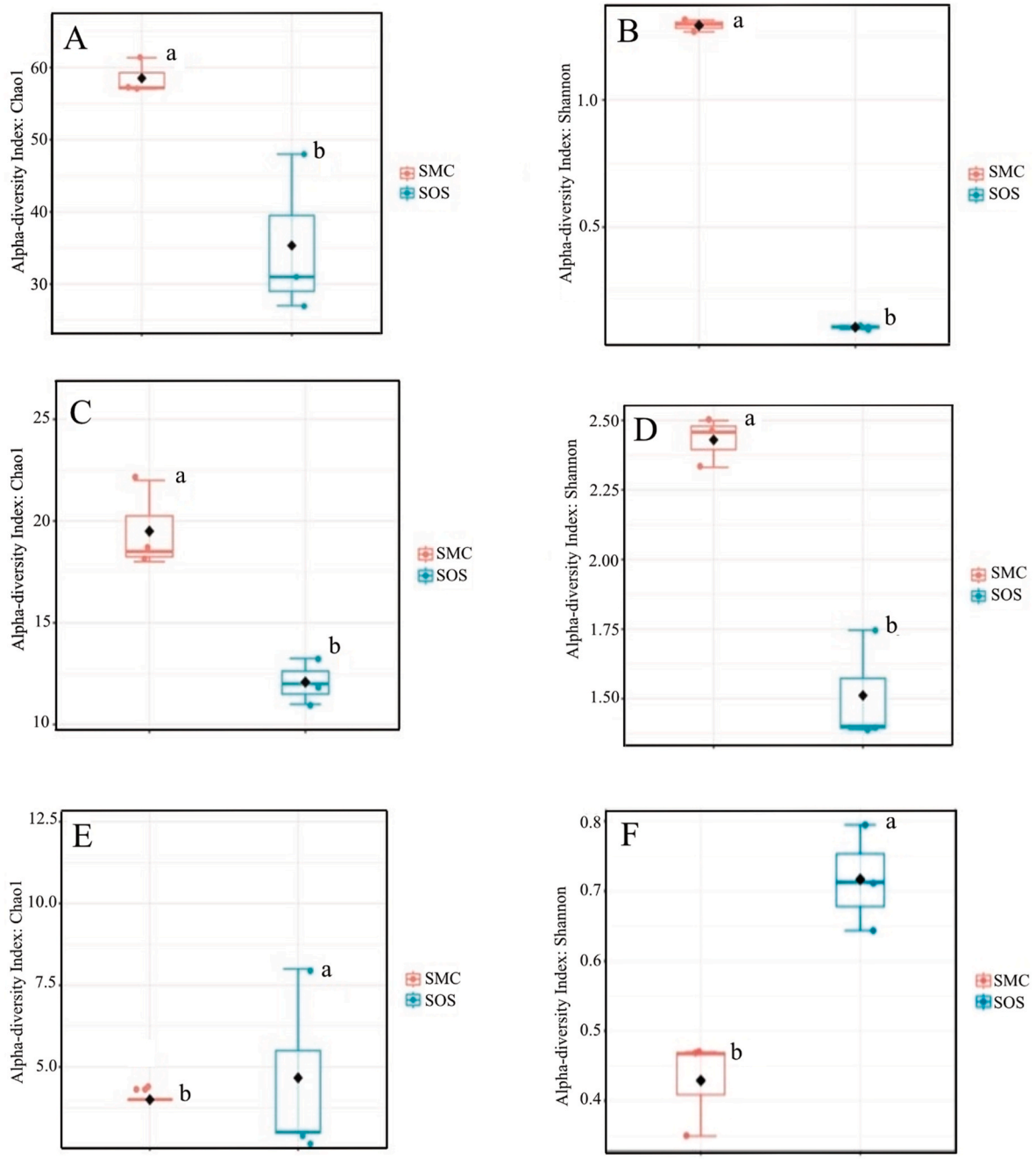

Fig. 2. Alpha diversity of (A–B) bacterial, (C–D) fungal and (E–F) oomycete communities in spent mushroom compost (SMC) or spent oyster substrate (SOS) as indicated by the diversity Chao1indices (A, C, E) and Shannon (B, D,F). Letters above the boxplots indicate significant differences between treatments with $p < 0.05$. Mean ± standard deviation are shown, n = 3.

genera such as *Iodophanus*, *Agaicus*, *Trichurus*, *Crassicarpon*, *Trichoderma*, and *Penicillium* (Fig. 4B). The oomycete communities in SMC were dominated by Phythiales, while the SOS samples were dominated by Phythiales and Lagenidiales (Fig. 4C).

Our findings revealed variations in microbial diversity and composition (Supplement SFig. 1) based on the type of the cultivated fungus, which was related to the type of the substrate used in the cultivation process. As indicated by Suwannarach et al. (2022), the microbial communities and type of substrate used in the cultivation of edible fungi have an impact on the quality of the fungi. SMC contains fermentation-composted material, while SOS is mostly based on straw material. Such cultivation conditions vary in their biodegradable and microbial profiles (Suwannarach et al., 2022). The phyla Proteobacteria and Bacteroidetes featured prominently in both SMC and SOS microbial communities, albeit in variable concentrations (Fig. 3). Such phyla are frequently found in oyster and white mushroom composts, containing beneficial bacteria such as *Pseudomonas* and *Bacillus*, known to promote plant growth (Tsotetsi et al., 2022). In terms of fungal communities, those in SMC were richer and more varied, with potential benefits for plant growth promotion and antagonism towards plant pathogens (Fig. 4). Some genera, such as *Trichoderma*, have been cited for their plant pathogen suppression qualities (Q. Liu et al., 2022). The fungal genus *Pleurotus*, found in SOS communities, is crucial for nutrient degradation, highlighting its potential for plant nutrition and bioremediation. However, the obtained results from the diversity studies is in line with the studies by Braat et al. (2022), which indicated the

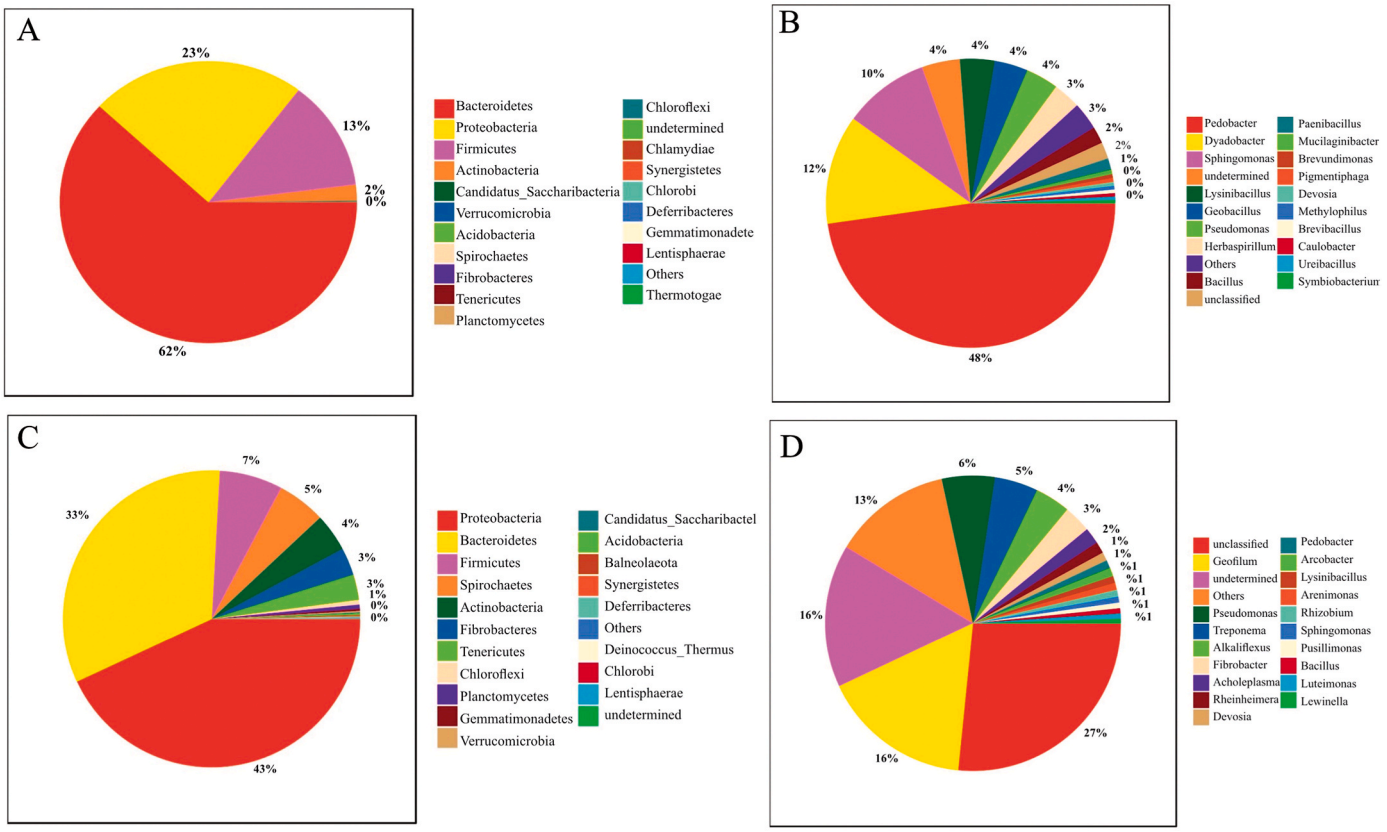

Fig. 3. Relative abundance in (%) of the dominant bacterial communities in SMC samples at the (A) phylum and (B) genus levels and in SOS samples at the (C) phylum and (D) genus levels. n = 3.

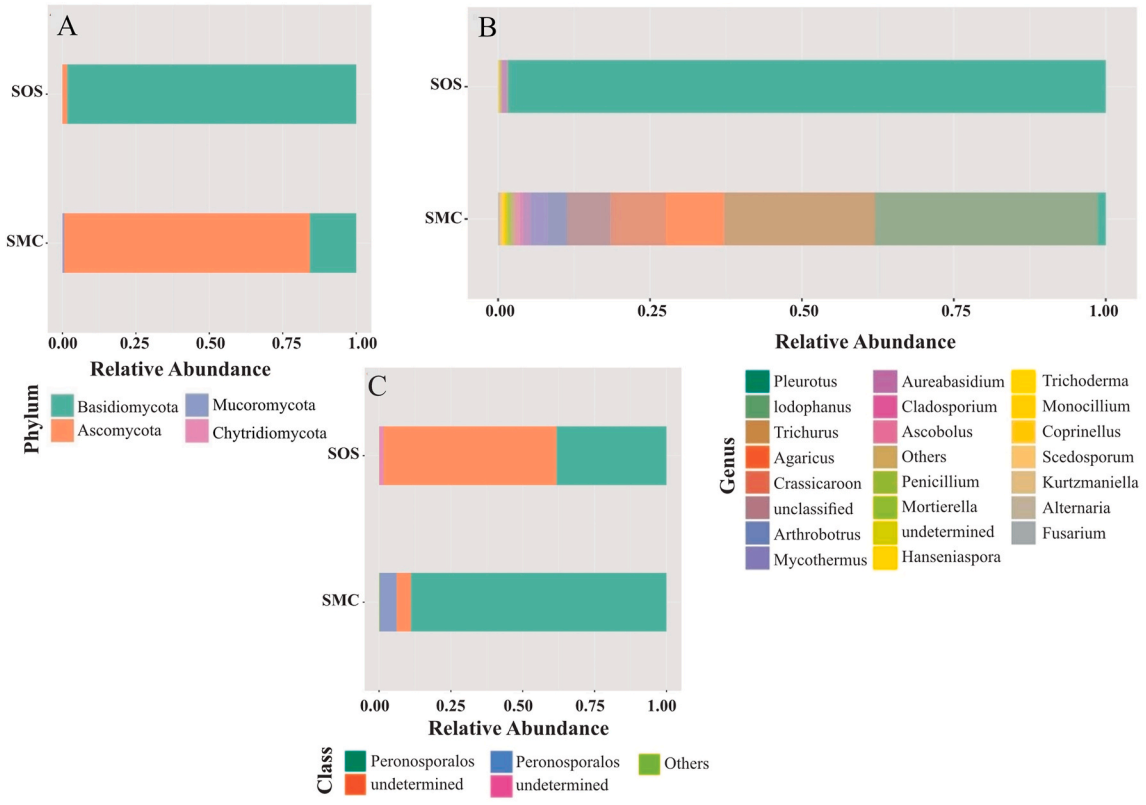

Fig. 4. Relative abundance in (%) of the dominant fungal communities in the samples of spent mushroom compost (SMC) and spent oyster substrate (SOS) at the (A) phyla and (B) genus levels and (C) the abundance of oomycete communities at genus levels. n = 3.

occurrence of such microbial groups during the cultivation of the mushrooms. This would also indicate the impact of substrate on the microbial diversity rather than the composting period.

However, the dominant *Phythiales* and *Lagenidiales* genera in the oomycete analyses is a cause for concern. Phythiales includes pathogenic fungi related to plant root diseases such as *Pythium* spp. (Chen et al., 2017) and parasitic pathogens (Schnepf and Deichgraber, 1978) harmful to plants. Their presence requires further investigation.

3.4. Microbial composition

PCA based on PLFA mole percentages revealed distinct profiles between SMC and SOS (Fig. 5). In total, 28 PLFAs were identified: i14:0, 14:00, i15:0, a15:0, 15:00, i16:0,16:0, 16:1ω9, 16:1ω7t, 16:1ω7c, 10Me16:0, 16:1ω5, i17:0, a17:0, 17:0, 17:1, 17:1ω8, 10Me17:0, br18:0, cy17:0, 18:0, 18:1, 18:1ω9, 18:1ω7, 10Me18:0, 18:2ω6, cy19:0, and 20:0 (Fig. 5B). The first and second principal components (PC1 and PC2) accounted for 67% and 28% of the total variation, respectively (Fig. 5A).

Compared with SOS, SMC exhibited an increased relative abundance of Gram-positive (G^+) bacterial indicators (such as i15:0, a15:0, i16:0, i17:0, and a17:0), Gram-negative (G^-) bacterial indicators (such as 16:1ω7c, 17:1ω8, cy17:0, 18:1ω7c, and cy19), fungal indicators (16:1ω9 and 18:1ω9), *Actinomycetes* indicators (10Me16:0, 10Me18:0), general bacteria indicators (15:0, 16:0, 16:1ω7t, 16:1ω5, 17:0, and 18:0), and eukaryote indicators (20:0). SOS communities displayed a reduced variety of PLFAs, but they presented a higher relative abundance of the fungal indicator 18:2ω6, *Actinomycetes* indicator 10Me17:0, G^+ indicator i14:0, and bacterial indicator br18:0. This is in line with the results of Suwannarach et al. (2022), highlighting the dominance of aerobic bacteria and G^+ in the composted material used for cultivation of *Agaricus* species.

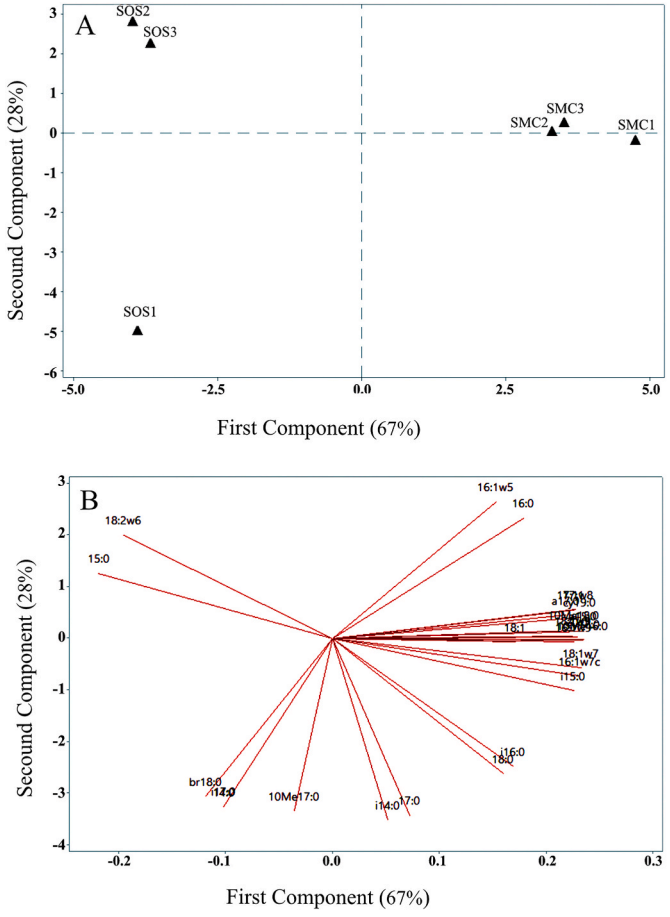

Fig. 5. (A) Phospholipid fatty acid (PLFA) profiles of samples collected from spent mushroom compost (SMC) and spent oyster substrate (SOS), as determined by principal component (PC) analyses. (B) The specific fatty acids identified in SMC and SOS, n = 3.

The PLFA profiles suggest a shift in microbial structure towards a fungal-dominant profile in SOS, and a bacterial-dominant profile in SMC, inclusive of both G^+ and G^- bacteria as well as *Actinomycetes* (Fig. 5). The emergence of PLFA profiles associated with G^+ and G^- bacterial communities has been observed in compost and after the introduction of SMC to soil (Hřebečková et al., 2020; Zheng et al., 2020; Suwannarach et al., 2022). This suggests that the growth environment in SMC can support a bacteria-centric structure with the potential for enhancing disease suppression against plant pathogens. However, the fatty acids 16:1ω9 and 18:1ω9 present in SOS can be of bacterial origin as well, while 18:2ω6 is of almost solely fungal origin. This might suggest that SOS has a larger fungal biomass but with lower diversity, which correlates with the results from the diversity analyses, where the genus *Pleurotus*, the cultivated oyster mushroom, was particularly dominant in SOS (Fig. 4B). These findings advocate enhanced microbial stabilization in composted SMC with respect to the bacterial-to-fungal ratio compared with SOS. The shift towards bacterial composition has also been highlighted by Hong-Wei et al. (2020) as an indicator of disease suppressive conditions.

Moreover, the prominent abundance of oomycetes in SOS combined with the dominance of fungal PLFA hints at a community structure that carries a heightened risk of the proliferation of root pathogens, potentially undermining their use in plant cultivation. This requires further clarification.

3.5. Microbial activity

Phenotypic microarray analyses revealed distinct metabolic profiles for both SMC and SOS, based on the utilization of nutrient sources (Fig. 6). Specifically, SMC profiles diverged from SOS in terms of carbon (Fig. 6A), nitrogen (Fig. 6B), and phosphorus (Fig. 6C) utilization patterns, with greater differentiation occurring on Dim1 than Dim2 for all three sources. The differences in the functional structure between the microbial communities of the investigated samples was a result of the type of the substrate used to cultivate the edible fungi (Suwannarach et al., 2022). The *Agaricus* species in the SMC samples are secondary decomposer fungi grown on aerobic fermented compost with richer organic matter and nutrient availability, while the *Pleurotus* species in the SOS samples can grow on non-degraded sterile lignocellulosic substrates, thereby with different nutrient availability (Suwannarach et al., 2022).

Heat map analyses (Supplement SFig. 2), in contrast, highlighted shared utilization patterns between SMC and SOS. For carbon sources, microbial communities in both SMC and SOS utilized pthreonine, propanedoil, myo-inositol, uridine, melibiose, glactonic acid, adonitol, sacharic acid, galactonic acid, cellobiose, maltose, malic acid, and glucosamine (Supplement SFig. 2A). For nitrogen sources, the microbial communities utilized compounds such as D,L-lactamide, acetyl-D-glucose, ethylenediamine, thymidine, D-serine, glycine, histidine, uric acid, urea, asparagine, adenosine, tyramine, and lactamide (Supplement SFig. 2B). Phosphorus sources included monophosphoridin, hypophosphate, monophosphamide, taurine, cystamine, thiourea, phosphoethylene, mannose, cyclic monsine, thiophosphate, and sulfate (Supplement SFig. 2C). The nutrient utilization patterns underscored the varied activities in the composted SMC and SOS. The shared carbon and nitrogen profiles suggested an assimilation pattern by microbial communities favoring complex sources (Mchunu et al., 2013). For example, cellobiose utilization hints at cellulase activity related to cellulose degradation (Mchunu et al., 2013). Similarly, urea utilization might indicate urease production. Malic acid utilization has been shown in other studies to boost nutrient uptake and enhance fruit quality (Si et al., 2022).

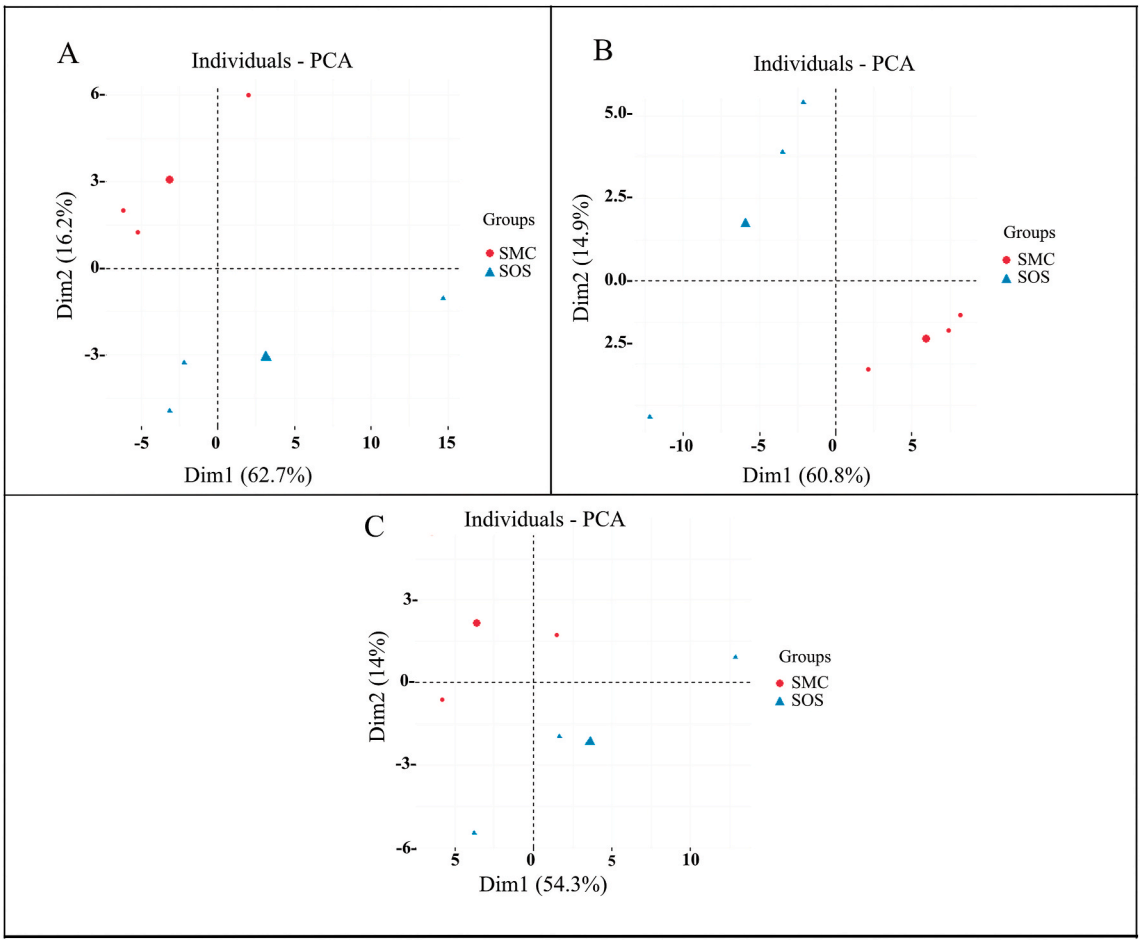

Fig. 6. Utilization profiles of (A) carbon sources, (B) nitrogen sources and (C) phosphorus sources by the microbial communities found in samples of spent mushroom compost (SMC) and spent oyster substrate (SOS), as determined by principal component analyses with Dim 1 and Dim 2 as the first and second component respectively.

Regarding bacterial isolates and their enzymatic roles, ten isolates were collected and characterized (Table 2). Within the context of these isolates, *Pseudomonas* spp. dominated in SMC, while *Bacillus* spp. were predominant in SOS. All the isolated strains from both SMC and SOS demonstrated cellulase, protease, xylanase, and phosphate activity (Table 2). The presence of *Pseudomonas* and *Bacillus* aligns with other studies on compost material (Suwannarach et al., 2022). Extracellular enzyme production helps microorganisms with nutrient acquisition and macromolecule degradation, for example of cellulose, hemicellulose, and lignin. Hence, the enzyme production in our samples indicates microbial activity, corroborating plant nutrient availability in SMC and SOS. Both SMC and SOS shared production of the enzymes phosphatase, cellulase, and xylanase, despite the varied substrates used in the cultivation of the edible fungi (Suwannarach et al., 2022). However, enzyme production may not be solely attributed to the mushroom mycelium but could also involve the bacterial communities present in the spent substrate (Y. Liu et al., 2022). Studies performed by Thai et al. (2022), demonstrated variations in extracellular enzyme production throughout mushroom cultivation, with a noted decline at the cultivation period's end. Consequently, the increased enzyme production observed in our study could enhance the stabilization of microbial profiles, attributed to the effects of composting. Antagonistic assays based on the dual-culture tests revealed that bacterial isolates *in vitro* could inhibit fungal pathogens such as *Botrytis cinerea*, *Rhizoctonia solani*, *Phytophthora colocasiae*, *Phytophthora infestans*, *Phytophthora cactorum*, and *Alternaria alternata* (Table 3, supplement SFig. 3B). Isolates 4

Table 2
The identification of pure bacterial isolates from spent mushroom compost (SMC) and spent oyster substrate (SOS) and their enzyme activities. (+) indicates positive and (−) indicates negative activities.

Isolate number	Species identification	Source	Protease activity	Phosphatase activity	Cellulase activity	Xylanase activity
1	*Bacillus altitudinis*	SOS	_	+	+	+
2	*Pseudomonas* spp.	SMC	+	+	+	+
3	*Bacillus circulans*	SOS	+	+	+	+
4	*Pseudomonas plecoglossicida*	SMC	+	+	+	+
5	*Pseudomonas abietaniphila*	SOS	+	+	+	+
6	*Pseudomonas putida* or *Pseudomonas plecoglossicida*	SMC	+	+	+	+
7	*Bacillus licheniformis*	SOS	_	+	+	+
8	*Pseudomonas putida*	SMC	+	+	+	+
9	*Pseudomonas*	SMC	+	+	+	−
0	*Enterobacter* spp.	SMC	+	−	+	−

Table 3
Antagonistic activity expressed as inhibition in (%) of radial growth in the plant pathogens *Botrytis cinerea*, *Rhizoctonia solani*, *Phytophthora colocasiae*, *Phytophthora infestans*, *Phytophthora cactorum* and *Alternaria alternata* by the bacterial isolates 1−10 from spent mushroom compost or spent oyster substrate. Significant differences between treatments was with $p < 0.05$. Mean ± standard deviation are shown, n = 3.

Isolate number	*Botrytis cinerea*	*Rhizoctonia solani*	*Phytophthora colocasiae*	*Phytophthora infestans*	*Phytophthora cactorum*	*Alternaria alternata*
1	40 ± 0,1^{c}	43 ± 0,12^{b}	0 ± 0,0	40 ± 0,12^{c}	47 ± 0,11^{b}	19 ± 0,1 ^{d}
2	35 ± 0,16 ^{d}	37 ± 0,15 ^{d}	47 ± 0,13^{a}	55 ± 0,14^{b}	42 ± 0,11^{c}	40 ± 0,13^{c}
3	37 ± 0,14	34 ± 0,13 ^{d}	33 ± 0,14 ^{d}	38 ± 0,13^{c}	45 ± 0,12^{b}	47 ± 0,14^{b}
4	**55 ± 0,1**^{a}	**50 ± 0,12**^{a}	**49 ± 0,11**^{b}	**60 ± 0,11**^{a}	**50 ± 0,11**^{a}	**60 ± 0,11**^{a}
5	43 ± 0,11^{b}	40 ± 0,14^{c}	19 ± 0,12^{e}	25 ± 0,12^{e}	45 ± 0,12^{b}	36 ± 0,13^{c}
6	29 ± 0,13^{e}	0 ± 0,0	10 ± 0,14^{f}	30 ± 0,12 ^{d}	45 ± 0,12^{b}	20 ± 0,11 ^{d}
7	45 ± 0,11^{b}	40 ± 0,11^{c}	30 ± 0,11 ^{d}	40 ± 0,1^{c}	45 ± 0,13^{b}	45 ± 0,1^{b}
8	**55 ± 0,1**^{a}	**50 ± 0,12**^{a}	**53 ± 0,11**^{a}	**60 ± 0,14**^{a}	**50 ± 0,12**^{a}	**60 ± 0,12**^{a}
9	25 ± 0,1^{e}	0 ± 0,0	0 ± 0,0	50 ± 0,11^{b}	20 ± 0,1 ^{d}	40 ± 0,11^{c}
10	35 ± 0,12 ^{d}	0 ± 0,0	43 ± 0,13^{c}	48 ± 0,12^{b}	19 ± 0,11 ^{d}	38 ± 0,11^{c}

and 8 were particularly potent, reducing the growth of all tested pathogens by about 60% (Table 3). For other isolates, the inhibition ranged from 0% to 50% (Table 4, supplement SFig. 3A). Further studies on isolates 4 and 8, using DLA, revealed their biocontrol efficacy against *P. infestans* on potato leaves (Table 4, supplement SFig. 4). These isolates also showed significant disease resistance against *P. colocasiae* on taro leaves (Fig. 7B–D), and *B. cinerea* on both tomato and grape fruits (Table 4, supplement SFig. 5). Several studies have highlighted the pathogen-suppressive potential of strains such as *Pseudomonas putida* (Elsharkawy et al., 2023) and *Pseudomonas plecoglossicida* (Tiana et al., 2017). However, the results obtained in the current study highlight the potential of SMC and SOS samples to host bacterial strains with suppressive characteristics towards pathogens that commonly occur in horticultural production systems, both on plant roots, such as *Phytophthora* ssp. (Erwin and Ribeiro, 1996), *Rhizoctonia solani* (Yang and Li, 2012), and *Alternaria alternata* (Ali et al., 2023), and on fruits, such as *Botrytis cinerea* (Petrasch et al., 2019).

Seedling tests indicated that inoculation with both isolates 4 and 8 led to enhanced plant height and leaf count in tomato plants compared with controls (Table 5, supplement SFig. 6). Dry weight measurements of stems and roots were also better for inoculated plants (Table 5, supplement SFig. 6), and the chlorophyll content in inoculated tomato plants was superior to control plants (Table 5, supplement SFig. 7). The impact of isolate 4, identified as *P. plecoglossicida*, on plant growth, might be attributable to its role in nutrient availability and phosphorus solubilization. In addition, application of organic fertilizers through SMC or biochar has been shown to improve plant chemical and physiological characters with respect to enhanced root structure (Martín et al., 2023) and the production of photosynthetic pigments (Guan et al., 2023). However, more investigations in these areas are needed in order to strengthen the disease suppression impact of these applications.

In recent years, the bioremediation of contaminants such as heavy metals and antibiotics has emerged as an important topic of environmental research. Recognizing this, our study introduces the prospect of utilizing SMC and SOS as novel agents in the fight against pollution. Although these substrates are well-known for their roles in agriculture and waste management, their potential for bioremediation remains largely unexplored.

Table 4
The effect of bacterial strains 4 and 8 isolated from spent mushroom composts against *Phytophthora infestans* on potato leaves, *Phytophthora colocasiae* on taro leaves, *Botrytis cinerea* on tomato or grape fruits using detached leaf/fruit assay. Letters indicate significant differences between treatments with $p < 0.05$. Mean ± standard deviation are shown, n = 6.

	Mean area of infection (cm^2)			
	Potato	Taro	Tomato	Grape
SK4	0.38 ± 0.9^{a}	0.33 ± 0.2^{a}	0.28 ± 0.4^{a}	0.35 ± 0.3^{a}
SK8	0.17 ± 0.3^{a}	0.51 ± 0.4^{a}	0.75 ± 0.8^{a}	0.5 ± 0.2^{a}
Control	2.66 ± 1.3^{b}	1.67 ± 0.3^{b}	1.81 ± 1.1^{b}	1.28 ± 0.7^{b}

Our observations of the microbial communities and activities supported by SMC and SOS indicate promising directions for future research in leveraging these substrates to mitigate environmental pollutants. Notably, studies like those by Wang et al. (2023a,b) highlight the efficacy of nitrogen-retaining microbial agents in swine compost for enhancing heavy metal resistance genes and reducing toxicity. Similarly, Xu et al. (2021) have highlighted the effectiveness of Pleurotus species in mycoremediation, particularly against cadmium, suggesting a broader potential application for mushroom-based substrates.

Our findings notably identified an elevation in aluminum levels across both SMC and SOS, signaling the need for detailed examination as highlighted in Table 1. Furthermore, the decrease in trace metal concentrations, specifically copper and molybdenum, suggests that microbial activity within the compost may facilitate the reduction of heavy metal levels. This indicates a beneficial role of microbial processes in mitigating environmental pollution.

Additionally, the significant rise in iron content (Table 1) underscores its importance for plant growth, warranting further investigation into how the inclusion of SMC and SOS in soil amendments affects nutrient absorption. Moreover, as indicated by Wang et al. (2023a,b), the spread of antibiotics and antibiotic resistance genes in compost with manure, such as chicken manure, is a matter of concern. While this area has not been explored in our study, it represents a crucial area for future research to address.

4. Conclusions

This study highlights the impact of composting of spent material after the harvest of either white or oyster mushrooms, and sheds light on the role the microbial communities found in SMC and SOS can play in enhancing plant growth and mitigating plant disease. Our findings emphasize the viability of SMC and SOS as a tool in horticultural practices, offering the dual benefits of nutrient supply and pathogen protection. Additionally, they advocate a more eco-friendly approach by reducing the reliance on peat, and synthetic fertilizers and pesticides. The diverse microbial profiles found within SMC and SOS support the incorporation of both into horticultural systems. While the microbial richness of SMC was superior to SOS regarding boosting plant growth and defense, SOS still holds promise, particularly for biodegradation, based on its predominant fungal communities. The bacterial strains in both substrates have potential for producing enzymes that boost plant nutrient uptake. However, the composting process was shown to significantly influence the composition and function of microbial communities, suggesting a trend towards microbial stabilization.

Our findings underscore the important possibility of using microbial communities to address horticultural challenges, but they also warrant corroboration through *in vivo* studies to provide a more comprehensive understanding of pathogen inhibition and plant nutrition enhancement. Additionally, the potential risks arising from any pathogenic presence as well as aspects related to environmental pollution require further study.

Table 5
Impact of bacterial strains 4 and 8 isolated from spent mushroom composts in comparison with control on chlorophyll content, dry and fresh weight of treatments, height and number of leaves of tomato plants, after seed inoculation with bacterial strains from spent mushroom composts. Letters indicate significant differences between treatments with $p < 0.05$. Mean ± standard deviation are shown, n= 6.

	Chlorophyll	Fresh weight stem	Fresh weight root	Dry weight stem	Dry weight root	Height	No. leaves
SK4	23.24±2.8 [a]	143±21.2 [a]	14.26±2 [a]	15.51±2.7 [a]	2.35±0.3 [a]	78±6.7 [a]	17.5±1.9 [a]
SK8	22.99±3.3 [a]	141.71±16.3 [a]	14.63±2.7 [a]	15.01±1.4 [a]	2.26±0.3 [a]	76.83±8.6 [a]	17.33±1.3 [a]
Control	17.83±2.2 [b]	117.38±11 [b]	10.93±2.2 [b]	10.71±2.7 [b]	1.83±0.1 [b]	67.25±4.7 [b]	12.66±1.9 [b]

Nonetheless, our research provides a robust baseline for developing sustainable horticultural systems enriched with SMC or SOS, and the groundwork for further studies to investigate the interplay of these microbial characteristics with system-specific abiotic and biotic factors.

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Ethics approval

There were no ethical aspects to this study.

Consent for publication

This material is authorized for publication by all the authors, who have agreed to its submission.

CRediT authorship contribution statement

Samar Khalil: Writing – original draft, Project administration, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Preeti Panda:** Writing – review & editing, Methodology, Formal analysis. **Farideh Ghadamgahi:** Writing – review & editing, Methodology. **Ana Barreiro:** Writing – review & editing, Methodology. **Anna Karin Rosberg:** Writing – review & editing, Methodology. **Maria Karlsson:** Writing – review & editing, Methodology. **Ramesh R. Vetukuri:** Writing – review & editing, Methodology, Funding acquisition, Data curation.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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