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Harnessing the Mycosphere: Metagenomic Profiling of Mushroom Microbiomes and Spent Substrates for Plant Disease Suppression

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Abstract

Soil-borne phytopathogens, including *Fusarium oxysporum*, *Rhizoctonia solani*, and *Sclerotinia sclerotiorum*, cause 20–40% annual global crop yield losses, necessitating sustainable alternatives to synthetic fungicides that have driven environmental degradation and resistance emergence. This review synthesizes current knowledge on metagenomic profiling of mushroom-associated microbiomes and spent mushroom substrate (SMS) as biological tools for pathogen suppression. Mushroom-forming fungi create distinct ecological niches the mycosphere and hyphosphere that selectively enrich antagonistic bacterial taxa including *Bacillus*, *Pseudomonas*, and *Streptomyces* through carbon exudate-mediated recruitment. Advanced shotgun metagenomic sequencing, coupled with bioinformatic platforms including antiSMASH for biosynthetic gene cluster identification and CAZy for carbohydrate-active enzyme annotation, has revolutionized our understanding of these suppressive communities. SMS, a globally abundant agricultural byproduct generating 4–5 kg per kilogram of harvested mushrooms, functions through multiple mechanisms: physical adsorption of phytotoxins such as fusaric acid, chemical antimicrobial compound release, and stimulation of copiotrophic microbial communities that intensify nutrient competition against pathogens. Synthetic microbial consortia (SynComs), rationally designed from mushroom-associated microbiomes, offer functional redundancy and ecological stability superior to single-strain biocontrol agents. Despite promising laboratory and greenhouse results, field translation faces challenges including ecological competence, soil heterogeneity, and raw SMS stabilization requirements. This review provides a comprehensive framework integrating metagenomic technologies, ecological principles, and practical applications for harnessing mushroom microbiomes in sustainable agriculture.

Keywords: Metagenomics, Spent Mushroom Substrate, Biological Suppression, Mycosphere, Soil-Borne Pathogens, Synthetic Microbial Consortia

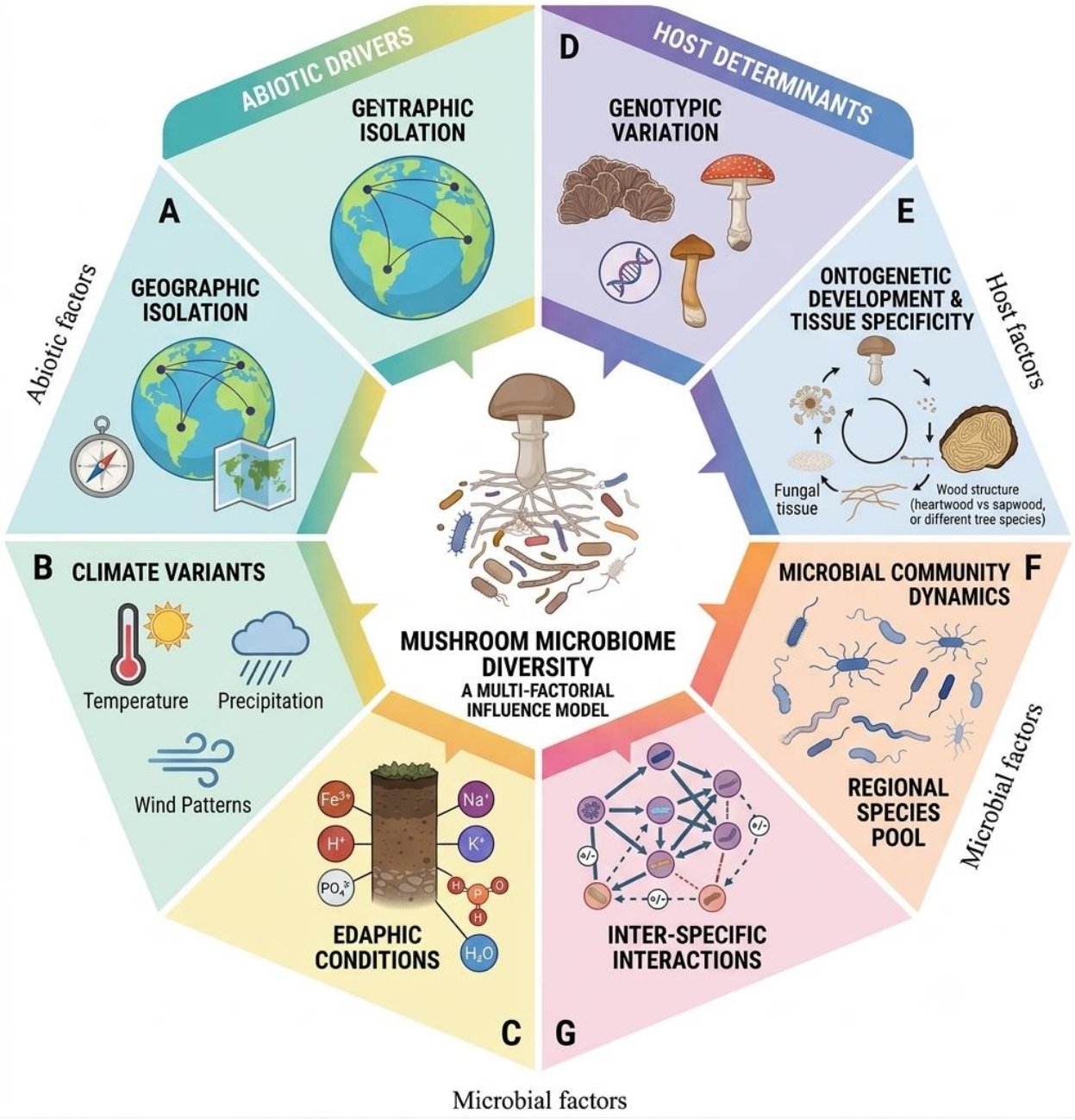
1. Introduction

Soil-borne phytopathogens including destructive fungal and oomycete species such as Fusarium oxysporum, Rhizoctonia solani, Sclerotinia sclerotiorum, Pythium ultimum, and Colletotrichum gloeosporioides pose a chronic threat to global agricultural biosecurity. These pathogens infect susceptible host crops through root tissues, disrupting vascular function, inducing systemic chlorosis, and causing severe root rot or wilt, which collectively result in annual global crop yield losses ranging between 20% and 40% (Hossain et al., 2025). For decades, agricultural management of soil-borne diseases has depended heavily on synthetic chemical fungicides and chemical soil fumigation. However, the prolonged application of these synthetic inputs has driven substantial environmental degradation, accelerated the emergence of fungicide-resistant pathogen strains, and severely disrupted native soil microbial ecosystems. The non-target toxicological effects of chemical pesticides diminish the structural stability of the soil microbiome, impairing essential biogeochemical cycles and compromising intrinsic plant-soil immune feedback loops (Matthiessen & Kirkegaard, 2006).

In response to these ecological and regulatory challenges, research has focused on harnessing the natural soil microbiome for sustainable disease control. Suppressive soils microbial ecosystems in which phytopathogens either fail to establish, establish but fail to produce disease, or cause initial disease that progressively declines demonstrate the efficacy of microbial-mediated plant defense. Within this framework, macrofungi (mushrooms) and their associated substrates have emerged as powerful ecological centers 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 mushroom industry generates immense volumes of residual organic material known as spent mushroom substrate (SMS) or spent mushroom compost (SMC). Globally, mushroom cultivation produces approximately 4 to 5 kilograms of SMS for every single kilogram of fresh fruiting bodies harvested. Historically treated as an agricultural waste product with high disposal costs and potential environmental risks, SMS is now recognized as a valuable bioresource rich in organic carbon, residual fungal mycelium, structural carbohydrates, trace minerals, and an abundant, highly active microbiota (Martín et al., 2023). When recycled into agroecosystems as a soil amendment, SMS alters the chemical architecture of soil organic carbon (SOC) and enriches beneficial copiotrophic and antagonistic microbial taxa capable of suppressing soil-borne pathogens through multiple complementary mechanisms (Herrero-Hernández et al., 2022).

Figure 1: Multi-Factorial Influence Model of Mushroom Microbiome Diversity Across Abiotic, Host, and Microbial Drivers.



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

The ecological influence of mushroom-forming fungi extends well beyond the visible fruiting body into the surrounding matrix, creating distinct, highly active microhabitats known as the mycosphere and the hyphosphere. The mycosphere encompasses the bulk soil zone hydrodynamically and chemically modulated by dense fungal mycelial networks. Within this domain, the hyphosphere represents the micro-scale interface immediately surrounding hyphal cell walls. These microhabitats act as dynamic biochemical marketplaces characterized by intense metabolic crosstalk, carbon flow, and selective microbial recruitment (Jacob et al., 2025).

Fungal hyphae continuously release carbon-rich exudates consisting of organic acids, polyols, monosaccharides, and bioactive signaling molecules. These exudates establish steep nutrient gradients

that differentiate the hyphosphere from the surrounding bulk soil, creating an ecological niche that selectively enriches specific bacterial lineages. Metagenomic and metatranscriptomic profiling reveal that the hyphosphere interface filters the indigenous soil microflora, rejecting opportunistic taxa while actively recruiting keystone bacterial symbionts (Joshi & Joshi, 2025). Specialized bacterial taxa including epiphytic and endohyphal species from genera such as Pseudomonas, Bacillus, Streptomyces, Burkholderia, and Collimonas consistently colonize these hyphal surfaces. In exchange for fungal carbon, these hyphosphere-associated bacteria execute vital ecological functions, including inorganic mineral solubilization, nitrogen mineralization, production of growth-promoting phytohormones, and the synthesis of broad-spectrum antimicrobial secondary metabolites that safeguard the host fungal network against antagonistic invaders (Herrera et al., 2020).

The physical architecture of the fungal mycelial network acts as a spatial conduit for bacterial dispersal across unsaturated soil matrices, a phenomenon described as the fungal superhighway. Soil pores frequently experience drying events that break liquid bridges, immobilizing flagellated bacteria. Fungal hyphae bridge these air-filled gaps, releasing a continuous hydration film along their outer cell walls that allows motile bacteria to swim along the hyphal surface. This directional bacterial migration enables the rapid colonization of newly exposed organic micro-substrates and spatially restricted nutrient zones that would otherwise remain inaccessible to isolated prokaryotes (Zhang et al., 2018).

However, high-resolution microfluidic studies and stable isotope tracing experiments demonstrate that transport dynamics along these fungal pathways are strictly regulated. For instance, experiments tracking arbuscular mycorrhizal (AM) fungal hyphae (Rhizophagus irregularis) across spatial barriers reveal that while hyphae readily explore mineral nitrogen compartments, their capacity to physically vector specialized, organic-nitrogen-mineralizing bacteria across uncolonized soil zones is intrinsically limited by low bacterial spatial mobility and stringent host selection. Consequently, efficient nutrient turnover and pathogen exclusion within the hyphosphere require localized co-existence and functional synergy rather than reliance on long-distance bacterial transport (Richter et al., 2022).

3. Metagenomic Frameworks and Bioinformatic Annotation Systems

Resolving the metabolic complexity and taxonomic composition of mushroom-associated microbiomes requires advanced metagenomic sequencing methodologies paired with targeted bioinformatic pipelines. Traditional microbial profiling relied primarily on targeted amplicon sequencing of marker genes, specifically the 16S rRNA gene for bacteria and archaea and the Internal Transcribed Spacer (ITS) region for fungi. While amplicon sequencing offers a cost-effective strategy for assessing community alpha and beta diversity and tracking broad taxonomic shifts, it suffers from inherent PCR amplification biases, limited taxonomic resolution, and an inability to directly measure functional genetic capacity (Banerjee et al., 2026).

To overcome these constraints, shotgun metagenomic sequencing has emerged as the definitive standard for profiling suppressive mycospheres. Shotgun metagenomics begins with total environmental DNA extraction directly from raw environmental samples such as soil, mycosphere microhabitats, or spent mushroom substrates. The extracted DNA undergoes high-throughput sequencing via short-read or long-read platforms, followed by computational quality control, host DNA decontamination, and de novo sequence assembly (Watts & Hurwitz, 2020). High-depth shotgun sequencing enables metagenomic binning algorithms to reconstruct high-quality Metagenome-Assembled Genomes (MAGs). MAG construction allows for species- and strain-level taxonomic identification according to Genome Taxonomy Database (GTDB) standards, uncovering 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 standard computational tool used to identify, annotate, and characterize Biosynthetic Gene Clusters (BGCs) within metagenomic assemblies and isolated microbial genomes (Dong & Strous, 2019). BGCs are physically clustered groups of genes that collectively encode the enzymatic machinery required to synthesize secondary metabolites. antiSMASH screens contigs for core catalytic domains associated with Non-Ribosomal Peptide Synthetases (NRPS), Polyketide Synthases (PKS, Type I, II, and III), NRPS-like hybrid complexes, terpene synthases, ribosomally synthesized and post-translationally modified peptides (RiPPs), phenazine biosynthesis clusters, and siderophore synthetases. Quantification of these BGCs in mycosphere metagenomes directly correlates with the chemical potential of the resident microflora to synthesize lipopeptides (e.g., surfactin, iturin, fengycin), phenazines, and iron-chelating molecules that suppress phytopathogens (Medema et al., 2014).

The CAZy (Carbohydrate-Active EnZymes) database classifies enzymes that synthesize, modify, or cleave glycosidic bonds. CAZy annotation categorizes 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). In mushroom-associated microbiomes, CAZy profiling quantifies the lignocellulolytic and chitinolytic capacity of the soil ecosystem. High abundances of GH18 and GH19 chitinase gene families, along with GH16 β-1,3-glucanases, serve as direct functional markers for fungal cell wall degradation, underpinning the capacity 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 nitrogen compounds into bioavailable forms.	Azotobacter chroococcum, 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).

Chemically, SMS represents a partially digested, highly modified lignocellulosic matrix. Raw substrates typically composed of cereal straw (wheat, rice), wood sawdust, sugarcane bagasse, cotton seed hulls, poultry manure, and gypsum undergo extensive enzymatic breakdown by the fungal mycelium during vegetative growth and fruiting cycles (Vasilakis et al.,2023). Consequently, SMS exhibits a high dry-weight organic matter content (typically 40% to 60%), reduced bulk density, high porosity, elevated water-holding capacity, and a nutrient profile containing total and available nitrogen, phosphorus, potassium, calcium, magnesium, and essential trace elements. Air-dried Pleurotus ostreatus SMS, for example, comprises approximately 38% to 40% cellulose, 18% to 20% hemicellulose, 14% to 16% lignin, 5% crude protein derived from residual fungal mycelium, and 10% mineral ash (Yagüe et al., 2025).

The physical, chemical, and biological properties of SMS vary depending on the mushroom species cultivated, the initial raw substrate composition, and the duration of the cropping cycle. Substrates derived from Pleurotus species (P. ostreatus, P. eryngii, P. florida, P. sajor-caju), Agaricus bisporus, and Lentinula edodes possess distinct biochemical structures that shape the soil microbiome and drive pathogen suppression through unique pathways (Parihar et al., 2026).

A core mechanism by which SMS suppresses soil-borne diseases involves the physical and chemical adsorption of pathogen-derived phytotoxins. Many destructive phytopathogenic fungi secrete toxic secondary metabolites that act as virulence factors during plant infection. A prime example is fusaric acid (FA), a potent phytotoxin secreted by Fusarium oxysporum species (such as Fusarium oxysporum f. sp. cucumerinum, FOC, the causal agent of cucumber Fusarium wilt) (Sun et al., 2026). Fusaric acid impairs host cell membrane permeability, induces severe leaf chlorosis, and causes rapid vascular

wilting. Furthermore, elevated fusaric acid levels disrupt the native host root microbiome, exerting toxic selective pressure that drastically reduces bacterial richness, alters community composition, and suppresses beneficial, FA-sensitive 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 chemical constituents exert direct toxic effects on phytopathogenic fungi, inhibiting conidial germination and hyphal elongation in pathogens such as Rhizoctonia solani, Fusarium oxysporum, and Colletotrichum gloeosporioides. In vitro dual-culture assays using antagonistic bacteria isolated directly from Pleurotus SMS demonstrate mycelial growth inhibition reaching 62.2% against Rhizoctonia solani and 76.6% against Colletotrichum gloeosporioides (Martínez et al., 2017).

Furthermore, the short- and long-term application of SMS fundamentally alters Soil Organic Carbon (SOC) chemical fractions and microbial community assembly. Solid-state Nuclear Magnetic Resonance (^{13}C CPMAS NMR) spectroscopy demonstrates that different SMS types introduce distinct functional carbon groups into the soil matrix. Substrates rich in residual plant fibers, such as Pleurotus eryngii SMS (SMS-PE), elevate the relative abundance of decomposable O-alkyl carbon and carbohydrate carbon fractions in SOC (Quisth et al., 2025). This influx of labile carbohydrate carbon stimulates fast-growing copiotrophic soil bacteria, specifically elevating the relative abundance of the bacterial phylum Proteobacteria and the fungal phyla Basidiomycota and Ascomycota. Functional metabolic predictions indicate that SMS-PE application upregulates total carbohydrate metabolism in the soil microbiome, driving intense nutrient competition that suppresses the prevalence of plant-pathogenic fungi, particularly Fusarium spp (Mesgar et al., 2024).

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	Bacillus spp., Pseudomonas spp., Nitrospirae,	Rhizoctonia solani,	Direct enzymatic antibiosis, secretion of bronopol, dual-

	(14–16%), rich in B, Fe, Cu, N, and K.	Deinococcus-Thermus	Colletotrichum gloeosporioides	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

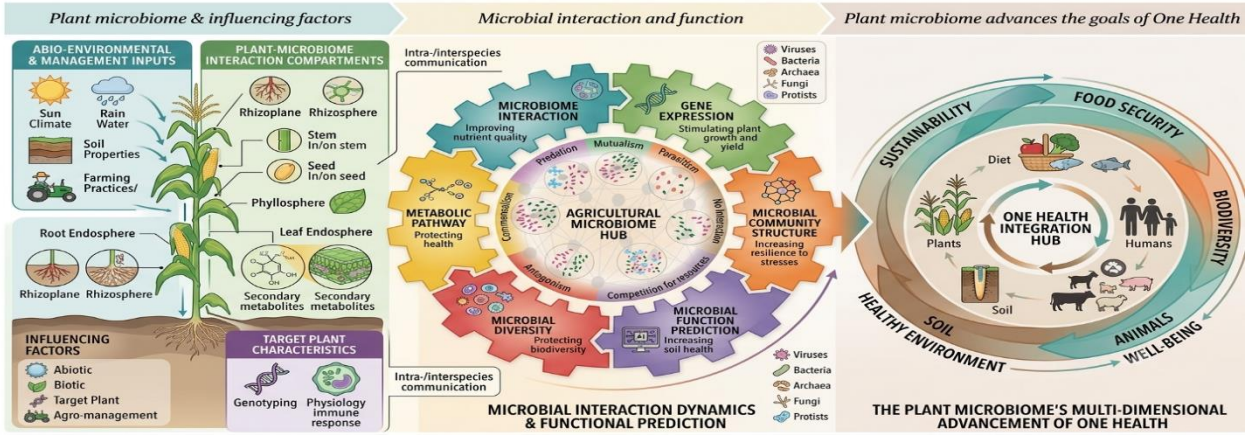
The biological suppression of soil-borne plant pathogens in mushroom-amended and mycosphere-rich soils operates through two distinct ecological frameworks: General Suppression and Specific Suppression. General Suppression results from the collective metabolic activity and competitive intensity of the total soil microbial biomass. When high volumes of organic amendments such as SMS are incorporated into soil, the sudden influx of organic carbon and nutrients triggers a rapid surge in total microbial respiration, enzymatic activity, and biomass accumulation a phenomenon referred to as the soil burn rate (Bello et al., 2025). Pathogenic fungal propagules, resting chlamydospores, and sclerotia require exogenous carbon and nitrogen signals to break dormancy and initiate germ-tube elongation toward host plant roots. The hyper-active saprotrophic microflora rapidly depletes bioavailable simple sugars, amino acids, and oxygen in the root zone, starving pathogen propagules and preventing successful infection. General suppression is non-specific, operates across broad pathogen taxa, and correlates directly with total soil microbial activity (Allen, 1976).

Specific Suppression, in contrast, occurs when individual microbial species or defined sub-assemblages of microorganisms deploy targeted antagonistic mechanisms against a specific phytopathogen. Specific suppression is potent, transferable between soils, and driven by specialized biochemical pathways involving direct antagonism, indirect competition, and host plant immune priming (Horton et al., 2019).

Direct antagonism encompasses biochemical warfare mediated by antibiosis and enzymatic lysis. Mushroom-associated bacteria predominantly strains of Bacillus, Pseudomonas, and Streptomyces possess extensive BGCs dedicated to antibiotic synthesis. Bacillus species synthesize families of cyclic lipopeptides, including surfactins, iturins, and fengycins. Iturins and fengycins directly insert into fungal cell membranes, forming transmembrane ion-conducting channels that cause rapid leakage of intracellular K^+ions, osmotic collapse, and cell lysis. Fluorescent Pseudomonas strains secrete broad-spectrum antifungal agents such as phenazine-1-carboxylic acid (PCA), 2,4-diacetylphloroglucinol (DAPG), pyoluteorin, and pyrrolnitrin, which disrupt fungal mitochondrial electron transport chains and inhibit cell division (Rai & Prasad, 2023). Concurrently, antagonistic microbes secrete extracellular cell wall-degrading enzymes (CWDEs). The cell walls of true fungi consist primarily of a structural matrix of cross-linked chitin (β-1,4-N-acetylglucosamine) and β-1,3-glucans, whereas oomycetes contain cellulose and β-glucans. Antagonistic fungi such as Trichoderma spp. and Chaetomium globosum, alongside bacteria like Actinoplanes and Lysobacter, secrete high concentrations of chitinases (EC 3.2.1.14), β-1,3-glucanases (EC 3.2.1.39), and proteases. These enzymes digest the structural framework of phytopathogenic hyphae, causing hyphal swelling, tip bursting, cytoplasm vacuolization, and complete cell lysis, which represents a primary mechanism of mycoparasitism (Cao et al., 2009).

Indirect antagonism relies on physical space occupancy, biofilm shielding, and nutrient sequestration that deprives pathogens of resources essential for survival and host colonization. Beneficial bacteria and yeasts in the mycosphere rapidly adhere to root surfaces and hyphal networks, forming dense biofilm matrices composed of extracellular polymeric substances (EPS) that physically block phytopathogenic hyphae from accessing host infection sites. A primary chemical mechanism of indirect suppression is high-affinity iron sequestration mediated by microbial siderophores (Lahlali et al., 2022). Iron (Fe^{3+}) is an essential cofactor for microbial growth, respiration, and pathogenicity; however, its bioavailability in aerobic soils at neutral or alkaline pH is extremely low. Beneficial pseudomonads and actinobacteria secrete high-affinity Fe^{3+}-chelating ligands, such as pyoverdines, pyochellins, and enterobactins, which possess stability constants for ferric iron that far exceed those of fungal phytopathogens. Siderophore-producing bacteria rapidly bind and deplete free Fe^{3+}in the rhizosphere, creating a localized iron-starvation environment that halts phytopathogenic spore germination and suppresses hyphal growth (Soares, 2022).

Figure 2: Conceptual Framework of Plant–Microbiome Interaction Compartments, Functional Interaction Dynamics, and the Multi-Dimensional Advancement of One Health.



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

While individual biological control agents (BCAs) such as single strains of Bacillus subtilis or Trichoderma harzianum frequently demonstrate high efficacy under controlled in vitro and greenhouse conditions, their performance in commercial agricultural fields is notoriously inconsistent (Desai et al., 2002). Single-strain inoculants often fail to establish permanent populations in open field soils due to competitive exclusion by native microflora, adverse edaphic conditions, fluctuations in temperature and moisture, and an inability to maintain high antibiotic gene expression across variable soil environments. Furthermore, a single microbial strain typically possesses a narrow spectrum of target pathogens, limiting its utility in soils harboring complex, multi-pathogen complexes (Khare & Arora, 2014).

To overcome these ecological limitations, agricultural biotechnology has shifted toward the design and deployment of Synthetic Microbial Communities (SynComs). A SynCom is a deliberately assembled consortium of known, characterized microbial strains designed to mimic the structural stability, functional redundancy, and ecological synergy of natural suppressive microbiomes while drastically reducing ecosystem complexity (Khan, 2024).

The construction of effective mushroom-derived SynComs relies on a sequence of ecological and bioinformatic design principles. The workflow begins with natural suppressive microbiomes or rich mycosphere matrices, which undergo metagenomic sequencing and culturomic strain isolation. Co-occurrence network analysis identifies core microbiome members and hub taxa highly connected microbial nodes that exert disproportionate influence over microbiome assembly and stabilization. Incorporating hub taxa ensures consortium structural cohesion. Next, candidate strains are evaluated through in silico bioinformatic screening (antiSMASH, CAZy, KEGG pathway mapping) to select strains with complementary, non-overlapping mechanisms of action (De Barba et al., 2017). For example, a multi-genus consortium combining a lipopeptide-producing Bacillus strain, a pyoverdine-secreting Pseudomonas strain, a chitinolytic Streptomyces strain, and a mycoparasitic Trichoderma fungus covers multiple ecological niches simultaneously. This functional redundancy ensures that if abiotic stress compromises one strain, alternative consortium members maintain pathogen suppression. Crucially, candidates are screened in vitro and in silico to eliminate intra-consortium antagonism. Mixing strains that secrete mutually toxic antibiotics causes intra-consortium interference competition, collapsing the SynCom. Advanced SynCom design pairs strains that utilize distinct carbon sources (niche partitioning) and communicate synergistically through cross-feeding or quorum-sensing signals (Hajji-Hedfi et al., 2026).

Recent experimental case studies underscore the efficacy of mushroom- and plant-associated SynComs. Research investigating Morchella sextelata (morel mushroom) cultivated under pathogen challenge identified Bacillus subtilis strain A9 as a core endophytic symbiont. Metagenomic and metabolomic profiling revealed that applying strain A9 suppressed rot disease caused by Lecanicillium aphanocladii. Integration of B. subtilis A9 induced metabolomic shifts in the host, upregulating stress-resistant and antimicrobial metabolites including L-proline, ketoleucine, and pelargonic acid, thereby restoring host health. In subterranean fruit-bearing systems, comparative metagenomics revealed that specific organ microbiomes filter unique bacterial protective shields (Qin et al., 2025). Assembly of a three-strain Bacillus SynCom isolated from geocarposphere soil demonstrated superior growth inhibition and disease control against fungal rot (Aspergillus spp.) compared to individual single-strain applications. Transcriptomic profiling revealed that the consortium synchronized molecular mechanisms regulating fungal cell wall degradation and cell division arrest (Liu et al., 2025). Similarly, ten distinct SynCom formulations composed of 14 bacterial isolates (spanning Bacillus, Pseudomonas, Streptomyces, and Trichoderma) were engineered to protect wheat crops against Rhizoctonia solani AG8 root rot. Seven of the ten SynComs successfully prevented pathogen infection in vivo, exhibiting

volatile organic compound (VOC) synthesis, cell-free supernatant enzymatic toxicity, and robust root colonization (Prigigallo et al., 2022).

7. Translational Challenges, Field Application, and Industrial Horizons

Despite the promising performance of spent mushroom substrates and synthetic microbial consortia under laboratory and controlled greenhouse conditions, translating these biological technologies into broad-acre agricultural practice presents operational and ecological challenges (Nuralykyzy et al., 2025).

The primary obstacle facing field-applied microbial inoculants is ecological competence the ability of an introduced strain or SynCom to survive, establish, compete, and maintain functional activity within a complex, non-sterile soil ecosystem. Field soils present extreme heterogeneity in soil texture, pH, organic matter content, salinity, temperature, moisture dynamics, and indigenous microbial competition (Umar et al., 2026). An inoculant engineered for optimal performance in neutral, nutrient-rich potting media may experience rapid population mortality when introduced into acidic, low-carbon, or drought-stressed field soils. Furthermore, indigenous soil microflora exert intense competitive exclusion, frequently preventing introduced SynCom strains from colonizing host root tissues (Bharti et al., 2020).

While spent mushroom substrate serves as an organic soil amendment, raw, untreated SMS presents operational and phytosanitary risks. Untreated SMS directly removed from mushroom cultivation facilities frequently harbors high residual moisture, uncomposted organic matter, weed seeds, and opportunistic mold spores, including human or plant pathogens such as Trichoderma green mold. If applied directly to crops without stabilization, raw SMS can undergo anaerobic putrefaction, generating phytotoxic organic acids, high electrical conductivity (EC) salinity spikes, and temporary nitrogen immobilization that severely stunts crop growth (Kousar et al., 2024).

8. Conclusions

Mushroom-associated microbiomes and spent mushroom substrates represent a transformative, ecologically sustainable paradigm for biological suppression of soil-borne phytopathogens. The integration of advanced shotgun metagenomic sequencing with targeted bioinformatic annotation platforms including antiSMASH for biosynthetic gene cluster characterization and CAZy for carbohydrate-active enzyme profiling has enabled unprecedented resolution of the taxonomic composition and functional metabolic potential resident within suppressive mycospheres. These analytical frameworks reveal that macrofungi actively engineer their surrounding microhabitats through carbon exudation, selective bacterial recruitment, and physical hyphal networks that function as dispersal superhighways, creating ecological zones of intense antagonistic activity against pathogenic fungi and oomycetes. Spent mushroom substrate, historically considered agricultural waste, has emerged as a valuable bioresource capable of suppressing disease through complementary chemical, physical, and biological mechanisms including phytotoxin adsorption, antimicrobial compound secretion, and stimulation of copiotrophic microbial communities that drive general and specific suppression. The chemical and microbial composition of SMS varies significantly across mushroom species, enabling targeted application strategies for specific pathogen complexes. Synthetic microbial consortia engineered from mushroom-associated beneficial strains address the ecological limitations of single-strain inoculants by incorporating functional redundancy, niche partitioning, and synergistic interactions that enhance survival and activity in diverse 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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