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METAGENOMIC PROFILING OF MUSHROOM-ASSOCIATED MICROBIOMES FOR BIOLOGICAL SUPPRESSION OF SOIL-BORNE PLANT PATHOGENS

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Abstract

A soil-borne phytopathogenic fungi, F. oxysporum, R. solani, and S. sclerotiorum, are responsible for 20–40% losses in global annual crop yields, and sustainable strategies for managing these fungi are required as synthetic fungicides had caused environmental pollution and resistance development. This review summarizes the present knowledge on metagenomic analyses of mushroom-associated microbiomes as well as spent mushroom substrate (SMS) as bgcolor="mintcream" biological agents for suppression of pathogens. Mushroom-forming fungi produce defined ecological niches the mycosphere and hyphosphere which preferentially harbor antagonistic bacterial groups such as Bacillus, Pseudomonas, and Streptomyces recruited by the carbon exudate. The high-throughput technology of shotgun metagenomic sequencing combined with the bioinformatic pipeline, such as antiSMASH for the identification of biosynthetic gene clusters and CAZy for the annotation of carbohydrate-active enzymes, has transformed our knowledge on these suppressive communities. SMS, the world's most common agro-waste generated at a rate of 4–5 kg of SMS per kg of harvested mushrooms, acts by different means: physical removal of phytotoxins such as fusaric acid, chemical antimicrobial compounds liberation, and activation of copiotrophic microbial contending with pathogens for nutriments. Synthetic microbial consortia (SynComs) derived from mushroom-associated microbiomes through a rational design, provide higher functional redundancy and ecological stability compared to single strain biocontrol agents. However, translating the promising laboratory and greenhouse outcomes to the field is challenging due to issues such as ecological fitness, variability in soil, and stability of crude SMS. This review presents a comprehensive conceptual framework based on integrating metagenomic approaches, ecological concepts, and practical exploitation for the use of mushrooms microbiomes in sustainable agriculture.

1. INTRODUCTION

Soil-borne phytopathogens, including harmful fungal and oomycete strains such as Fusarium oxysporum, Rhizoctonia solani, Sclerotinia sclerotiorum, Pythium ultimum, and Colletotrichum gloeosporioides, are the constant menace for agricultural biosecurity at a global scale. These pathogens enter their susceptible host crops via root tissue, they interfere with vascular function, trigger systemic chlorosis, and result in extreme root rot or wilt of the crop, which induces a loss of 20-40% of the crops harvested annually worldwide (Hossain et al., 2025). Decades of agriculture treatment for soilborne disease have relied substantially on synthetic chemical fungicides and soil fumigation with chemicals. Nonetheless, the extended usage of these synthetic agents has led to significant environmental pollution and to the erosion of effectiveness due to the selection of fungicide-resistant pathogen populations as well as to the disruption of native soil microbial communities. Side effects of toxicity to non-target organisms, namely the soil microbial community (Francis et al., 2005; Ekholm et al., 2008), leading to damage of key elements in the biogeochemical cycles and plant—soil feedbacks of innate immune system (Matthiessen & Kirkegaard, 2006).

In response to this ecology and regulatory predicament, studies have been prioritized to exploit the natural soil microbiome for disease control that is sustainable. Suppressive soil microbial communities, where phytopathogens cannot establish, establish but do not cause disease, or cause initial disease followed by decline, provide a compelling example of microbially-mediated plant protection. On these grounds, macrofungi (mushrooms) and their living substrates are considered as potent ecological hubs for pathogen suppression (Jansson et al., 2023). Cultivated edible and medicinal mushrooms, including Agaricus bisporus, Pleurotus ostreatus, Pleurotus eryngii, Lentinula edodes, and Morchella sextelata, form intricate mutualistic, commensal, and saprotrophic relationships with specialized bacterial and microfungal communities (Cateni et al., 2021).

The production of the mushroom industry results in enormous amount of residual organic material called as spent mushroom substrate (SMS) or spent mushroom compost (SMC). About 4 to 5 kg of SMS is generated by the worldwide mushroom production for every 1 kg of fresh fruiting bodies (Jensen et al., 2016). Traditionally considered as a disposal problem in the form of agricultural waste with high disposal cost and risk of environmental contamination, SMS is emerging as a functional bio resource rich in organic carbon, residual fungal mycelium, structural carbohydrates, trace minerals, and an extraordinary, very active microbiota (Martín et al., 2023). When reused in agroecosystems at the soil level as an amendment of soil, SMS modifies the SOC chemistry and stimulates a set of beneficial, copiotrophic and antagonistic microbial groups with the potential to suppress soil-borne pathogens via a suite of complementary mechanisms (Herrero-Hernández et al., 2022).

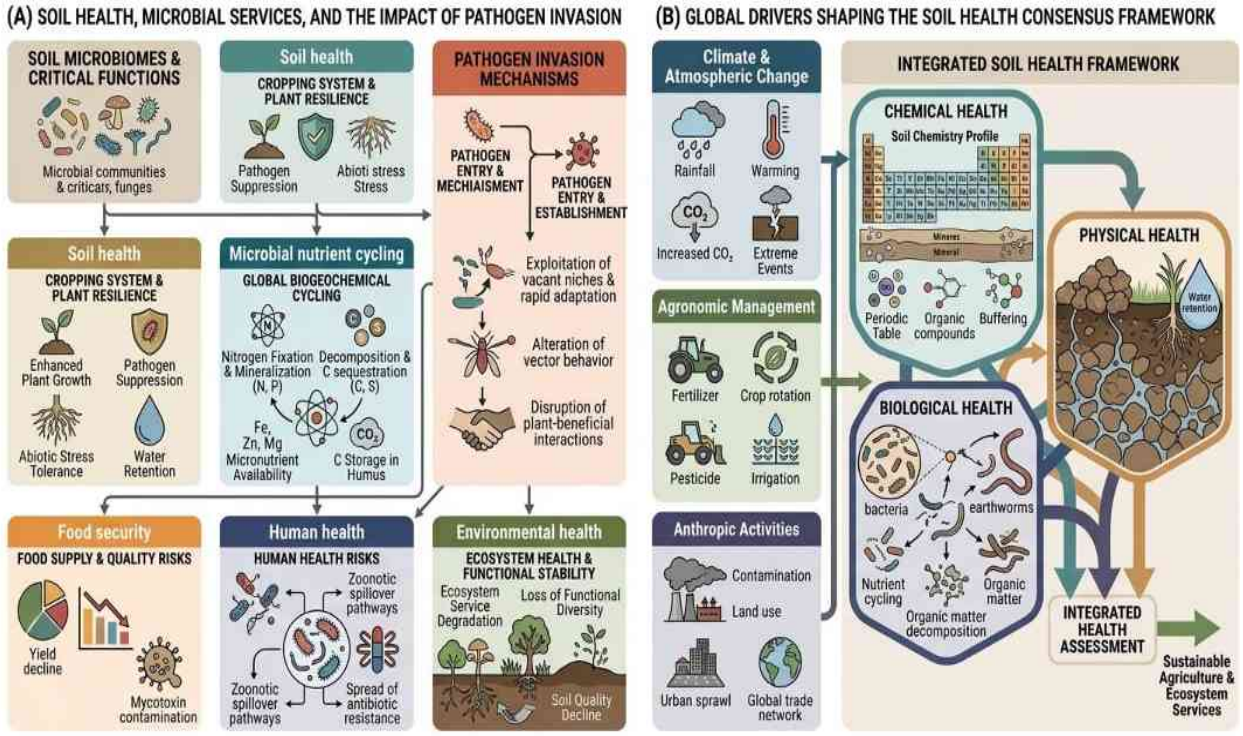


Figure 1: Multidimensional Impacts of Pathogen Invasion on Soil Microbial Services and Global Drivers Shaping the Integrated Soil Health Framework.

2. Spatial and Ecological Niche Dynamics of the Mycosphere and Hyphosphere

The impact of mushroom-forming fungi on their environment goes far beyond the air-exposed fruiting body as they modify the surrounding substrate, or soil, and this modification is dubbed the mycosphere or the hyphosphere (for fungi and bacteria, respectively). Hydrodynamically and chemically, the mycosphere includes that part of the bulk soil that is affected by mycelial networks, either because of water flow or by chemicals or exudates. In this microenvironment, the hyphosphere is the nano-scale interface around hyphal cell walls.Direct sunlight and UV radiation are excluded from these very small spaces.Jacob et al., 2025 have described these microniches as "dynamic-bio-chemical barges in metabolic exchanges, carbon flow and selective microbial recruitment."

Fungal hyphae constantly exude carbonaceous products (i.e., organic acids, polyols, monosaccharides, and bioactive signaling compounds) of their metabolism. These exudates set up nutrient gradients that distinguish the hyphosphere from the rest of the bulk soil, and form an ecological environment that preferentially selects for certain bacterial lineages. Hyphosphere interfaces have been shown by metagenomic and metatranscriptomic analyses to exert a selective pressure on the native soil microflora, which discourages putative taxa, but also recruit keystone bacterial endo (Joshi & Joshi, 2025). Specialized bacterial taxa including epiphytic and endohyphal species from the genera Pseudomonas, Bacillus, Streptomyces, Burkholderia, and Collimonas, continually colonizes these hyphal surfaces. In return for fungal carbon, these hyphosphere-dwelling bacteria perform essential ecological services such as solubilization of recalcitrant inorganic minerals, mineralization of nitrogen, synthesis of growth-promoting phytohormones, and production of broad-spectrum antimicrobial secondary

metabolites that protect the host fungal network from antagonistic invaders (Herrera et al, 2020).

The fungal mycelial network serves as a spatial conduit for bacterial dispersal in unsaturated soil matrices, a process termed the fungal superhighway. Soil pores often undergo drying events, which result in the formation of air-filled gaps and flagellated bacteria become immobilized. Fungal hyphae form bridges over these air-filled gaps and release a constant hydration layer on their outer cell walls, which allows motile bacteria to swim along the hypha. This directed bacterial dispersal facilitates the rapid colonization of newly available organic micro-substrates and spatially restricted nutrient niches that would otherwise be inaccessible to non-motile prokaryotes (Zhang et al., 2018).

However, high-resolution microfluidic studies and stable isotope tracing experiments show that transport along these fungal pathways is tightly regulated. For example, studies that have tracked arbuscular mycorrhizal (AM) fungal hyphae (Rhizophagus irregularis) across spatial barriers have shown that while hyphae readily explore mineral nitrogen compartments, the ability of hyphae to physically transport specialized organic-nitrogen-mineralizing bacteria into uncolonized soil zones is an inherent trade-off between low bacterial spatial mobility and strong host selection. Thus, efficient nutrient cycling and pathogen exclusion within the hyphosphere requires localized co-existence and functional complementarity, not long-range bacterial transport (Richter et al., 2022).

3. Metagenomic Frameworks and Bioinformatic Annotation Systems

The metabolic complexity and the taxonomic composition of a mushroom-associated microbiome can be unraveled only through the application of sophisticated metagenomic sequencing techniques in combination with appropriate bioinformatic tools. Microbial profiling has traditionally been based on targeted amplicon sequencing of marker genes, mainly the 16S rRNA gene for bacteria and archaea and the Internal Transcribed Spacer (ITS) region for fungi. When it comes to general diversity assessment along with monitoring major taxa dynamics at the community level, amplicon sequencing is a very effective and inexpensive approach, but it is affected by unavoidable PCR amplification bias, provides limited taxonomic resolution, and cannot be used to directly infer functional genetic potential (Banerjee et al., 2026).

To surpass the limitations of this approach, shotgun metagenomic sequencing has become the ultimate method to profile suppressive mycospheres. Shotgun metagenomics is an approach in which total DNA is extracted from the environmental samples such as soil, mycosphere microhabitats, or spent mushroom substrates, etc. and sequenced without culture (Converse et al., 2019). The isolated DNA is subjected to high-throughput sequencing using short-read or long-read platforms, and then computational quality control, host DNA decontamination, and de novo sequence assembly are performed (Watts & Hurwitz, 2020). High-depth shotgun sequencing allows metagenomic binning methods to produce high-quality Metagenome-Assembled Genomes (MAGs). Construction of MAGs enables species- and strain-level taxonomic classification according to GTDB standards and reveals previously uncultivated keystone taxa that drive soil suppressiveness (Kim et al., 2026).

Concurrently, assembled contigs are processed through targeted functional annotation pipelines to map metabolic pathways and biosynthetic potential. In the context of biological pathogen suppression, three core bioinformatic databases serve as primary analytical systems:

The antiSMASH (Antibiotics and Secondary Metabolite Analysis Shell) platform is the computational tool of choice for the identification, annotation, and characterization of Biosynthetic Gene Clusters (BGCs) in metagenomic assemblies and isolated microbial genomes (Dong & Strous, 2019). BGCs are physically clustered genes that work together to produce the enzymes needed to make secondary metabolites. antiSMASH tests contigs for core catalytic domains of Non-Ribosomal Peptide

Synthetases (NRPS), Polyketide Synthases (PKS, Type I, II, and III), NRPS-like hybrid clusters, terpene synthases, ribosomally synthesized and post-translationally modified peptides (RiPPs), phenazine biosynthesis clusters, and siderophore synthetases. These BGCs are quantified in mycosphere metagenomes as a direct proxy for the chemical potential of the associated microflora to produce lipopeptides (e.g., surfactin, iturin, fengycin), phenazines, and iron-chelating compounds that inhibit phytopathogens (Medema et al., 2014).

The CAZy (Carbohydrate-Active EnZymes) database classifies enzymes involved in the synthesis, modification, or cleavage of glycosidic bonds. CAZy annotation classifies enzymes into structural families: Glycoside Hydrolases (GHs), Carbohydrate Esterases (CEs), Polysaccharide Lyases (PLs), GlycosylTransferases (GTs), and Auxiliary Activities (AAs, including lytic polysaccharide monooxygenases) (Cantarel et al., 2009). CAZy profiling provides a quantitative measure of the lignocellulolytic and chitinolytic potential of the soil ecosystem in mushroom-associated microbiomes. Elevated levels of GH18 and GH19 chitinase gene families, as well as GH16 β-1,3-glucanases, are direct functional indicators of fungal cell wall degradation and thus the ability of the microbiome to

Table 1: Metagenomic Profiling Pipelines, Key Bioinformatic Databases, and Functional Gene Targets in Suppressive Mycospheres

Bioinformatic Platform / Database	Target Genomic / Functional Module	Biological Mechanism / Output	Key Associated Microorganisms
antiSMASH	Non-Ribosomal Peptide Synthetases (NRPS)	Synthesis of cyclic lipopeptides (surfactin, iturin, fengycin) causing fungal membrane disruption.	Bacillus subtilis, Bacillus velezensis, Paenibacillus spp.
antiSMASH	Polyketide Synthases (PKS Type I, II, III)	Synthesis of broad-spectrum polyketide antibiotics, macrolides, and antifungal agents.	Streptomyces spp., Pseudomonas fluorescens, Burkholderia spp.
antiSMASH	Siderophore Synthetase Clusters	Production of high-affinity iron chelators (pyoverdine, enterobactin) driving competition for Fe^{3+}.	Pseudomonas spp., Streptomyces spp., Enterobacter spp.
CAZy Database	Glycoside Hydrolases (GH18, GH19 Chitinases)	Enzymatic hydrolysis of chitin (β-1,4-N-acetylglucosamine) in fungal pathogen cell walls.	Trichoderma spp., Actinoplanes spp., Chaetomium globosum
CAZy Database	GH16, GH55 (β-1,3-Glucanases)	Enzymatic cleavage of structural glucan linkages in phytopathogenic fungal and oomycete cell walls.	Bacillus spp., Lysobacter spp., Trichoderma harzianum
KEGG Pathway	Phosphonate & Phosphate Solubilization (pqqABCDE)	Organic acid synthesis (gluconic acid) solubilizing bound inorganic phosphorus for root uptake.	Pseudomonas spp., Paenibacillus mucilaginosus, Azotobacter spp.
KEGG Pathway	Nitrogen Fixation & Mineralization (nifH, gdh)	Conversion of atmospheric $N_2$and mineralization of organic	Azotobacter chroococcum,

		nitrogen compounds into bioavailable forms.	Rhizobiales, Actinomadura spp.

4. Spent Mushroom Substrate (SMS) as a Biocontrol Amendment

The industrial production of edible mushrooms generates massive quantities of spent mushroom substrate (SMS). With 4 to 5 kilograms of SMS generated for every kilogram of harvested mushroom, millions of metric tons of this organic material are produced globally each year. Historically, improper disposal of raw SMS through open dumping or uncontrolled landfilling created substantial environmental liabilities, including leachate runoff that eutrophicates aquatic ecosystems, high chemical oxygen demand (COD), and atmospheric emissions of hazardous gases resulting from uncontrolled anaerobic decomposition. However, recent research has reframed SMS as a bioresource for sustainable agriculture, soil remediation, and biological disease suppression (Sabaratnam et al., 2024).

From a chemical point of view, SMS is a partially fermented and extensively altered lignocellulosic substance. Raw materials such as cereal straw (wheat, rice), wood sawdust, sugarcane bagasse, cotton seed hulls, poultry manure and gyps are subjected to vigorous degradation by fungal mycelium during vegetative development as well as fruiting cycles (Vasilakis et al.,2023). As a result, SMS has a high dry-weight organic contents (usually 40% to 60%), low bulk density, high porosity, high water holding capacity and a nutrient composition of total and available nitrogen, phosphorus, potassium, calcium, magnesium and some vital trace elements. To air dry Pleurotus ostreatus SMS, for instance, it consists of 38 to40% cellulose, 18 to 20% hemicellulose, 14 to 16% lignin, 5% the crude protein contributed by residual fungal mycelium and 10% mineral ash (Yagüe et al., 2025).

The nature (physical, chemical and biological) of SMS depends on the type of mushroom grown, the raw substrate material and the duration of the cropping cycle. The biochemical composition of the substrates from Pleurotus spp. (P. ostreatus, P. eryngii, P. florida, P. sajor-caju), Agaricus bisporus and Lentinula edodes is different and they modulate the soil microbiome differently, leading to pathogen suppression via different mechanisms (Parihar et al., 2026).

Among the strategies used by SMS for suppressing soil-borne diseases is the physical and chemical binding of pathogen-produced phytotoxins. Among many plant-pathogenic fungi that produce toxic secondary metabolites as virulence factors during plant infection. A classic example is fusaric acid (FA), a strong phytotoxin produced by the Fusarium oxysporum group (e.g. Fusarium oxysporum f. Sp. cucumerinum, FOC, a causative of cucumber Fusarium wilt) (Sun et al., 2026). It decreases host cell membrane permeability and activates leaf chlorosis and vascular wilting rapidly. In addition, increased level of fusaric acid alters the native host root microbiome by applying toxic selective force, which significantly diminishes bacterial richness, shifts community composition, and downregulates probiotic, FA-susceptible commensal taxa such as Bacillus (Badiwe et al., 2025).

Amending soil with SMS derived from Lentinula edodes effectively mitigates fusaric acid-driven dysbiosis. The porous, lignocellulosic matrix and functionalized organic surfaces of L. edodes SMS physically adsorb fusaric acid, immobilizing the phytotoxin and lowering its bioavailability within the rhizosphere. By stripping fusaric acid from the root zone, SMS protects beneficial Bacillus populations from toxin-induced mortality. These preserved Bacillus strains then execute direct biological suppression against Fusarium hyphae (Liu et al., 2015). Experiments utilizing dual-compartment pot systems confirm that this protective effect is microbiome-dependent: while SMS physical adsorption reduces free toxin concentration in both sterile and non-sterile soils, sustained suppression of Fusarium wilt severity (upward of 50% reduction) occurs exclusively in

non-sterile systems where the native microbial community is stabilized and protected by the SMS amendment (Moročko, 2006).

In addition to toxin adsorption, water extracts from spent mushroom substrates contain natural antimicrobial compounds synthesized by the fungal mycelium and its associated microflora. Liquid chromatography-mass spectrometry (LC-MS) profiling of aqueous extracts from various Pleurotus substrates reveals species-specific bioactive chemical profiles. Pleurotus sajor-caju SMS contains high concentrations of 3-(o-chlorophenyl)-5-(p-tolyloxymethyl)-2-oxazolidone. Pleurotus sapidus SMS reveals the presence of cichoriin, ethiin, and cafestol palmitate. Pleurotus ostreatus SMS accumulates bronopol alongside organic antimicrobial agents (Ishihara et al., 2018). These enzymes have a poisonous action on phytopathogenic fungi and inhibit conidial germination and hyphal growth of pathogens such as Rhizoctonia solani, Fusarium oxysporum, and Colletotrichum gloeosporioides. In vitro assays for dual cultures of antagonistic bacteria, obtained from Pleurotus SMS, show mycelia growth suppression of up to 62.2% and 76.6% against Rhizoctonia solani and Colletotrichum gloeosporioides, respectively (Martínez et al., 2017).

In addition, both the short- and long-term treatments with SMS substantially impact SOC chemical fractions and microbial community composition. Different types of SMS contribute distinctive functional carbon groups to the soil matrix as demonstrated by Solid-state Nuclear Magnetic Resonance ((_^13)"C" CPMAS NMR) H3 Sesbjerg et al., 2017. Substrates with a high content of residual plant fibers, e.g., Pleurotus eryngii SMS (SMS-PE), increase the proportion of readily decomposable O-alkyl C and carbohydrate C fractions in SOC (Quisth et al., 2025). The burst of labile carbohydrate C further accelerates the growth of fast-growing copiotrophic soil bacteria, among the bacterial phylum Proteobacteria and the fungal phyla Basidiomycota and Ascomycota also show higher relative abundance. Predicted functions related to metabolism show that SMS-PE treatment à total carbohydrate metabolism in the soil microbiome, leading to strong nutrient competition and the reduction of plant-pathogenic fungi, mainly Fusarium spp (Mesgar et al. , 2024).

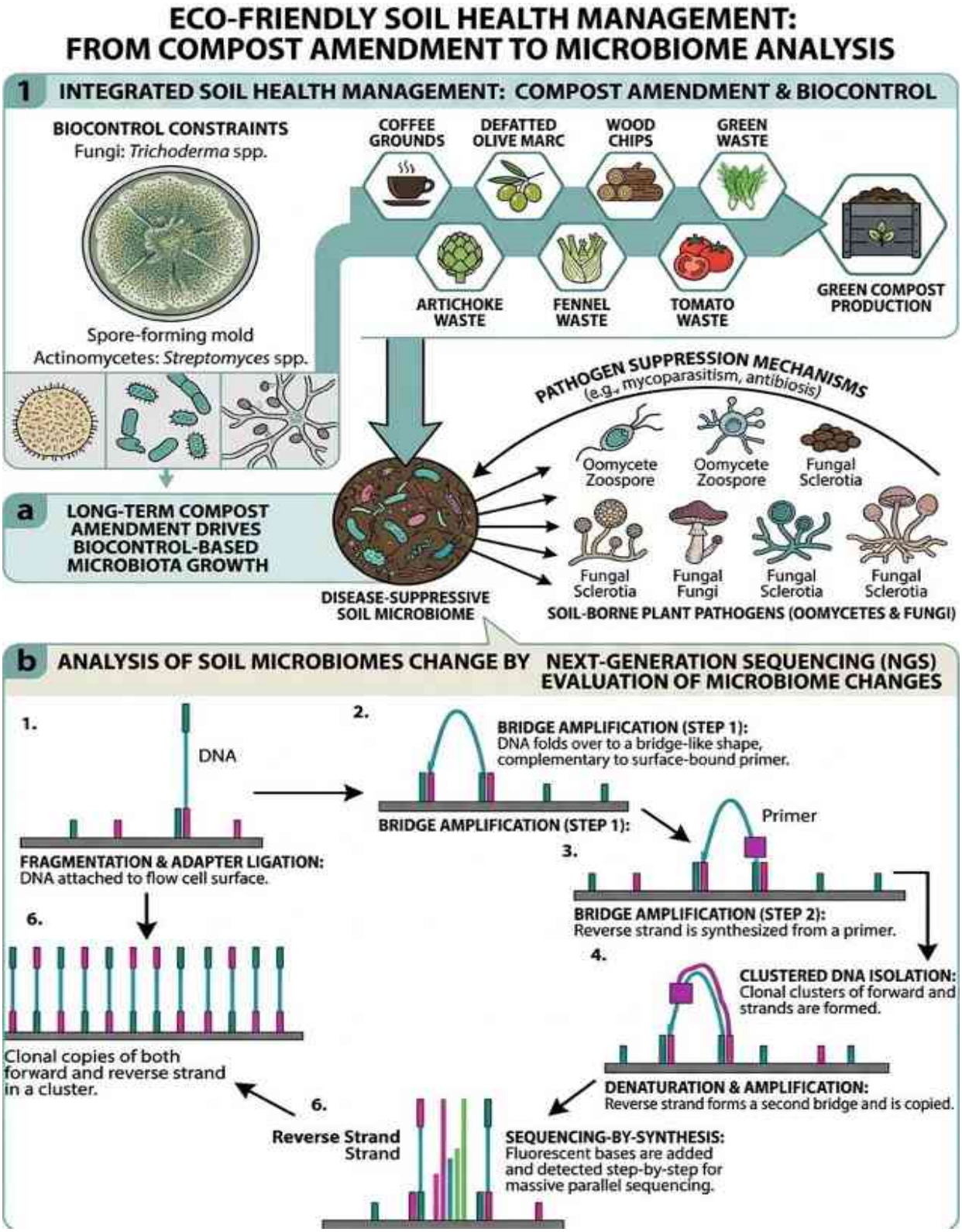


Figure 3: Integrated Framework of Organic Compost Amendment, Biological Pathogen Suppression Mechanisms, and Next-Generation Sequencing (NGS) Workflows for Soil Microbiome Evaluation.

Table 2: Comparative Analysis of Spent Mushroom Substrates Across Fungal Species: Chemical Profiling, Enriched Taxa, and Pathogen Suppression Spectrum

Mushroom Cultivar Source	SMS Chemical & Lignocellulosic Profile	Enriched Core Microbial Taxa	Target Soil-Borne Pathogen Spectrum	Primary Bioactive / Antagonistic Mechanism
Pleurotus ostreatus	High cellulose (38–40%), moderate lignin (14–16%), rich in B, Fe, Cu, N, and K.	Bacillus spp., Pseudomonas spp., Nitrospirae, Deinococcus-Thermus	Rhizoctonia solani, Colletotrichum gloeosporioides	Direct enzymatic antibiosis, secretion of bronopol, dual-culture growth inhibition.
Pleurotus eryngii	High O-alkyl C, abundant labile carbohydrate carbon, high C:N ratio.	Proteobacteria, Basidiomycota, Ascomycota, Thermomyces spp.	Fusarium oxysporum f. sp. cucumerinum, Fusarium wilt species	Upregulation of carbohydrate metabolism, intense copiotrophic resource competition.
Agaricus bisporus	High aromatic carbon, elevated humic acid, high calcium and organic N content.	Firmicutes, Actinobacteria (Actinomadura, Ilumatobacter, Actinoplanes)	Fusarium oxysporum, Sclerotinia sclerotiorum, soil-borne nematodes	Chemical stabilization of aromatic SOC, sustained secretion of lipopeptides and secondary antibiotics.
Lentinula edodes	Porous lignocellulosic matrix, high residual mycelial protein and fungal chitin.	Bacillus spp., Chaetomium globosum, Arthrobotrys amerospora	Fusarium oxysporum (Fusaric acid-producing strains)	Physical adsorption and immobilization of fusaric acid phytotoxins; protection of beneficial root commensals.
Agaricus subrufescens	Humified organic matter, elevated trace mineral and phosphorus levels.	Chaetomium globosum, Arthrobotrys amerospora, Chrysosporium spp.	Nematodes (Meloidogyne spp.), fungal root rot pathogens	Mycoparasitism, nematophagous predation, synthesis of chaetoglobosin A and lytic enzymes.

5. Mechanisms of Biological Pathogen Suppression

Biological suppression of soil-borne plant pathogens in mushroom-amended and mycosphere-rich soils operates via two distinct ecological frameworks: General Suppression and Specific Suppression. General Suppression arises from the aggregate metabolic activity and competitive intensity of the entire soil microbial biomass. High levels of organic amendments to soil (e.g., SMS) provide a massive influx of organic carbon and nutrients that cause an immediate increase in total microbial respiration, enzymatic activity, and biomass build-up a process known as the soil burn rate (Bello et al., 2025). Pathogenic fungal propagules, resting chlamydospores, and sclerotia need exogenous carbon and nitrogen signals to exit dormancy and begin germ-tube growth towards host plant roots. The hyper-active saprotrophic microflora quickly consumes bioavailable simple sugars, amino acids, and oxygen in the root zone, starving pathogen propagules and preventing successful infection. General suppression is non-specific, effective against a wide range of pathogen groups, and

directly related to total soil microbial activity (Allen, 1976).

In contrast, Specific Suppression is when individual microbial species or specific assemblages of microorganisms use targeted antagonistic mechanisms against a particular phytopathogen. Specific suppression is strong, portable between soils, and driven by unique biochemical pathways of direct antagonism, indirect competition, and host plant immune priming (Horton et al., 2019).

Direct antagonism also involves biochem warfare in the form of antibiosis and enzymatic degradation. Bacteria associated with mushrooms mainly belonging to Bacillus, Pseudomonas and Streptomyces have large BGCs for production of antibiotics. Species of Bacillus produce several families of cyclic lipopeptides such as surfactins, iturins and fengycins. Iturins and fengycins penetrate the fungal cell membranes and are involved in the formation of transmembrane ion-permeable pores that elicit a rapid intracellular flow of "K"+ ions followed by osmotic shock and cell lysis. These agents interfere directly with fungal mitochondrial electron transport chains and also inhibit cell division (Rai & Prasad, 2023): Fluorescent Pseudomonas isolates produce several broad-spectrum antifungal metabolites including phenazine-1-carboxylic acid (PCA), 2,4-diacetylphloroglucinole (DAPG), pyoluteorin and pyrrolnitrin with mode of action disruption of fungal mitochondrial electron transport chain. At the same time, antagonistic bacteria produce extracellular cell wall hydrolyzing enzymes (CWDEs). The cell walls of true fungi are mainly composed of a structural matrix of cross linked chitin (β-1,4-N-acetylglucosamine) and β-1,3 glucans while the walls of oomycetes are made of cellulose and β-glucans. Antagonistic fungi like Trichoderma spp. and Chaetomium globosum and bacteria such as Actinoplanes and Lysobacter produce significant levels of chitinase (EC 3.2.1.14), β-1,3-glucanase (EC 3.2.1.39) and proteases. These enzymes break down the cell wall of phytopathogenic hyphae, inducing swelling, bursting of hyphae tips, vacuolization of cytoplasm and complete cell lysis and this is one of the principle mechanisms of mycoparasitism (Cao et al. , 2009).

The indirect antagonism is mediated through occupation of space, biofilm protection, and nutrient depletion that leads to the exclusion of the pathogen from resources required for its survival and dispersal in the host. Microorganisms (bacteria and yeast) in the mycosphere attach to the surface of roots and hyphae rapidly, establish on root surface and form complex biofilm matrices consisting of adsorbed extracellular polymeric substances (EPS) that physically impede phytopathogenic hyphae from penetration of host infection sites. One of the main chemical bases of indirect suppression is the high affinity iron sequestration with microbial siderophores (Lahlali et al., 2022). Iron (Fe^{3+}) is a vital cofactor for microbial metabolism and pathogenesis; however, its availability in the aerobic soil under neutral or alkaline pH is limited. Good pseudomonad and actinobacterial candidates produce high-affinity $^{3+}$-chelating ligands (e.g. pyoverdines, pyochellines, and enterobactins) whose binding constants for ferric iron are much higher as those of fungal phytopathogens. Siderophore bacteria quickly adhere to and reduce free Fe^{3+} in the rhizosphere, inducing a iron deficiency in the area, which has been shown to stops the spore germination of phytopathogenic fungi and slow hypha growth (Soares, 2022).

6. Synthetic Microbial Consortia (SynComs) Derived from Mushroom Microbiomes

Although individual BCAs, such as single strains of Bacillus subtilis or Trichoderma harzianum, often show good efficacy under in vitro and greenhouse conditions, their performance in commercial agriculture is known to be highly variable (Desai et al., 2002). Single-strain inoculants are often unable to establish stable populations in soils under open field conditions due to competitive exclusion by indigenous microflora, hydrothermal soil stress, temporal variation, and difficulty to sustain high level of antibiotic gene expression in diverse soil environments. In addition, a single microbial strain would usually have a relatively limited range

of target pathogens, and hence it might not be very effective in soils with complex, multi-pathogen complexes (Khare & Arora, 2014).

To break these ecological constraints, agricultural biotechnology now embraces the synthesis and application of Synthetic Microbial Communities (SynComs). A SynCom is a synthetic community composed of a defined, characterized set of microbial strains that simulates the structural stability, functional redundancy and ecological synergy of natural suppressive microbiomes with a substantial reduction in complexity of the ecosystem (Khan, 2024).

Effective mushroom-derived SynComs are built using a series of ecological and bioinformatic design principles. The process starts with natural suppressive microbiomes or rich mycosphere matrices, which are then metagenomically sequenced and culturomically strain isolated. Core microbiome members and hub taxa (highly connected microbial nodes that disproportionately influence microbiome assembly and stabilization) are identified using co-occurrence network analysis. Hub taxa are added to guarantee the structural integrity of the consortium. After this, candidate strains are tested in silico with bioinformatic screening (antiSMASH, CAZy, KEGG pathway mapping) to choose strains with complementary, non-overlapping mechanisms of action (De Barba et al., 2017). A multi-genus consortium, for instance, comprising a lipopeptide-producing Bacillus strain, a pyoverdine-secreting Pseudomonas strain, a chitinolytic Streptomyces strain, and a mycoparasitic Trichoderma fungus, fills several ecological niches at once. Functional redundancy guarantees that other consortium members will continue to suppress pathogens if abiotic stress compromises one strain. Most importantly, candidates are tested in vitro and in silico for intra-consortium antagonism. Mixing strains that produce mutually toxic antibiotics leads to intra-consortium interference competition, causing the SynCom to collapse. Advanced SynCom design involves strains that have different carbon sources (niche partitioning) and interact synergistically via cross-feeding or quorum-sensing signals (Hajji-Hedfi et al., 2026).

);

/* **Synonyms & Contrasts** */

Recent experimental case studies highlight the efficacy of mushroom- and plant-associated SynComs. Bacillus subtilis strain A9 was selected as a core endophytic symbiont among the research on Morchella sextelata (morel mushroom) cultivated under pathogen pressure. Metagenomic and metabolomic analysis showed that the treatment of strain A9 highly inhibited the rot disease caused by Lecanicillium aphanocladii. The integration of B. subtilis A9 mediated metabolomic changes in the host by increasing the production of stress-resistant and antimicrobial metabolites such as L-proline, ketoleucine, and pelargonic acid and that returned the host to a healthy state. Comparative metagenomics analysis further revealed that the structured organ microbiomes act as filters shaping distinct bacterial protective shields (Qin et al., 2025) in subterranean fruit-bearing systems. Synthetic three-strain Bacillus SynCom isolated from geocarposphere soil exhibited highest proliferation inhibition capacity and disease suppression against fungal rot (Aspergillus spp.) over single-strain treatments. Transcriptomic analysis demonstrated that the consortia coordinated the activity of molecular pathways responsible for fungal cell wall degradation and cell division arrest (Liu et al., 2025). In a similar way, ten distinct SynComs created from 14 bacterial isolates (including Bacillus, Pseudomonas, Streptomyces, and Trichoderma) were constructed for a wheat crop coconservation strategy against Rhizoctonia solani AG8 root rot. Seven of the 10 SynComs exhibited volatile organic compound (VOC) production, enzymatic toxicity of cell free supernatant and strong root colonization, preventing pathogen infection in vivo (Prigigallo et al., 2022).

7. Translational Challenges, Field Application, and Industrial Horizons

Although the results of laboratorial and controlled greenhouse studies with SMS and tSynCom look promising, scaling these biological solutions to broadÂ-scale conventional agriculture will present operational and environmental challenges (Nuralykyzy et al., 2025).

A central limitation to applied microbial inoculants, under field conditions, is ecological competence The capacity of an inoculant strain or SynCom to survive, establish, compete and maintain functional activity in a complex, nonsterile soil system. Soils at field sites exhibit very high variability in soil texture, pH, organic matter content, salinity, temperature, moisture regimes, and indigenous microbial pressure (Umar et al., 2026). An inoculant designed for optimal performance in neutral, nutrient-rich potting soils may suffer rapid population collapse in acidic, low-carbon, or drought-stressed field soils. In addition, native soil microflora apply strong competitive exclusion often leading to introduced SynCom strains not being able to colonize host root tissues (Bharti et al., 2020).

When spent mushroom substrate is used as an organic soil conditioner raw untreated SMS is a business and phytosanitary hazard. Untreated SMS obtained directly from the mushroom growing houses often contains high moisture, non-composted organic matter, seeds of some crops and fungal spores of the genus Trichoderma (green mold) an opportunistic pathogen of humans and a potential pathogen of plants. If raw SMS is applied to crops without prior stabilization it can ferment in anaerobic conditions, form and release phytotoxic organic acids, cause a rise in electrical conductivity (EC) acting as salt stress, lead to a temporary dibiation of nitrogen which could cause stunting in crops (Kousar et al., 2024).

8. Conclusions

Mushroom-associated microbiomes and spent mushroom substrates (SMS) provide a transformative, ecologically sustainable model for biological control of soil-borne phytopathogens. Innovative shotgun metagenomic sequencing technologies integrated with bioinformatic annotation platforms like antiSMASH (for biosynthetic gene cluster characterization) and CAZy (for carbohydrate-active enzyme profiling) have provided the highest resolution taxonomic and functional metabolic potential of resident microbial communities within suppressive mycospheres to date. These frameworks have demonstrated that macrofungi are keystone species that shape their surrounding microbial communities via carbon exudation, selective bacterial recruitment, and physical hyphal networks that serve as dispersal superhighways, forming ecological zones of intense antagonistic activity against pathogenic fungi and oomycetes. Spent mushroom substrate, once viewed as agricultural waste, is now an important bioresource for disease control, with complementary chemical, physical, and biological mechanisms of suppression, including phytotoxin adsorption, antimicrobial compound secretion, and stimulation of copiotrophic microbial communities responsible for both general and specific suppression. SMS chemical and microbial composition is highly heterogeneous among mushroom species, allowing targeted strategies for specific pathogen complexes. Engineered microbial consortia comprising beneficial strains associated with mushrooms overcome the ecological constraints of single-strain inoculants by incorporating functional redundancy, niche partitioning, and synergistic interactions that enhance survival and activity across heterogeneous field environments. However, successful translation of these biological technologies from laboratory to broad-acre agriculture requires overcoming challenges related to ecological competence, soil heterogeneity, raw SMS stabilization, and formulation scalability. Future research directions should prioritize field-scale validation studies, development of strain-specific molecular markers for monitoring SynCom establishment, and refinement of SMS processing protocols to ensure consistent, standardized products for sustainable agricultural implementation.

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