Response of soil fungal communities associated with different soil fractions to tillage practices and crop rotation

by

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Dedication

To my parents and my forever love . . .

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List of abbreviations

AM	Arbuscular mycorrhiza
AMF	Arbuscular mycorrhizal fungi
ANOVA	Analysis of variance
ASV	Amplicon sequence variant
CAP	Principal coordinate analysis
CT	Conventional tillage
dNTP	Deoxynucleotide triphosphate
DT	Deep tillage
HSD	Honestly significant difference
HTS	High-throughput sequencing
INSD	International nucleotide sequence databases
ITS	Internal transcribed spacer
LFC	$\log_2$ fold change
LSA	Large soil aggregates
LSU	Large subunit
NMDS	Non-metric multidimensional scaling
PCoA	Principal Coordinate Analysis
PCR	Polymerase chain reaction
PERMANOVA	permutational multivariate analysis of variance
POM	Particulate organic matter
QS	Quorum Sensing
qPCR	Quantitative polymerase chain reaction
RB	Raised bed tillage practice
SRA	Sequence Read Archive
SSA	Small soil aggregates
SSU	Small subunit
VT	Vertical tillage

Abstract

This thesis investigates the effects of agricultural practices on soil microbial communities, with a specific focus on understanding how different tillage practices and crop rotation impact soil fungal communities within the distinct soil fractions. A two consecutive year study (2021 and 2022) was conducted in three fields located in Manitoba, Canada, each undergoing a different phase a crop rotation cycle that included corn, canola, and soybean. Additionally, the research examined multiple tillage practices that included conventional tillage, deep tillage, vertical tillage, and raised bed practice. To comprehensively assess soil fungal microbial communities, next-generation sequencing of the internal transcribed spacer region within nuclear ribosomal DNA was employed. Our study confirmed that tillage practices and crop rotation shape the structure of the soil fungal communities within the different soil fractions. In both years, Ascomycota and Basidiomycota were recognized as the prevailing fungal phyla in all tillage practices and crop rotations. These two phyla are widely recognized as the primary classical fungal decomposers and pathotrophs in soils. Furthermore, while tillage practices did not exert a significant impact on soil fungal alpha diversity, the highest predicted average fungal richness, as measured by Observed, Shannon, and Simpson alpha diversity indices, was associated with vertical tillage in 2021 and conventional tillage in 2022. Moreover, there was a significant difference in soil fungal alpha diversity among plots within different fields in 2021. Predictions based on both Shannon and Simpson indices indicated that plots in soybean in 2021 and plots in corn in 2022 were anticipated to exhibit the highest average fungal richness. We also noted a significant difference in soil fungal alpha diversity among different soil fractions in both years of our study. The relative abundance of numerous amplicon sequence variants exhibited significant difference in response to both tillage practices and crop rotation. Specifically, soils subjected to conventional tillage practices demonstrated a significantly lower relative abundance of plant pathogens within the phylum Ascomycota and a higher relative abundance of saprotrophic fungi. Our investigation further revealed that the relative abundance of beneficial soil fungi, particularly arbuscular mycorrhizal fungi, was higher in soils under the vertical tillage practice. In plots within the corn field, we also noted a markedly higher relative abundance of arbuscular mycorrhizal fungi. Conversely, plots within canola fields exhibited a higher relative abundance of saprotrophic fungi, including members of the phyla Ascomycota,

Basidiomycota, and Mortierellomycota. Our results suggest that tillage and crop rotation influence soil fungal community distribution. This study provides insights into the intricate relationships between agricultural practices and soil fungal communities, shedding light on the dynamics of these critical components of agricultural ecosystems in Manitoba.

1 General Introduction

Soils are the most diverse and essential ecosystem on the planet (Roger-Estrade et al., 2010). The functions carried out by soil organisms have substantial direct and indirect impacts on various aspects of agriculture, including on crop growth, the efficiency of nutrient cycling, the prevalence of soil and residue-borne pests and diseases, water management, and the long-term sustainability of soil productivity (Hartmann and Six 2023). Additionally, soil organisms play a crucial role in determining the resistance and resilience of agroecosystems when faced with abiotic disturbance and stress (Brussaard et al., 2007). Extensive land conversion, mechanical disruption of soil, excessive fertilization, intensive irrigation, and improper pesticide use have collectively contributed to soil degradation, environmental pollution, eutrophication, and the release of greenhouse gases. As a result, intensive agriculture is widely recognized as one of the most significant threats to global biodiversity and the provision of ecosystem services (Capelle et al., 2012; TSiafouli et al., 2015; Bevivino and Dalmastri 2017; Zabel et al., 2019; Abdul Rahman et al., 2021). Therefore, it is imperative to implement sustainable agricultural management practices that can meet the world's food demand while concurrently safeguarding biodiversity and maintaining the functioning of ecosystems (Mehrabi et al., 2018).

Soil biological activity is primarily concentrated in the topsoil, which can vary in depth from a few centimeters to as much as 40 centimeters and may extend even deeper in some specific ecosystems. Biological components, such as plant roots and soil organisms, occupy a relatively small fraction, accounting for less than 0.5% of the total soil volume. Among the biotic components of soil, soil microorganisms play a substantial role in driving the majority of biological activities. Soil microbes play a dynamic role in preserving soil fertility and the recycling of nutrients. They are responsible for breaking down organic matter introduced into the soil and are actively involved in key biogeochemical cycles. This microbial activity not only enhances plant health but also contributes to increased crop yields (Aislabie et la., 2013; Sagova-Mareckova et al., 2023). Indeed, specific soil microorganisms produce compounds that trigger the innate defense mechanisms of plants, thereby enhancing their resistance to pathogens. The participation of microorganisms in various soil processes makes them well-suited for providing a comprehensive assessment of soil

quality and health, a dimension that cannot be fully gauged through physical or chemical measures alone (Schlatter et al., 2017; Dini-Andreote 2020; Chen et al., 2023).

Industrial agriculture places its emphasis on maximizing productivity through the cultivation of high-yielding crops in monocultures or limited crop rotations, while heavily relying on agrochemical inputs and employing intensive soil management practices. This approach is frequently linked to adverse environmental consequences, including soil degradation, water contamination, the release of greenhouse gases, and the reduction of biodiversity (Tilman et al., 2001). Adapting agricultural management strategies to strike a balance between crop yield and the safeguarding of natural resources is essential for maintaining food production to meet the needs of a growing and progressively more demanding global population (Hartmann et al., 2015). Agricultural practices, including mechanical soil management (such as shallow and deep tillage) (Kraut-Cohen et al., 2020), crop management (involving crop rotations, cover cropping, residue retention, intercropping, and agroforestry) (Orrù et al., 2021), and the use of external inputs (like mineral and organic fertilizers, organic amendments, pesticides, and biologicals) (Walder et al., 2022), can significantly impact the composition and activity of the soil microbiome. For instance, research findings indicate that shifting from conventional tillage to no-tillage practices lead to decreased soil physical disruption and a more significant rise in fungal biomass compared to bacterial biomass. This outcome is likely linked to the improved preservation of fungal hyphae integrity in the soil. Soils under no-tillage practices typically exhibit cooler temperatures, a distinct distribution of plant debris, variations in air and water distribution, and other differences compared to conventionally tilled soils (Orrù et al., 2021). Furthermore, rotation with different crops has the potential to stimulate the development of specific microbial communities, including arbuscular mycorrhizal fungi (AMF), and oligotrophic or metabolically versatile fungal groups that can break down complex carbon compounds introduced into the soil when different crops are incorporated (Cloutier et al., 2020). This can lead to a reduction in soil-borne pathogens and an increase in disease-suppressive microorganisms.

Utilizing molecular studies based on metagenomics and metabarcoding, researchers have been able to map the complete range of soil microbial diversity, providing a more comprehensive understanding of the specific microbial mechanisms that influence soil processes. These advanced

techniques allow for a detailed analysis of microbial communities, their functional roles, and their interactions, contributing invaluable insights to field such as agriculture, environmental science, and microbiology. Specifically, DNA-based and RNA-based analyses of the soil microbiome have become increasingly prevalent, significantly expanding our knowledge of the phylogenetic and taxonomic makeup of soil microbial communities (Fierer 2017). It is now widely acknowledged that traditional culture-based methods significantly underestimate soil microbial diversity. Soils are known to harbor a wide range of microbial taxa from all three domains of life, with the majority of these microorganisms remaining uncharacterized (Tedersoo et al., 2014).

The recent advancements in high-throughput sequencing (HTS) technologies have enabled a more in-depth characterization of soil microbial communities. Metagenomics studies employ massively parallel sequencing techniques to comprehensively analyze communities within the soil microbiome, minimizing inherent biases. Among the massively parallel sequencing platforms, the MiSeq (Illumina Inc.) is a widely recognized and prominent platform. It operates using sequencing-by-synthesis technology, making use of fluorescently labeled dNTPs (deoxyribonucleotide triphosphates). Currently, MiSeq sequencing is the primary choice for metabarcoding studies of fungi and other organisms. Its paired-end approach allows the sequencing of amplicons up to approximately 550 bases in length (MiSeq 2×300).

Sequencing the internal transcribed spacer (ITS) region of the nuclear ribosomal RNA (rRNA) operon is a fundamental practice in species identification using HTS-based metabarcoding. The ITS represents the primary fungal DNA barcode (Nilsson et al., 2019).

For several important groups of plant pathogens and endophytes, the ITS region doesn't offer sufficient accuracy for identification at the species level. However, the more rapidly evolving ITS region is recommended by many studies for its higher variability to prevent the underestimation of diversity in sampled communities. In contrast, the more slowly evolving small subunit (SSU) (18S) and large subunit (LSU) (28S) nuclear rRNA genes, lack sufficient variation in sequences between species within these fungal groups, hindering robust determination (Nilsson et al., 2019). Most HTS-based studies typically focus on either the ITS1 or ITS2 subregion, which spans around 250–400 bases, because the full ITS region typically covers 500–700 bases. The ITS2 subregion

offers several advantages, including reduced length variation and more universal primer sites, which leads to lower taxonomic bias compared to ITS1 (Bazzicalupo et al., 2013; Tedersoo et al., 2015; Sommermann et al., 2018; Nilsson et al., 2019; Kauserud 2023).

The purpose of this study was to determine:

1) Firstly, how the choice of crop rotation sequence and contrasting tillage systems influence soil fungal community composition (i.e., the identities of the organisms that are present) and structure (i.e., patterns of relative abundance among the organisms that are present). The central hypothesis in our study was that using different tillage systems along with crop rotation sequence would cause shifts in soil fungal community structure (alpha-diversity), composition, and abundance of specific fungal functional groups which may potentially affect the soil quality. Additionally, we also test the hypothesis that soil disturbance through tillage systems might hinder the establishment and expansion of dominant fungal plant pathogens, and potentially leading to increased species richness over time in disturbed soil. Using amplicon sequencing of the internal transcribed spacer region (ITS) gene, we aimed to show the shifts in fungal community in soil over two consecutive years and the positive association of fungal pathotroph abundance within the crop rotation sequence (corn, canola, and soybean) and different tillage systems (conventional tillage, deep tillage, vertical tillage, and raised bed).
2) Secondly, the role of different soil fractions in shaping the composition of fungal communities under different management systems. More specifically, our goal was to investigate the characteristics of soil fungal community structure and composition within distinct-sized soil fractions (large fraction and small fraction) and compared that with particulate organic matter across a range of management intensities. We tested the hypothesis: soil fungal communities found in soil fractions not only differ within the size-distinct soil fractions, but also from those inhabiting particulate organic matter. We expected distinct-sized soil fractions would support greater diversity by providing access to complex and various organic matter in the soil spaces, and would increase soil fungal niche space, favoring coexistence of many fungal taxa in the soil fractions. The effects of specific crop species and/or tillage practices on soil fungal communities can be more significant in

specific fractions. For instance, the arrangement of soil aggregates and soil organic matter, which is closely linked to the existence and abundance of microbial communities, is shaped by various types of tillage systems. Furthermore, the identity of plant species can exert a significant influence on the microbes associated with them both during plant growth and in the initial stages of residue decomposition. However, as the residue undergoes more extensive decomposition, the distinct signature of the original crop species may gradually diminish.

This two-year study aimed to analyze how contrasting tillage treatments and crop rotation sequences influence soil fungal communities in Portage la Prairie, Manitoba. Soil samples were collected from plots within three field, each representing a distinct phase of the crop rotation cycle (canola, corn, and soybean) during a specific year. These plots underwent four different tillage treatments: conventional tillage (CT), vertical tillage (VT), deep tillage (DT), and raised bed (RB). Fungal communities in these samples were then compared using next-generation amplicon DNA sequencing with a MiSeq sequencer, targeting the internal transcribed spacer 2 (ITS2) region of the rDNA as the fungal marker.

2 Literature Review

2.1 Ecological Importance of Soil

Soil, water, and air constitute three fundamental natural resources that play a crucial role in sustaining most life forms. The key to achieving economic stability or facing devastation often lies in our ability to effectively manage our soil resources. For instance, the soil acts as a vital source of nutrients for plant growth, which, in turn, is essential for sustaining both animal and human nutrition. Moreover, soil serves as a medium for the recycling and detoxification of organic materials, as well as facilitating the recycling of numerous nutrients and global gases. A robust and healthy soil establishes a critical connection to the overall well-being of plants, animals, and humans alike. Throughout history, we have witnessed the dire consequences of mismanaging the soil resource base, leading to adverse outcomes such as poverty, malnutrition, and economic calamity (Bezdicek et al., 1996).

Physically, soils consist of minerals and organic particles that exhibit a range of sizes and composition that directly influence soil ecosystems. In fact, the majority of nutrients found in soils are present in minerals and organic matter. Minerals possess unique and consistent physical properties along with distinct chemical compositions. The arrangement and types of minerals present in the soil matrix influence pore size distribution, porosity, and overall soil structure. The pores in the soil, for instance, hold both air and water, acting as conduits for the movement of these elements. Furthermore, these pores offer pathways for small creatures to traverse, serving as runways, and provide avenues for roots to elongate and develop.

Soil organic matter is comprised of the recent remains of plants, microbes, and animals, along with resilient organic compounds formed through decomposition processes. Soil organic matter originates primarily from the production of higher plants, which act as the main source of organic material. Soil organisms then consume and decompose this organic material, leading to the accumulation of diverse and variable organic matter in the soil through the process of decomposition. As soil microorganisms decompose organic matter they release essential nutrient ions into the soil water, making these ions available for another cycle of plant growth.

Finally, soil acts as a microbial reservoir. Within this ecosystem, an immense number of biological processes are continuously at work, interconnecting soil, plant, animal, and human health and maintaining ecosystem stability and resilience (Bardgett and van der Putten, 2014; Bender et al., 2016; Dini-Andreote, 2020; Walder et al., 2022). For example, the microbial communities present in soil, specifically bacteria and fungi, are intricately associated with the major functioning of plants (Singh et al., 2020). Their communities form symbiotic relationships with plant roots that improve nutrient uptake and enhance crop growth. The plant microbiota, which includes microorganisms originating from the soil, can also find their way into the gut microbiota of both humans and animals and become a part of their gut microbiota (Young et al., 2011; Nyanza et al., 2014) (Figure 2.1). Soil microorganisms in the gut aid in breaking down complex plant materials in the digestive systems, facilitating nutrient absorption and contributing to the host's nutrition and well-being (Banerjee and van der Heijden 2023). Overall, the soil and its diverse microbial population play a vital role in shaping microbial communities within other organisms, serving as the cornerstone for maintaining ecological equilibrium and biodiversity throughout the entire ecosystem.

2.2 Microbiome Research

Microbiome research has experienced rapid evolution in recent decades, capturing significant scientific and public interest. However, due to the diverse range of disciplines exploring this subject, we are lacking a clear commonly agreed definition of the term "microbiome" (Berg et al., 2020). The field of microbiome research originated in environmental microbiology and started back in the seventeenth century (Berg et al., 2020).

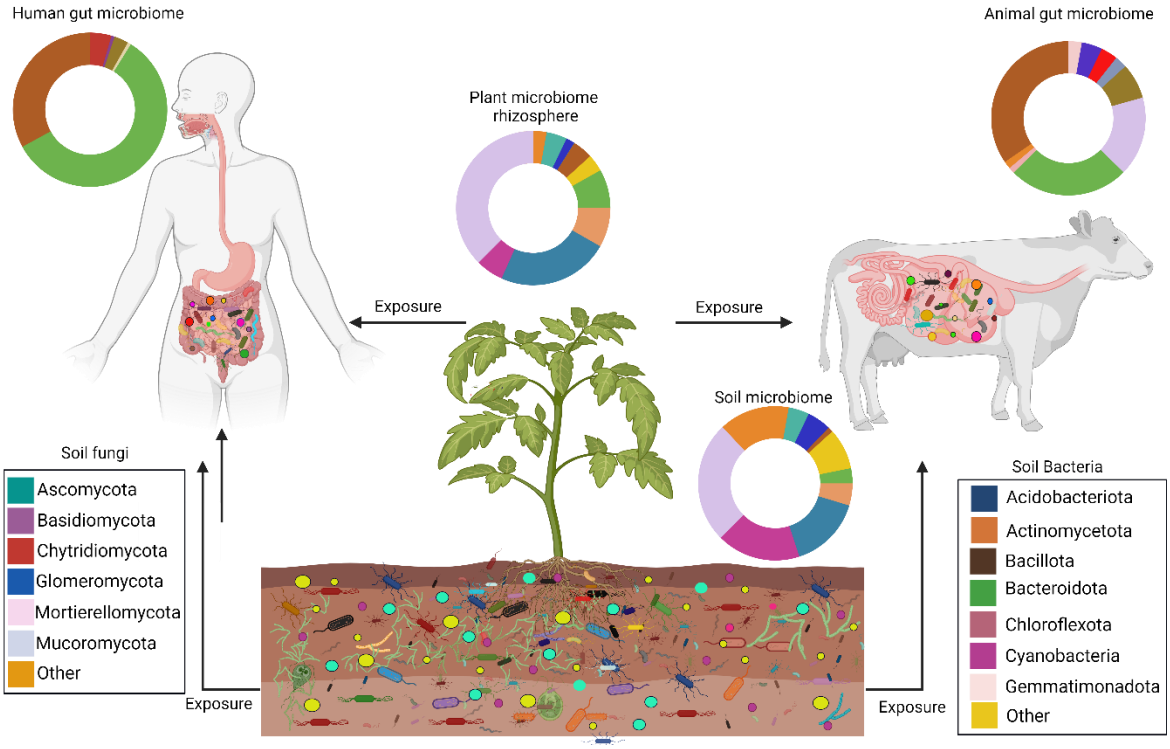

Figure 2. 1. The interconnected relationship among microbiomes in soil, plants, animals, and humans. Microorganisms serve as the bridging connection between the health of soil, plants, animals, and humans, while microbial communities establish links between these diverse ecosystems. Among these ecosystems, soil houses the Earth's most diverse and intricate microbiome, making it a vital reservoir of microorganisms. Figure adapted from Banerjee and van der Heijden (2023) by BioRender.com (2023).

The invention of the initial microscopes opened up the exploration of a previously unknown world, leading to the identification of microorganisms. The ability to observe the previously unseen world broadened the perspectives and understanding of researchers in the seventeenth century. Antonie van Leeuwenhoek examined a variety of bacteria with different shapes, fungi, and protozoa—referred to as animalcules—primarily collected from water, mud, and dental plaque samples. Over time, it has evolved into an interdisciplinary platform encompassing numerous field, including agriculture, food science, biotechnology, bioeconomy, mathematics (informatics, statistics, and modeling), plant pathology, and human medicine (Berg et al., 2020).

In 1988, Whipps and colleagues investigating the ecology of rhizosphere microorganisms, introduced the initial definition of the term "microbiome" (Whipps et al., 1988). They described the term "microbiome" by combining two words "micro" and "biome," referring to a "distinct

microbial community" thriving in a "specifically defined habitat with unique physio-chemical characteristics," which they likened to a "theatre of activity". This definition marks a significant advancement in the understanding of microbial communities, as it defines a microbial community with unique properties and its intricate interactions with the environment, leading to the creation of specific ecological niches. Nevertheless, numerous other definitions of microbiome have been published in the past few decades. The definition most commonly cited, attributed to Lederberg, characterizes microbiomes within an ecological context as a community of commensal, symbiotic, and pathogenic microorganisms existing within a body space or other environmental setting (Lederberg and Mccray 2001).

2.3 Soil Microbiome

Soils represent highly diverse biological environments, constituting roughly 25% of worldwide species diversity (Decaëns 2010; Coleman and Wall 2015; Trap et al., 2016; Dubey et al., 2019; Banerjee and van der Heijden 2023). Tens of millions of species of bacteria, fungi, archaea, viruses and microeukaryotes coexist beneath the surface, yet only a mere few hundred thousand have undergone comprehensive characterization (Fierer 2017). One gram of soil has the capacity to accommodate around 6000 distinct bacterial genomes, along with several meters of fungal hyphae, as well as a tens of thousands of protists and archaea cells, trillions of viruses, and nematodes (Leake et al., 2004; Hoorman 2011; Tecon and Or 2017; Williamson et al., 2017). A growing body of research suggests that microbial communities in the soil are associated with the major functioning of plants, animals, and human health (Table 2.1) (Singh et al., 2020). In an agricultural ecosystem, for instance, soil nutrient cycling, the dynamics of organic matter, soil structure, the transformation and storage of carbon, and many other ecosystem services are a few examples of the direct and indirect effects of the soil microbiome on a broad array of biological functions occurring in these ecosystems (Figure 2.2) (Sprent 2001; Kibblewhite et al., 2008; Harris 2009; Kumar et al., 2013; Hashem et al. 2017; Trived et al., 2020; Banerjee and van der Heijden 2023).

Table 2. 1. Some soil microbial taxa and their biological contributions to ecosystem functioning (modified from Banerjee and van der Heijden 2023

Soil microbial impact	Types of function	Example of participating group	References
Direct impact on soil health			
Nutrient uptake and circulation	*Nitrogen fixation*	*Rhizobium, Bradyrhizobium*	Fierer 2017
	Nitrification	*Nitrosomonas, Nitrobacter, Nitrospira,*	
	Denitrification	*Pseudomonas*, *Trichoderma*, *Fusarium*	
	Phosphate solubilization	*Pseudomonas*, *Bacillus*, *Serratia*, *Penicillium*	
	Siderophore formation	*Bacillus, Fusarium*, *Pseudomonas*, *Serratia*	
Exchanges of greenhouse gases	Incomplete denitrification Methane production Microbial respiration	*Pseudomonas, Methanosarcina, Methanobacterium*	Banerjee and van der Heijden 2023 Fierer 2017
Water purification	Nutrients absorption Uptake amino acids and chemical substances from infiltrating water	Diverse range of soil microorganisms.	Bünemann et al., 2018 Wall et al., 2015
Soil structure and soil erosion	Soil aggregation formation Soil particles gluing	Diverse range of soil microorganisms.	Six et al., 2018
Carbon sequestration	SOM stabilization	Diverse range of soil microorganisms.	Naylor et al., 2020
Soil organic matter dynamics	Decomposition Detoxification of chemical residue	Diverse range of soil microorganisms. *Bacillus, Pseudomonas, Achromobacter*	Anthony et al., 2020
Indirect impact on soil health			
Biocontrol of microbial communities	Disease suppressive soils	*Pseudomonas, Streptomyces, Paenibacillus*	Expósito et al., 2017

Table 2.1. continued. Some soil microbial taxa and their biological contributions to ecosystem functioning (modified from Banerjee and van der Heijden 2023)

Soil microbial impact	Types of function	Example of participating group	References
Antibiotic resistance gene	Plasmid transfer	Wide array of microorganisms	Kahn 2017
		Direct impact on plant health	
Crop yield and nutritional status	Nitrogen-fixing bacteria Mycorrhizal fungi Endophytes	*Rhizobium, Bradyrhizobium, Glomeraceae, Gigasporaceae, Trichoderma*	Trivedi et al., 2020
Stimulation of plant growth	Hormone production Micronutrients	*Burkholderia, Bacillus, Trichoderma*	Banerjee and van der Heijden 2023
Soil-borne plant pathogens	Detrimental impacts on plants	*Sclerotinia*, *Rhizoctonia*, *Fusarium*	Wei et al., 2015
Pathogen suppression	Antagonistic towards pathogens Triggers systemic resistance	*Pseudomonas*, *Enterobacter*, *Bacillus*, *Sarocladium*	Berendsen et al., 2012
Regulation of hormones	Cytokinins, gibberellins	*Pseudomonas*, *Enterobacter*, *Bacillus*,	Backer et al., 2018
Seed germination	Regulation of dormancy	*Azospirillum*, *Cellulosimicrobium*, *Bacillus*	Banerjee and van der Heijden 2023
Improved water absorption	Mycorrhizae are able to extract water from small soil micropores	*Glomus, Paraglomus, Diversispora*	Van der Heijden et al., 2008
		Indirect impact on plant health	
Disruption of signals	Breakdown of homoserine lactones	*Bacillus thuringiensis*	Banerjee and van der Heijden 2023
Leaf nutrient concentration	Microbial growth and metabolism	Wide array of microorganisms	Banerjee and van der Heijden 2023
		Direct impact on animal health	
Food resource	Some vertebrates Nematodes	*Aphelenchoides, Rhabditis, Protorhabditis*	Lerner 1996
Pathogenic source	Malignant Blackleg	*Clostridium, Burkholderia, Chlamydia*	Lerner 1996

Table 2.1. continued. Some soil microbial taxa and their biological contributions to ecosystem functioning (modified from Banerjee and van der Heijden 2023)

Soil microbial impact	Types of function	Example of participating group	References
	Indirect impact on animal health		
Mitigation of harmful substances	Animals including cows	Microbes capable of breaking down toxins.	Peixoto et al., 2021
Adjustment to environment shifts	Microbes capable of breaking down toxins	Wide range of microorganisms	Peixoto et al., 2021
	Direct impact on human health		
Food resource	Truffles, root crops	Ectomycorrhizal fungi	Van der Heijden et al., 2008
Pathogenic resource / Allergies	*Fungal meningitis*	*Exserohilum rostratum*	Wall et al., 2015
	Ringworm infection	*Trichophyton rubrum*	Brevik and Brevik 2012
	Diarrhoea and dysentery	Protists	Steffan et al., 2018
	Gastroenteritis	*Escherichia coli*	Steffan et al., 2018
	Abundance of allergens in the soil	Wide array of microorganisms	Wall et al., 2015
	Indirect impact on human health		
Antibiotic-resistant microbe	Direct or indirect source	Not assessed	Banerjee and van der Heijden 2023
Airborne dust	Coccidioidomycosis	*Coccidioides*	Brevik et al., 2020

The structure of the soil microbiome exhibits substantial variation across different ecosystems and even within smaller soil habitats. However, bacteria and fungi usually hold sway over the microbial biomass and diversity in soil, with their diversity surpassing other microbial groups by several orders of magnitude (Fierer, 2017). The enriched communities of fungal and bacteria play central roles in contributing to plant health through various mechanisms. Of the roughly 29 essential compounds required by plants, 18 are sourced from the soil, and soil microbial communities, particularly bacteria and fungi, play the main role in delivering these compounds to plants (Banerjee and van der Heijden 2023). Common genera of soil fungi, such as those from the Glomeromycota, can establish themselves not only on plant surfaces but can also associate themselves within a soil microflora, a phenomenon known as "Mycorrhiza." Arbuscular mycorrhiza (AM) stands as the earliest documented interaction between a fungus and its plant host in terms of mycorrhizal associations (Redecker et al., 2000).

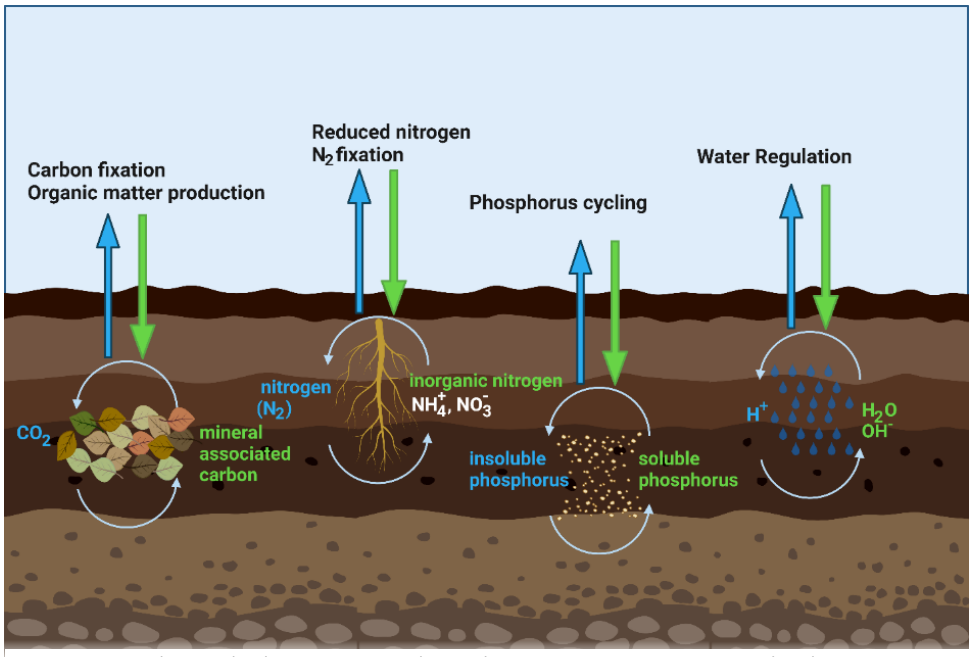

Figure 2. 2. Soil microbial communities have the capacity to generate and utilize trace gases present in the atmosphere (such as hydrogen, carbon dioxide, nitric oxide, nitrous oxide, methane, and various volatile organic compounds). They also play a role in shaping soil acidity, controlling the dynamics of soil carbon, and facilitating the cycling of essential nutrients (like iron, sulfur, phosphorus, and nitrogen) within the soil's layers (Fierer 2017). Moreover, soil microbial communities also have the potential to influence soil water dynamics. It's worth highlighting that numerous additional processes, facilitated by soil microbial communities, are not depicted in Figure 2.2 but can hold significance in ecosystem-level functions Figure adapted from Fierer (2017) by BioRender.com (2023).

2.3.1 Soil Microbiome; Rhizosphere

The rhizosphere is an area surrounding plant roots, where root exudates play a significant role in regulating interactions within the soil microbiome. Microorganisms in the rhizosphere are capable of perceiving and interpreting signals generated by their own kind, other microbes, and plants. They can influence their plant hosts by releasing signaling molecules (Figure 2.3) (Pantigoso et al., 2022). The primary results of this communication include the activation of plant immunity, enhancement of stress tolerance, overall improvement in growth and health, and better nutrition. Some examples of these signaling molecules are N-acyl homoserine lactones (Bailly and Weisskopf, 2012), diffusible signal factors (Kakkar et al., 2015), diketopiperazines (Oldroyd, 2013), molecules resembling phytohormones (Ortiz-Castro et al., 2011), and volatile organic compounds (Xu et al., 2015). This interaction with the plant can occur either at the individual strain level or at the level of the entire microbiome. Individual microbes and their communities can establish interactions with plants that range from being beneficial, neutral, to detrimental (Figure 2.3). Research on plant-microbe communication has primarily focused on soil bacteria and fungi. However, other soil microbial communication processes in nematodes and protists are also being uncovered and studied (Geisen et al., 2018; Manosalva et al., 2015).

The primary signaling mechanisms observed in the rhizosphere can be categorized into three groups based on the communication pathway and the participants involved:

1. Microbe-to-microbe: Microbial intraspecies and interspecies signaling primarily occur through the synthesis and detection of nducers, a mechanism commonly referred to as Quorum Sensing (QS). Autoinducers play a pivotal role in either triggering or inhibiting the transcription of a wide array of QS-regulated genes, which encompass functions such as biofilm formation, chemotaxis, and the expression of virulence factors. This enables microorganisms within microbial communities to monitor their cell density and synchronize collective changes in behavior (Fuqua et al., 2001; Venturi and Keel, 2016). One extensively studied class of QS molecules is N-acyl homoserine lactones (AHL), which act on Gram-negative bacteria, including renowned soil bacteria genera like *Pseudomonas* spp., *Burkholderia* spp., and *Serratia* spp (Pantigoso et al., 2022). In addition to QS inducer molecules, several other compounds, including oxalic acid, trehalose, glucose, and

thiamine, have been identified as signaling molecules in various communication processes. For example, studies have shown that the mycorrhizal fungus *Laccaria bicolor* releases trehalose, which acts as a chemoattractant for the mycorrhiza helper bacterium *Pseudomonas fluorescens*. In return, the mycorrhiza helper bacterium secretes thiamine, which facilitates the growth of the mycorrhizal fungus (Hassani et al., 2018). Overall, such interactions within soil microbial communities can assist microbes in persisting in nutrient-poor environments, accessing recalcitrant compounds that are challenging to break down, eliminating toxic metabolites, or engaging in electron exchange processes.

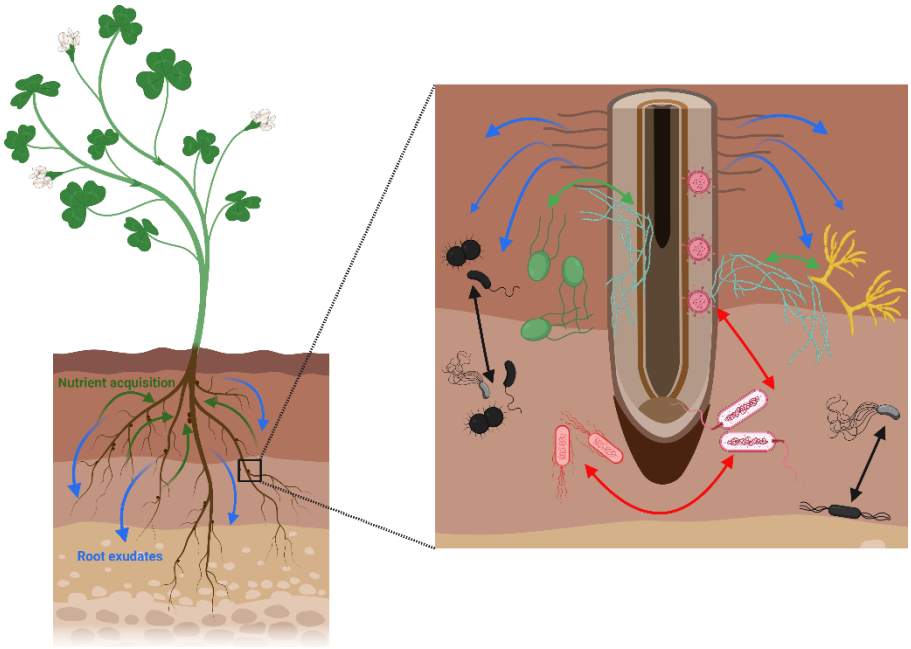

Figure 2. 3. Overview of processes of plant microbe association assembly. Plant-microbial associations involve inter-species interactions, which can be either positive (in green), like cooperation, negative (in red), such as competition, or neutral (in black), where both organisms do not impact each other. Root exudates (in blue) have the potential to influence the rhizosphere microbiome or specific microbial groups capable of solubilizing or mineralizing essential plant nutrients, such as nitrogen, phosphorus, and iron. This, in turn, enhances nutrient acquisition for plants. Figure generated by BioRender.com (2023).

2. Plant-to-microbe: Many root exudates, which are commonly found in most plants, have been identified as signaling molecules that communicate with soil microbes (Pantigoso et al., 2022) (Figure 2.3). Plants utilize dedicated pattern recognition receptors to identify the rhizosphere microbes associated with them (Venturi and Keel, 2016). The transmission of signals from plants to microorganisms through plant-secreted molecules has been demonstrated to play a role in various beneficial interactions with plants (Mathesius et al.,

2003; Mhlongo et al., 2018). Studies on signaling between plants and rhizosphere microorganisms have primarily focused on close symbiotic relationships, with a particular emphasis on associations involving mycorrhizal fungi (Hassan and Mathesius, 2012). For instance, in nutrient-deficient conditions, the host plant increases the production of strigolactones to stimulate the development of mycorrhizal fungi and facilitate the establishment of symbiosis as a means of acquiring nutrients (Aliche et al., 2020). Many studies are also indicating that various chemical compounds exuded by plants and present in the rhizosphere can function as both food sources and signaling molecules for a wide range of microorganisms. For example, plant growth-promoting rhizobacterium *Pseudomonas protegens* possesses four potential chemoreceptors for amino acids, which augment its ability to perform chemotaxis toward amino acids (Hida et al., 2020).

3. Microbe-to-plant: Microorganisms generate signaling molecules that are detectable by plants and can impact their development, gene expression, as well as their immune and stress responses (Badri and Vivanco, 2009; Venturi and Keel, 2016). Rhizosphere microorganisms initiate plant responses through microbial elicitors referred to as microbe-associated molecular patterns such as substances like lipopolysaccharide, peptidoglycans, flagellin, and chitin (Millet et al., 2010). Root-associated microbes, whether free-living, symbiotic, or endophytic, have the capacity to produce a wide array of these molecules. These molecules serve as triggers for systemic defense responses, also referred to as induced systemic resistance (ISR). Microbe-associated molecular patterns can also induce systemic acquired resistance (SAR) a response primarily induced by pathogens (Conrath et al., 2015; Pieterse et al., 2014). Overall, rhizosphere microbes contribute to stress mitigation in plants by modulating nutritional and hormonal equilibrium, fostering systemic tolerance to both biotic and abiotic stresses, and promoting plant growth (Egamberdieva et al., 2017).

Fungi and bacteria in the soil play a crucial role in preserving soil health and fertility through diverse interactions, both among themselves and with potential hosts. According to studies, plants that are healthy and show no symptoms maintain intricate relationships with their rhizosphere microbiota, which in turn contribute to supporting the overall performance of the plants (Hassani

et al., 2018). Indeed, fungi and bacteria in the soil not only facilitate numerous vital soil processes but also reacts to both biotic and abiotic conditions within the soil ecosystems. In addition, consistent alterations in soil microbial communities have been linked to shifts in important soil functions such as phosphorus availability, plant health, soil pH, soil moisture levels and overall ecosystem dynamics (Delgado-Baquerizo et al., 2018; Isobe et al., 2020, Fierer et al., 2021). It is frequently possible to pinpoint specific microbial taxa or functional genes linked to distinct soil processes, such as nitrification, methane generation, carbon sequestration, and cellulose breakdown. A predictive understanding of how these communities respond to their environment within agroecosystems is therefore of great interest.

2.3.2 Key Functions of Soil Microbiome in Agroecosystems

Soil microbial communities do not all possess equivalent functions in the agroecosystems; they can distinctly vary in their impacts on soil processes as well as their reactions to environmental circumstances. Within soil microbial communities, bacteria and fungi play the most significant role in ecosystem functions because of their abundance, their species diversity and the multiplicity of their metabolic activities. For example, they are main contributors to global C and N cycling and are responsible for about 90% of all organic matter decomposition in the soil (McGuire and Treseder 2010).

Soil biodiversity in general, and microbial diversity in particular, plays a pivotal role in steering the soil mechanisms that are crucial for maintaining agricultural productivity (Hartmann and Six 2023). Microbial communities have main responsibility for the soil's capacity to maintain homeostasis by eliminating pollutants and delivering essential ecosystem regulatory and supportive functions, including soil fertility, resilience, and the ability to withstand various stresses (Saccá et al., 2017). The key roles played by the soil microbiome in agroecosystems are described as fellow:

2.3.2.1 Nutrient Cycling

Microbes break down organic matter to release inorganic nutrients accessible to plants, modify nutrient availability through processes involving oxidation, reduction, solubilization, and chelation, and both store and release nutrients within necromass (Falkowski et al., 2008; Saccá et al., 2017). These mechanisms facilitate worldwide nutrient cycling and govern approximately 90% of the energy flow within soil ecosystems. For example, the presence of nitrogen compounds, a primary factor limiting plant growth in agroecosystems, is regulated by an intricate web of microorganisms with diverse metabolic capabilities (Figure 2.4).

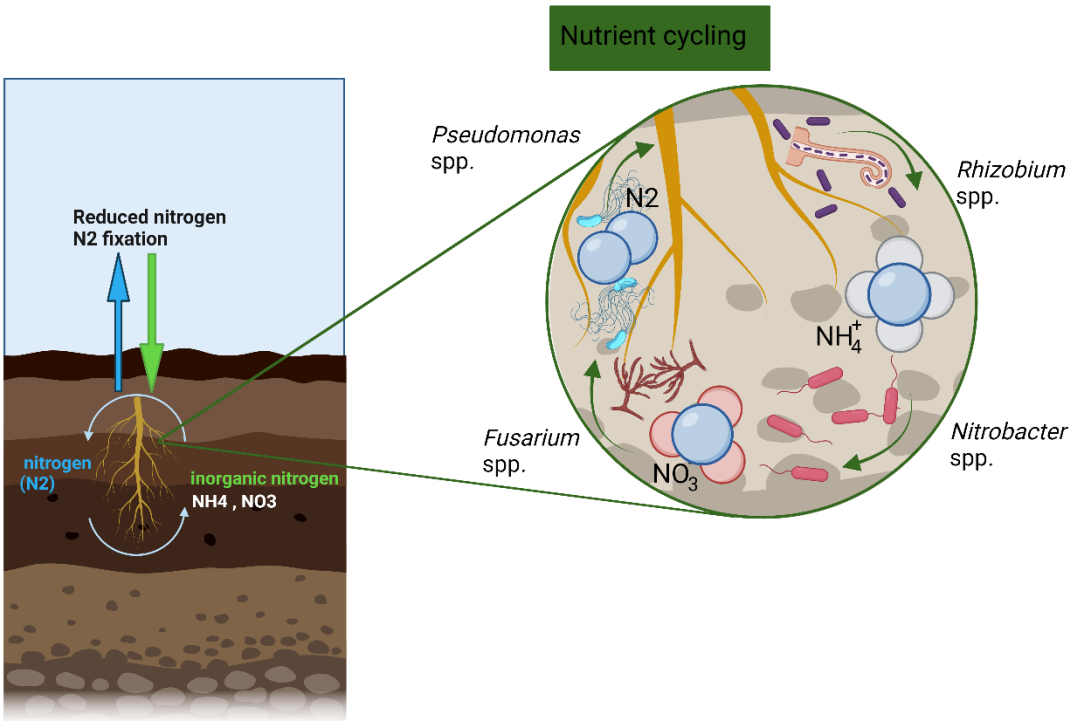

Figure 2. 4. Through the nutrient cycling orchestrated by soil microorganisms, plants obtain inorganic sources of nitrogen, such as NH_4^+ and NO_3^-. Biological nitrogen fixation is conducted by a diverse array of bacteria that form symbiotic relationships with plants, such as *Rhizobium*, as well as by independent, free-living bacteria like *Azotobacter*. In adequately aerated and neutral soils, ammonium, produced by nitrifying bacteria such as *Pseudomonas*, rapidly converts into nitrate, which plants can readily access, through ammonia- and nitrite-oxidizing bacteria such as *Nitrospira* and *Nitrobacter* (Hartmann and Six 2023). Ultimately, nitrate is transformed into dinitrogen when exposed to low levels of oxygen pressure, a process carried out by fungal genera like *Fusarium*, *Cylindrocarpon*, and *Trichosporon*. Figure generated by BioRender.com (2023).

The sustainability and productivity of agro-ecosystems heavily rely on the efficiency of nutrient turnover and recycling. This relies on the effective interplay between plants and soil, where microbial populations play a pivotal role.

Carbon, nitrogen, and phosphorus stand as some of the most crucial elements that soil microorganisms, especially bacteria and fungi, engage in cycling within soil ecosystems. These components play a crucial role in the creation of biomolecules like amino acids, proteins, DNA, and RNA. A significant portion of plant nutrients originate from the breakdown of minerals through weathering processes. Mineral weathering by soil bacteria and fungi plays a significant role in ion cycling and plant nutrition. For instance, phosphorus is not widely prevalent in the environment, and its accessibility is additionally limited due to its tendency to precipitate in the presence of divalent and trivalent cations under neutral and alkaline pH conditions (Aislabie et la., 2013). Soil microorganisms carry out the transformation of phosphorus through two primary mechanisms. In the first, they convert organic phosphorus into inorganic phosphate via a process facilitated by phosphatase enzymes, which are synthesized by many bacteria and fungi. In the second approach, they convert insoluble and immobilized phosphorus into soluble or mobile forms through the generation of organic acids (Figure 2.2). For instance, mycorrhizal fungi produce oxalate to extract phosphate from insoluble sources of phosphorus. This mobilization of phosphorus by fungal symbionts represents a significant strategy that enables plants to surpass limitations posed by phosphorus scarcity. Many ectomycorrhizal basidiomycetous fungi possess phosphate transporters with a strong affinity, which are activated in extraradical hyphae as a response to phosphorus deficiency (Plassard and Dell 2010).

2.3.2.2 Plant Growth; Abiotic Stresses; Disease and Pest Regulation

Soil microorganisms engage with plants, promoting growth and enhancing their resilience against biotic and abiotic stresses. The wide range of interactions between roots and microbes leads to the creation of a dynamic environment in the soil wherin microbial communities also engage with one another (Barea et al., 2005). A growing body of evidence suggests that plants actively recruit microorganisms in the rhizosphere that promote their growth (Table 2.2). Once established, these plant-growth-promoting microorganisms have the capacity to stimulate plant growth. Their role extends beyond nutrient transformation and transportation, encompassing a regulatory network

of phytohormones such as auxins, cytokinins, gibberellins, abscisic acid, and ethylene. These hormones influence fundamental traits in the host plant like cell division and development (Company et al., 2019). In particular, fungi like *Trichoderma* and *Laccaria* possess the ability to generate or modify phytohormone concentrations within the rhizosphere. This capability also allows them to regulate plant development and responses to stress (Eichmann et al., 2021). For instance, AMF enhance plant growth and stress tolerance by improved access to nutrients and water through their hyphal network and stimulation of root hair growth, increased root hydraulic conductivity by modulating the expression of root aquaporins, improved cell turgor through osmotic adjustments in plant cells, and enzymatic neutralization of reactive oxygen species in plant tissues (Le er al., 2019; Begum et al., 2019).

Table 2. 2. The involvement of soil microbes in promoting growth among cultivated plants.

Soil microbes	Role	Target plant	Stress	Reference
Pseudomonas sp.	Enhancing shoots biomass Increasing flowers quantity	*Petunia hybrida* *Impatiens wallerina* *Viola wittrockiana*	Drought Nutrient	Nordstedt et al.,2020
	Enhancing germination rate	*Arabidopsis thaliana* *Gossypium hirsutum*	Salinity	Chu et al., 2019
	Enhance biomass production Modify levels of abscisic acid Boost activity of antioxidant enzymes	*Lycopersicum esculentum*	Drought	Brilli et al., 2019
Trichoderma sp.	Enhance seed biomass Increase resilience to stresses	*Brassica napus*	Salinity Drought	Ennab et al., 2020
	Enhance stomatal conductance Elevate shoot dry weight Improve phosphorus uptake	*Solanum lycopersicum*	Drought	Khoshmanzar et al., 2020
	Enhance sulfur uptake Raise chlorophyll content Increase sucrose and sugar content	*Saccharum officinarum*	Drought	Scudeletti et al., 2021
Rhizobium sp.	Accumulate higher levels of proline, soluble sugars, and proteins Safeguard membrane systems	*Medicago sativa*	Low Temperature	Liu et al., 2019
Bacillus sp.	Enhance proline accumulation Elevate activities of antioxidant enzymes Raise chlorophyll content Increase carotenoid content Mitigate cell membrane damage	*Solanum lycopersicum*	Salinity	Yoo et al., 2019

Table 2. 2. continued. The involvement of soil microbes in promoting growth among cultivated plants.

Soil microbes	Role	Target plant	Stress	Reference
Bacillus sp.	Enhance growth and development Increase levels of chlorophyll and carotenoids Raise salicylic acid content Increase proline content Release Indole-3-acetic acid under stress	*Capsicum annuum*	Salinity Heavy metal Drought	Abdul Rahman et al., 2021
Aspergillus fumigatus	Improve disease suppression	*Theobroma cacao*	Disease suppression (*Phytophthora palmivora*)	Omoniyi Adebola Amadi 2010
Cladosporium spp.	Including plant signaling molecules	*Panax notoginseng*	Disease suppression (*Alternaria panax)*	Zheng et al., 2017
Beauveria spp.	Endophytic growth in plant tissues Growth promotion	Wide varieties of plants	Disease and insect pest suppression	Zheng et al., 2023
Metarhizium spp.	Endophytic growth in plant tissues Growth promotion	Wide varieties of plants	Disease and insect pest suppression	Zheng et al., 2023

Soil microorganisms have developed an extensive array of tactics to engage in belowground warfare, employing antibiosis and hyper-parasitism. Thereby, they function as biocontrol agents, managing the prevalence and spread of pests and pathogens. They can be located in the external environment, residing on the surface of plants as epiphytes, or within a microenvironment inside plant tissues as endophytes. To be considered to be an endophytic microbe, it is crucial that the interaction does not pose harm to the host; in cases where the microbe is a plant pathogen, the presence of disease symptoms should remain undetectable (Strobel, 2018; Carvalho et al., 2020). The earliest recorded example of an endophytic interaction between a fungus and its plant host is the arbuscular mycorrhiza (Saikkonen et al., 1998). Various types of fungi establish such relationships, yet in agriculture, the most significant ones are the AMF belonging to the Phylum Glomeromycota (Schüssler et al., 2001; Gosling et al., 2006). AMF engage in a symbiotic relationship with over 80% of terrestrial plant families. AMF benefit their host principally by increasing the effective underground surface area, facilitating uptake of water and nutrients. They are also able to promote plant growth by increasing uptake of relatively immobile phosphate ions (Smith and Read, 1997). In addition, mycorrhizas have been shown to play an important role in maintaining resilience against foliar-feeding insects (Gosling et al., 2006), enhanced tolerance to drought (Augé et al., 1994), and improved resistance to soil-borne pathogens (Lingua et al., 2002, Pozo et al., 2002).

Therefore, soil microorganisms exert advantageous regulatory influences on plant pathogens and pests (Saccá et al., 2017). Soil microorganisms associated with roots enhance plant health through direct and indirect means including competing with pathogens for both physical space and resources, as well as triggering systemic resistance and priming for heightened defense. Additionally, they employ direct defense responses like antibiosis (generating antibiotics, lytic enzymes, and volatiles) and hyper-parasitism. The antagonistic role against pathogens and pasts have been proved in a wide diversity of soil bacteria (i.e. *Streptomyces*, *Pseudomonas*, *Bacillus*) and fungi (i.e. *Trichoderma*, *Fusarium, Aspergillus, Cladosporium, Metarhizium, Beauveria*) (Cawoy et al., 2011; Palaniyandi et al., 2013; Ciancio et al., 2016; Saccá et al., 2017; Hartmann and Six 2023).

2.3.2.3 Biodegradation of Organic Waste and Pollution

The introduction of contaminants (such as chemicals, pharmaceuticals, heavy metals, and nanoparticles) into agricultural soil and groundwater occurs through various means, including pesticides, organic fertilizers (like manure, compost, and sewage sludge), irrigation water, and plastic waste. Soil contamination is a major threat to soil functions, and it is a global problem (CEC 2006). Soil microbes capable of breaking down organic pollutants like synthetic polymers or herbicides hold significant potential as a valuable resource for remediating polluted locations through bioremediation techniques. The majority of organic pollutants found in nature can be modified by metabolic processes, and even xenobiotics can frequently undergo transformation due to the wide-ranging substrate specificity exhibited by numerous enzymes.

The range of potential microbial agents capable of breaking down environmental pollutants is as varied as the pollutants themselves. These agents include bacterial genera like *Alcaligenes*, *Bacillus*, *Flavobacterium*, *Pseudomonas*, *Rhodococcus*, and *Streptomyces*, alongside fungal genera such as *Aspergillus*, *Fusarium*, *Penicillium*, and *Trichoderma* (Rolli et al., 2021).

2.3.2.4 Climate Regulation

Among the vital ecosystem services offered by soil microorganisms, climate regulation stands out, as bacteria and fungi have a crucial function in mediating carbon flux between the land and the atmosphere (Singh et al., 2010; Gougoulias et al., 2014; Saccá et al., 2017; Naylor et al., 2020; Hartmann and Six 2023). The process of capturing atmospheric carbon dioxide (CO_2) within the soil is primarily carried out by higher plants. While a small quantity of carbon assimilation is accomplished by autotrophic soil microorganisms, the greater significance lies in the indirect impacts of microbes on the cycling of soil carbon. These impacts arise from processes that influence the turnover and preservation of soil carbon (Six et al., 2006; Yuan et al., 2012; Berg et al., 2020). Most soil organic matter, for instance, is preserved as microbial necromass, which undergoes a gradual cycling due to its attachment to soil minerals and the creation of organic-mineral complexes trapped within soil aggregates (Hartmann and Six 2023). Soil microorganisms play a significant role in CO_2 emission from soil through processes like litter decomposition and heterotrophic respiration. Approximately half of the annual land-based carbon emissions, totaling about 120 Pg, stem from heterotrophic microbial respiration, resulting in emissions up to nine

times greater than the combined global anthropogenic sources each year (Crowther et al., 2015). There is substantial evidence highlighting the significant role of bacteria, fungi and archaea in mediating the exchange of carbon between soil and atmosphere. Facultative anaerobic methanogenic archaea such as *Methanosarcina*, *Methanosaeta* and aerobic methanotrophic bacteria such as *Methylomonas*, *Methylococcus* account for approximately two-thirds of the annual emissions of 500-600 Tg of methane (CH_4). It is also estimated that mycelium produced by mycorrhizal fungi within forest ecosystems contributed up to 60% of the autotrophic soil CO_2 efflux and approximately 25% of the total soil CO_2 efflux (Conrad 2009; Ekblad et al., 2013).

Nitrous oxide (N_2O) possesses significant environmental impact, being a potent greenhouse gas with a global warming potential 298 times greater than that of CO_2 on an equivalent mass basis. Additionally, it serves as a potent substance contributing to stratospheric ozone depletion (Hu et al., 2017). Traditional understanding attributes conventional N_2O emissions primarily to bacterial pathways. However, advancements in molecular techniques, such as metagenomic approaches, have revealed unexpectedly diverse N_2O-related metabolic pathways within other microbial domains, including fungi and archaea. Recent studies have illuminated the significant contribution of fungi to N_2O production, highlighting it as a shared characteristic among various fungal taxa (Marusenko et al., 2013). Fungal denitrifiers have been identified as the primary contributors to dryland N_2O emissions, particularly prevalent during dry seasons across a range of environmental conditions. For instance, in laboratory microcosms employing inhibition techniques, fungi were found to account for more than 70% of total N_2O production in semi-arid grassland and desert soils. Furthermore, they constituted 89% in grassland soils, 85% in additional grassland soils, and 79% in riparian soils (Hu et al., 2017).

2.4 Impacts of Agricultural Management on Soil Microbiomes

Agricultural practices disrupt the dynamics of microbial communities, as they have the potential to modify interactions between microbes. This influence extends to the prevalence of beneficial microorganisms, such as those engaged in nitrogen fixation or detoxification, and detrimental pathogens, which are expected to impact plant health (French et al., 2021). Tillage, crop domestication, the use of chemical inputs, water management adjustments have resulted in documented adverse effects on the structure and functioning of soil microbiomes (Table 3)

(Hussain et al., 2009; Wolmarans and Swart, 2014; Tsiafouli et al., 2015; Chen et al., 2020; French et al., 2021). In general, three primary detrimental outcomes have been identified in relation to soil microbial communities: (1) diminishing soil microbial diversity or total biomass, (2) modification of soil microbiome functionality, and (3) the disturbance of beneficial associations between plant or insect hosts and their symbiotic microorganisms.

Although the impacts of agricultural practices differ among distinct microorganism groups, the general trend includes decreased total biomass, reduced species richness, and diminished taxonomic diversity in microbiomes (Orrù et al., 2021). These alterations are broadly observed and often amplified in the agro-systems where there is heightened soil disturbance due to practices like tillage (Venter et al., 2016; Wang et al., 2020; French et al., 2021). Table 3 represents an overview of some recurring patterns that have emerged from years of research examining how agricultural management practices influence the composition and functioning of soil microbiomes.

In natural ecosystems, the structure of soil microbial communities can be altered by decisive factors such as soil type, climate, biotic interactions, as well as plant species and their diversity (Philippot et al. 2013). This alteration often is driven by complex interacting environmental factors. For example, the richness and diversity of soil microbiota heavily relies on the ecosystem types and exhibits a notable correlation with soil pH (Fierer and Jackson 2006). In the industrial agriculture, on the other hand, the emphasis is placed on optimizing productivity through the cultivation of high-yield crops in monocultures or basic rotations, while employing substantial amounts of agrochemical inputs and practicing intensive soil management techniques. This strategy is often associated with adverse environmental consequences, including soil deterioration, water contamination, the release of greenhouse gases, and a decline in biodiversity (Tilman et al., 2001). Agricultural management modifies the physical and chemical properties of soil, leading to a direct impact on the soil microbial life and composition of their communities (Bevivino and Dalmastri, 2017). For instance, tillage methods can lead to alterations in soil moisture levels (Kühling et al., 2017), soil aggregation patterns (Sapkota 2012), and the distribution of pore sizes (Gao et al., 2019). These changes subsequently impact the ability of soil microorganisms to access oxygen, water, and nutrients.

Table 2. 3. Summary of positive and negative consequences of important management practices on soil microbiomes.

Type of practice	Impacts on soil microbiomes	Impact site	References
Tillage	Reducing microbial variety and total biomass, Diminishing functional diversity of microorganisms Disturbing relationships with advantageous microorganisms.	Soil Rhizosphere	Kraut-Cohen et al., 2020
Organic soil amendments	Enhancing microbial diversity, abundance, and metabolic activity Enhancing the suppression of soil-borne pathogens Strengthening positive plant-soil feedback (e.g., in maintaining soil structure) Reducing or increasing microbial diversity	Soil Rhizosphere	Hartmann et al., 2015
Crop diversification	Boosting soil fertility Reducing or increasing microbial diversity Enhancing suppression of disease Promoting beneficial interactions between microbes and plants	Soil Rhizosphere	Misra et al., 2019
Water resource control	Reducing connections with advantageous root microorganisms Diminishing the presence of water-stress-tolerant microorganisms Rising levels of multidrug-resistant bacteria in soil Increasing microbial activity	Soil Rhizosphere	Mavrodi et al., 2018

2.4.1 Agricultural Management Practices; Soil Tillage Systems

Tillage is the mechanical manipulation of both soil and plant residue, typically with the purpose of creating a suitable seedbed for planting crop seeds. Tillage operations incorporate crop residues into the soil, enhance nutrient mineralization, and decrease the presence of weeds, pests, and pathogens (Delitte et al., 2021; Hobbs et al., 2008; Van den Putte et al., 2010). These actions lead to a short-term increase in crop yield. In addition, tillage is a prevalent agricultural technique that impacts soil structure, leading to raised bulk density and reduced porosity, consequently influencing biological attributes of soil. Extensive tillage alters microbial diversity by disrupting the physical variety within the soil environment (Acosta-Martínez et al. 2011).

Tillage methods are broadly classified as conventional tillage and conservation tillage (Unger 1990). As per the description offered by the Conservation Technology Information Center (CTIC 1995), conventional tillage, also known as aggressive tillage, encompasses tillage practices that result in less than 15% of residue being exposed after planting, or less than 560 kg ha^{-1} of small grain residue equivalent during the critical wind erosion phase. In contrast, conservation tillage, also known as reduced tillage, pertains to tillage methods that result in more than 15% crop residue (debris), or more than 560 kg ha^{-1} of small grain residue covering the soil surface (including minimal tillage techniques) (Daughtry et al. 2004; Li, 2013).

Conventional tillage (ploughing) exposes the deeper soil layers to the surface. This process leads to heightened turnover of macroaggregates, disturbing the established pore structure, ultimately promoting soil erosion. Additionally, it expedites the decomposition of soil organic matter (SOM) and contributes to a decrease in stocks of soil organic carbon (Six et al., 2002). These alterations in soil structure yield direct consequences for the physical environment where soil microbes reside. Fungi, in particular, often experience adverse impacts due to the disruption of their hyphal network (Orrù et al., 2021). Meanwhile, bacteria within microaggregates tend to experience comparatively lesser initial effects (Young and Ritz, 2000).

Conservation management strategies (such as direct seeding) aim to maintain the integrity of the soil structure, thereby achieving a reduction in soil erosion and the loss of soil organic carbon when compared with conventional tillage (Hartmann and Six, 2023). This strategy might result in a

decrease in crop yield, although the decline is usually modest and varies based on factors such as the type of crop, soil texture and climate (Pittelkow et al., 2015). Conservation tillage practices have been found to promote soil fungal communities including arbuscular mycorrhizae, mycoparasites and nematophagous fungi, whereas fungal saprotrophs and plant pathogens dominated under conventional tillage (Srour et al., 2020). Reduced tillage methods also appear to encourage microbial groups that have the capability to generate exopolysaccharides and lipopolysaccharides, essential biomolecules that play a key role in enhancing aggregate stability and resilience to desiccation (Costa et al., 2018).

Overall, a growing body of literature shows that all forms of tillage have the potential to disrupt soil structure, alter soil moisture and oxygen levels, affect soil compaction, and potentially impact both soil physicochemical characteristics and microbial attributes. Below, the key alterations in soil factors caused by tillage (soil compaction, soil aggregation, and soil moisture status) are listed and discussed with respect to impacts on soil microbial communities.

2.4.1.1 Soil Compaction and Soil Microbial Communities

Soil compaction is another undesirable but widespread side effect of agricultural intensification and has been recognized by the Food and Agriculture Organization of the United Nations (FAO) as one of the major threats to soil. Compaction is caused by compressive pressures exerted on the soil as a result of the escalating weight of heavily mechanized agricultural machinery and frequent in-field activities such as plowing, seeding, fertilizing, spraying, and harvesting. As a result of these field operations, substantial changes occur in the soil structure, including heightened bulk density, disruption of macroaggregates, diminished macropores, and reduced connectivity among pores. These alterations have direct implications for processes such as water infiltration, nutrient movement, oxygen diffusion, and penetration, as well as root space availability, ultimately influencing the physicochemical environment in which microorganisms thrive (Schäffer et al., 2008).

The primary factor by which compaction influences soil microbial communities seems to be the constraint imposed by pore space and oxygen availability, attributed to the rise in bulk density that consequently diminished air permeability and the diffusion of gases (Keller et al., 2017). Microbial species, both bacterial and archaeal, that possess the ability to metabolize in environments

characterized by a low partial pressure of oxygen, typically flourish under such circumstances. Indeed, bacterial and archaeal genera recognized for their anaerobic lifestyles, including *Desulfuromonas*, *Anaeromyxobacter*, *Geobacter*, *Anaerolinea*, *Longilinea*, *Intrasporangium*, *Dechlorosoma*, and *Methanosarcina*, exhibited a notable rise in their relative abundance in response to soil compaction (Longepierre et al., 2021).

2.4.1.2 Soil Aggregation Patterns and Soil Microbial Communities

Soil aggregate stability serves as a valuable indicator of soil structure (Mehra et al., 2018). Soils can be perceived as intricate three-dimensional arrangements comprising compacted aggregates and pore spaces (Wilpiszeski et al., 2019). Aggregates are composed of groupings of mineral particles, coated organic substances, polysaccharides, plant roots, and other living materials (Huang et al., 2015; Lucas et al., 2019; Zhou et al. 2020; Custodio et al., 2021). Soil aggregates, classified as either microaggregates (<250 µm) and macro-aggregates (ranging from 0.25 to 2 millimeters) which exhibit an uneven arrangement of mineral particles and nutrients (Asano and Wagai, 2014, 2015). These aggregates organize themselves hierarchically, forming networks of particles and pores that intermittently link during various occurrences. The formation of networks and connections between soil aggregates results in a fluctuating movement of water and nutrients, which can be accessed by soil organisms (Fox et al., 2018).

Soil management practices (e.g., tillage, crop rotation, and organic matter inputs) alter the soil structure and the size-class distribution of aggregates (Angers, 1992; Kushwaha et al., 2001; Six et al., 2000). In agricultural environments where the land undergoes intensified soil tillage, aggregates break apart, leading to the degradation of soil structure and making it susceptible to swift dispersion through wind or rain. The process of homogenization and seasonal mixing within the plowed layer of tilled soils results in the creation of fragile aggregates that can be easily dislodged by rainfall, irrespective of their size (Mehra et al., 2018). In undisturbed soils, on the other hand, both micro- and macroaggregates remain stable and exhibit slow turnover rates owing to their substantial content of soil organic matter. Under the no-tillage practices and reduced soil disturbance, the organic content in the soil remains undisturbed and is not exposed to microbial decomposition (Six et al., 2000). No-tillage practices result in reduced soil disturbance, which in turn restricts the diffusion of oxygen (O_2) (Paustian et al., 2000; Pulleman and Marinissen, 2004).

The restriction of oxygen diffusion can impact the oxygen availability within the soil aggregates, potentially influencing the microbial communities that rely on the porous networks formed by these aggregates for their activities and nutrient exchange (Custodio et al., 2021).

In contrast, under conventional tillage systems, soil macro-aggregates experience significant disruption, leading to the exposure of soil organic matter to microbial decomposition (Balesdent et al., 2000; Shepherd et al., 2001). Shepherd et al. (2001) discovered a reduction in the presence of soil microbial-binding agents surrounding aggregates following conventional tillage. Hence, any disruption to the arrangement of soil aggregates interferes with soil's physical, biological, and chemical processes. This disruption can influence both aggregate stability and soil organic matter content, ultimately exerting an impact on the entire system through three primary activities:

(1) alterations in the soil environment that directly influence microbial activity,
(2) modifications in soil structure due to compaction (*see section 2.4.1.1*),
(3) shifts in biodegradation rates upon the incorporation of aboveground residues into the soil.

Therefore, the arrangement of soil aggregates impacts the relationships among plants, microbes, and the soil matrix. In addition, during soil aggregate formation, microorganisms from the nearby bulk soil become enclosed within the aggregate structures. Once established, these soil aggregates form a distinct environment that can differ significantly from the surrounding soil (Bocking and Blyth, 2018). Hence, soil aggregates create an extensive array of physicochemical environments, establishing unique microhabitats for a variety of microbial communities (Custodio et al., 2021). For instance, within macro-aggregate structures, microbes come across pores characterized by decreased water content, facilitating greater rates of oxygen diffusion and limited accumulation of organic carbon. The limited diffusion of organic carbon due to the scarcity of water-filled pores can lead to the spatial separation of metabolic competitors within the macro-aggregates. This separation affects soil microbial diversity (Dechesne et al., 2008). In contrast, the pore spaces between micro-aggregates experience higher levels of water saturation. As a result, the rates of oxygen diffusion are limited, and there is a relatively abundant supply of carbon in solution in the pore spaces between micro-aggregates. This creates an environment with low oxygen levels,

promoting a more fermentative lifestyle for soil microorganisms (Neumann et al., 2013; Rabbi et al., 2016).

The influence of soil aggregates on the composition of microbial communities has been investigated in numerous research studies (Briar et al., 2011; Davinic et al., 2012; Chen et al., 2015; Bach et al., 2018; Wu et al., 2019; Wilpiszeski et al., 2019; Custodio et al., 2021; Li et al., 2023). Bach et al. (2018) conducted a comparison of soil fractions that mainly consisted of macro-aggregates or micro-aggregates from identical agricultural soils. The soil fraction composed of micro-aggregates exhibited a greater relative abundance of certain taxa, including the phylum Gemmatimonadetes, the Rubrobacteridae subdivision of *Actinobacteria*, the orders Sphingomonadales and Sphingobacteriales, as well as the fungal orders Onygenales and Chaetosphaeriales, along with the Trichosporonacae family. Conversely, the macro-aggregate fraction contained a higher proportion of filamentous fungi, particularly arbuscular mycorrhiza fungi from the phylum Glomeromycota (Bach et al., 2018).

2.4.1.3 Soil Moisture and Soil Microbial Communities

Soil moisture is one of the major factors influencing soil microbial community structure (Torsvik and Øvreås, 2002; Kaisermann et al 2015). A marginal reduction in soil moisture could impose stress on soil microbial communities, attributed to physical limitations influencing microbial habitats (Or et al., 2007). When considering soil aggregates, which serve as fundamental microbial habitats, the drying process exhibits non-uniformity, potentially leading to isolated pockets of drought-induced stress for soil microorganisms, particularly within larger pores (Ruamps et al., 2011).

Data from several studies indicate that the adoption of no-till practices conserves water due to factors such as reduced soil disturbance, an increased proportion of water retention and consistent pores, enhanced infiltration rates, and reduced evaporation. Additionally, the mulching effect of crop residue that remains covering the soil surface in no-till systems serves as a natural barrier, further reducing water evaporation and contributing to the overall water conservation benefits of these practices.

Fluctuations in soil moisture frequently result in changes in the proportional presence of various bacterial and fungal taxa that reside within the soil (Nguyen et al., 2018). Although fungal communities tend to exhibit greater adaptability to drought conditions (de Vries et al., 2018), likely due to their capability to accumulate osmoregulators (Ramirez et al., 2004), it is important to note that this resilience can vary depending on factors such as fungal species composition, and environmental context. The extensive network that filamentous fungi manage to establish within the soil enables these organisms to span a significant soil volume, granting them access to pockets of residual humidity present in the soil (Amend et al., 2016; Manzoni et al., 2012). However, diminished pore connectivity due to soil disturbance in long term tillage practice might bring about alterations in soil fungal community structure by reducing hyphal integrity in the soil. No-tillage practices generally result in higher levels of soil moisture and cooler soil temperatures compared to long term conventional tillage (Wang et al., 2017; Li et al., 2020). In addition, in these soils the distribution of plant debris is different, air and water distribution are different, and so on (Orrù et al., 2017).

2.4.2 Agricultural Management Practices; Cropping Systems

Crop management techniques like crop diversification and cover cropping offer strategies for positively impacting both soil structure and microbiome functions. The implementation of crop diversification, whether through crop rotation, intercropping, mixing cultivars, or adopting agroforestry methods, can contribute to biodiversity and maintain a healthy balance in ecosystems. These practices can also have favorable outcomes on crop yields. Positive effects on soil biodiversity and ecosystem function resulting from increased plant diversity are often attributed to complementarity effects, such as facilitation and niche differentiation (Hooper et al., 2005). Crop diversification has the direct capability to modify the soil microbiome by providing nutrients via root exudates and organic matter decomposition, while also creating physical niches on root surfaces (Hartmann and Six, 2022). Recent meta-analyses indicate that the implementation of cover cropping or crop rotations can result in enhancements in soil microbial diversity and richness. These improvements vary, ranging from 3% to 27%, when compared to monoculture systems or soils with lower levels of agricultural diversity (Venter et al., 2016; Kim et al., 2020).

There can also be indirect effects, as crop diversification can lead to changes in physical and chemical properties of the soil. At the level of soil structure, heightened diversity in root systems and modifications in soil organic matter and nutrient inputs via plant litter and rhizodeposition can influence porosity, aeration, and the stability of aggregates. This, in turn, can create a broader array of microbial niches (Galindo-Castañeda et al., 2022). Plants with deep-rooting characteristics have the ability to shift the distribution of nutrients, water, and oxygen across different depths within the soil profile (Galindo-Castañeda et al., 2022).

Crop management also can play a role in shaping the abundance and makeup of microbial communities, both directly through interactions between plants and microbes, and indirectly by influencing changes in soil structure and adjustments to physicochemical soil properties (Kong and Six, 2012; Kim et al., 2020). For instance, cover crops can yield favorable impacts on microbial abundance, activity, and diversity. Moreover, they can enhance functional redundancy and complementarity within microbial communities (Alahmad et al., 2019). Additionally, cover crops have the potential to stimulate some microbial communities, including arbuscular mycorrhizal fungi, oligotrophic bacteria, and versatile bacterial groups (Cloutier et al., 2020; Finney et al., 2017; Delitte et al., 2021). These bacteria are capable of breaking down intricate carbon compounds introduced into the soil when cover crops are incorporated (Delitte et al., 2021).

Enhancing crop diversity typically leads to an increase in microbial diversity (Eisenhauer et al., 2010; Stefan et al., 2021), although this outcome is not universally consistent, which in fact demonstrates the reliance of these effects on factors like specific plant species and the prevailing soil and climatic conditions (Stefan et al., 2021). A major advantage of implementing crop diversification is the ability to attract beneficial microorganisms and decrease pathogen levels by disrupting their lifecycle dependence on specific hosts and enlisting naturally occurring antagonists (Peralta et al., 2018). An extensive meta-analysis encompassing 54 field studies conducted globally revealed that the implementation of cover cropping increased subsequent AMF colonization of roots in summer cash crops by approximately 30%. The impact was most pronounced when the cover crop itself acted as a host for AMF (Bowles et al., 2017). In addition, the practice of crop diversification along with the application of organic soil amendments is associated with the development of "suppressive soils." These soils offer essential ecosystem services, including the

control of diseases, weeds, and insect pests (French et al., 2021). This phenomenon occurs through mechanisms such as the competition for root space occupied by microorganisms and the modulation of plant immune responses (Vukicevich et al., 2016). Recent research conducted in wheat, for instance, discovered that the enrichment of fungi with disease-protective properties in rhizosphere communities was dependent on the specific type of legume in a crop rotation. This finding implies that certain plant species or cultivars might be better suited than others to optimize disease protection (Esmaeili Taheri et al., 2016).

Numerous studies have established a strong connection between soil microbes and plants (Fry et al., 2018). First, there can be direct, specific connections between a plant species and a particular type of microbe, such as symbiotic interactions. Additionally, plants have the capability to modify soil chemical and physical conditions (López-Angulo et al., 2020). Some changes can result from above-ground mechanisms, such as the deposition of plant litter or shifts in microclimate influenced by the plant's canopy cover (Delgado-Baquerizo et al., 2018). Alternatively, some changes may arise from below-ground processes, including enhanced soil aeration and loosening due to root growth, the release of root exudates or plant signaling molecules, and alterations in ion uptake (Doornbos et al., 2012). Therefore, an increased variety of plants, and consequently, their roots, would result in a greater diversity of microenvironments, especially within the rhizosphere. The resulting diversity of microhabitats would subsequently contribute to the enhancement of microbial diversity which potentially leading to improved soil functioning (Wardle, 2006). As an example, Steinauer et al. (2015) demonstrated that an increase in plant diversity resulted in a notable rise in soil microbial biomass and enzymatic activity. Similarly, findings from a grassland biodiversity experiment indicated that greater plant diversity led to elevated microbial activity and enhanced carbon storage (Lange et al., 2015).

In conclusion, the practices of enhancing crop diversity both spatially and temporally, as well as implementing cover cropping, hold significant value in the context of enhancing both soil structure and microbial diversity. In turn, these practices yield positive effects on microbial diversity and functions, encompassing vital processes. However, further research is essential to gain a deeper comprehension of how crop diversification modifies the intricate microscale structural environment within the soil and the subsequent impacts on beneficial microorganisms. In addition,

it is crucial to enhance our understanding of how microbial interactions with diverse co-planted hosts influence the overall performance of cropping systems. This understanding is essential for identifying compatible crop combinations that align with specific microbial functions (Galindo-Castañeda et al., 2022).

2.4.3 Impact of Agricultural Management on Soil Fungal Communities

Environmental disruptions, both in a general context and specifically through diverse land uses and agricultural practices as mentioned earlier, result in modifications to the composition of microbial communities. Agricultural practice alterations in soil are recognized as a primary catalyst for shifts in biodiversity within different ecosystems (Bevivino and Dalmastri, 2017). Therefore, it is essential to understand how soil microorganisms react to anthropogenic disruptions. Typically, the reactions of microbial populations to disturbances can manifest as either resistance or resilience (Griffiths et al., 2001; Westergaard et al., 2001). When the microbial community remains unchanged following a disturbance, it is considered to be resistance. Otherwise, if the community undergoes changes but later recovery and reverts to its initial state, it is regarded as displaying resilience (Van Elsas et al. 2007).

Fungi thrive as prominent residents of soil owing to their remarkable adaptability and ability to assume diverse forms in reaction to challenging or adverse conditions. Due to their capacity for producing a diverse array of extracellular enzymes, fungi can effectively degrade various types of organic materials, participating in the decomposition of soil constituents and thus playing a role in the regulation of carbon and nutrient equilibrium (Zifcakova et al., 2016). The main contributions of the fungal community to the operation of the agroecosystem involve soil stabilization and nutrient cycling (Stromberger, 2005). Currently fungi in agroecosystems are recognized to fulfill numerous additional roles. These include engaging in competitive or mutualistic relationships with bacteria and other soil organisms, as well as performing various networking which make soil fungal biodiversity a central element in maintaining the health of managed soils (Orru et al., 2021).

The diversity and functioning of fungi are governed by a multitude of biotic factors (such as plants and other organisms) as well as abiotic factors (including soil pH, nutrient availability, moisture, salinity, soil texture, structure, and temperature) (Lauber et al. 2008; Herold et al. 2014). These factors themselves are regulated by implementation of agricultural practices, as mentioned earlier.

All agricultural practices, including the adoption of cover and rotational crops, utilization of composts, and implementation of tillage systems are likely to affect the different groups of soil fungi.

2.4.3.1 Soil Fungal Communities; Impact of Tillage Systems

Long-term tillage systems have been found to result in significant impacts on soil structure, organic matter distribution, and nutrient availability. Modified soil physical and chemical conditions in tillage practices establish distinct micro and macro-habitats for soil fungal communities, leading to shifts in their community structures (Orru et al., 2021). For instance, fungal diversity was generally greater in field that underwent conservative management practices, with notable increases observed in soils subjected to no-till cultivation (Wang et al., 2021). This condition supports the establishment and sustainability of hyphal networks, ultimately resulting in increased fungal biomass (Spedding et al., 2004). The impact of the tillage regimen on the hyphal networks of fungi can be exemplified by considering mycorrhizal fungi as a case study, particularly arbuscular mycorrhizal fungi. Numerous studies have indicated that tillage represents a significant stress factor resulting in a reduction in inoculum potential (Jasper et al., 1991; Singh 2003; Usuki et al., 2007). Fungal hyphae create extensive networks within cultivated soils and become active upon contact with seedlings. Tillage can fragment these networks, which may lead to a potential loss of cell contents (Orru et al., 2021). Furthermore, tillage impacts fungal sporulation by depositing the propagules on the soil surface, where they are exposed to elevated temperatures and increased competition from antagonistic microorganisms (Andrade et al., 2003). In no-till field, the mycorrhizal system tends to be more stable.

Practicing conventional tillage in agricultural lands often result in soil fungal communities dominated by aerobic microorganisms, conservation tillage practices, however, promote greater fungal population and activity, along with an increase in microbial biomass (Spedding et al., 2004; Wang et al., 2021). Therefore, distinct tillage approaches can influence soil fungal communities and their diversity through soil disturbances. This also impacts the function of the soil fungal communities within the ecosystem. A large number of research studies indicate that the implementation of tillage practices plays a significant role in shaping soil characteristics and influencing the composition of soil fungal communities (Wang et al., 2016; Wang et al., 2017; Xia

et al., 2018; Orru et al., 2021). Typically, soil sites with minimal disruption are characterized by a higher diversity within soil fungal communities, mainly saprotrophic fungi. No-till (NT) practices have also been observed to raise the relative proportion of symbiotic fungi (Schmidt et al., 2019; Hydbom and Olsson, 2021).

Conservation tillage practices, including no-tillage (NT), can positively impact soil fungal communities as it establishes new living conditions characterized by sufficient nutrition and reduced soil disturbance. In conservation tillage, when plant residues are left on or near the soil surface, they stimulate fungal growth and temporarily trap nutrients. In addition, conservation management promotes fungal community biodiversity by creating smaller, stable micro-niches within the environment that could accommodate species with diverse functional roles (Orru et al., 2021). These practices create an environment conducive to the establishment and sustenance of hyphal networks. Thus, in turn, contributes to a higher fungal biomass. In addition, reduced tillage leads to a decrease in the breakdown of hyphae, resulting in more stable fungal populations. This retention of fungal populations contributes to better soil nutrient retention and enhanced suppressive effects against pathogenic microorganisms (Goss and deVarennes, 2002).

2.4.3.2 Soil Fungal Communities; Soil Structure

The structural arrangement of soil particles and soil texture have been also proposed as factors contributing to the rich diversity of not only soil fungal communities but also all soil residents. This is due to their role in creating a wide array of environments for organisms, promoting distinct communities, and encouraging differentiation (Or et al., 2007; Tecon and Or, 2017; Vos et al., 2013; Zhou et al., 2002). The heterogeneity of soil structure can result in spatial variations in nutrient availability and other physicochemical properties. This has been demonstrated to contribute to heightened soil fungal diversity (Curd et al., 2018). Moreover, the structural heterogeneity within the soil environment results diverse spaces and spatial isolation, ultimately leading to an increase in soil fungal diversity (Wang and Or, 2012). Evidence suggests that soil microbes exhibit a preference for specific physical niches. These preferences might be due to physical or chemical attributes of specific minerals, as soil microbes tend to favor minerals that offer certain nutrients. For example, the intensified tilling of the soil can disrupt its structure, which subsequently reduces the diffusion and movement of phosphate ions (Deubel et al. 2011). Over the extended period of

using tillage practices, these alterations in phosphate dynamics could potentially influence the extent of AMF colonization in plant roots (Sheng et al., 2013).

As a reciprocal relationship existing in all ecosystems, all soil fungal activities, resulting in the alteration of soil structure, also influence the nature and extent of soil pore spaces available for microbial habitation. Alteration of pore distributions would result in alteration of the salient controls on other soil microbial communities, their composition and function, namely nutrient, water, and oxygen concentrations as well as predator/prey relations. This interconnectedness of ecosystems highlights how alterations in one aspect of the soil can trigger cascading effects on various other components.

2.4.3.3 Soil Fungal Communities; Nutrient Availability

Besides changing the soil structure and its consequence on uniformity of accessible microhabitats, anthropogenic activities also impact nutrient availability, consequently resulting in changes to soil microbial diversity (Schimel and Schaeffer, 2012; Sengupta and Dick, 2015). Implementation of long-term tillage practices disrupt soil structure, break down soil aggregates and mix various layers leading to increased aeration and decomposition rate of organic matter. This results in faster breakdown of plant residues and other organic materials, releasing nutrients like nitrogen, phosphorus, and carbon into the soil. Notably, the abundance of these nutrients, can undergo substantial modifications, impacting the composition of microbial communities within the soil (Schmidt et al., 2018).

Increasing the available resources for soil nitrogen, carbon and phosphorous concentrations is frequently associated with alterations in various attributes of the ectomycorrhizal fungi community (Lilleskov 2005). These alterations encompass reduced production of fruiting bodies, diminished diversity within the community, and changes in the relative abundance of ectomycorrhizal fungal community constituents.. This phenomenon can be attributed partially to the variation among ectomycorrhizal fungal taxa in their physiological capacities to exploit diverse nutrient compounds. Research findings additionally indicate substantial shifts within AMF communities in response to alterations in soil nitrogen levels (Egerton-Warburton and Allen, 2000). These studies offer multiple lines of evidence that as nitrogen availability increases, there is a parallel reduction in diversity. This decline is predominantly driven by the decrease or disappearance of large-spored genera

(such as *Scutellospora*, *Acaulospora*, and *Gigaspora*), in contrast to smaller-spored species like *Glomus*.

2.5 How Soil Fungal Communities can be Used to Assess Soil Quality and Health

Numerous endeavors have been made to define the characteristics of a high-quality soil. The concept of soil health represents the latest endeavor to define and quantify a type of ideal soil that facilitates favorable agricultural and environmental results (Norris et al., 2020; Fierer et al., 2021). The idea of soil health can be defined both in terms of its conceptual framework and its practical implementation (Lehmann et al., 2020). In a conceptual context, the U.S. Department of Agriculture Natural Resource Conservation Service (USDA-NRCS) provides the following definition: "Soil health, also referred to as soil quality, is defined as the continued capacity of soil to function as a vital living ecosystem that sustains plants, animals, and humans" (USDA–NRCS, 2018). The USDA-NRCS also presents a more practical definition by furnishing a set of essential soil health indicators, encompassing physical, chemical, and biological attributes (Lehmann et al., 2020).

The biological characteristics of soil, including a variety of microorganisms like bacteria, fungi, archaea, and protists, are intricately interconnected with multiple facets of soil quality and overall health (Bach et al., 2020; Fierer, 2017). As described in the earlier sections, soil microbial communities, particularly fungi, play a pivotal role in several dimensions of soil quality. They govern nutrient accessibility, the stability of soil aggregates, carbon sequestration, the breakdown of pollutants, the prevalence of plant diseases, and the promotion of plant growth. Considering the significant role that soil microbial communities play in various soil quality, it's logical to expect the existence of microbial indicators for assessing soil health.

Soil fungal communities not only play a pivotal role in driving numerous vital soil processes, but they also exhibit the capacity to adapt in response to both biotic and abiotic soil conditions. For instance, consistent alterations in soil fungal communities have been linked to variations in phosphorus (P) availability, soil pH, labile organic carbon pools, and levels of soil moisture (Isobe et al., 2020; Ramírez et al., 2020). It is often possible to identify specific fungal taxa or functional genes that are linked to distinct soil processes, such as denitrification and cellulose degradation (Shoun et al., 1992; Fierer, 2017). Rather than concentrating solely on specific microbial attributes (including particular fungal taxa or genes) as indicators of soil health, an alternative approach is to

employ these taxa or genes to detect shifts in soil characteristics and processes that are already recognized as significant components of soil health. This approach simplifies the process of obtaining pertinent microbial information and leads to more comprehensible results for assessing soil health (Fierer, 2017). Additionally, it is worth noting that microbial-based indicators of soil conditions are most valuable when they can be applied effectively to a diverse range of soil and ecosystem types. The fact that specific taxonomic groups show changes in response to phosphorus (P) availability at one location doesn't necessarily imply that these same groups serve as reliable indicators of P availability in various soils. While not all taxa or genes may be present in all soils, the identification of microbial bio-indicators necessitates confirmation that these taxa or genes are consistently linked to specific aspects of soil health within the relevant soil or ecosystem types. To identify these microbial bio-indicators, it is essential to conduct thorough, cross-site analyses of well-characterized soils.

2.6 Detection of Microbial Responses to Management

Since the resilience of a soil ecosystem in the face of significant disturbances partially depends on its microbial constituents, analyzing the composition and structure of soil fungal communities can provide enhanced insights into comprehending and influencing ecosystem processes (Bevivino and Dalmastri, 2017). Various methodologies can be pursued to investigate diversity and community structure of soil microorganisms, and to assess dynamic processes on a whole-community scale or within specific taxonomic groups. These approaches encompass identification and categorization, as well as functional characterization.

A major factor that influences the effectiveness of these methodologies is the strong preference of mycologists to heavily depend on culture-based techniques in their ecological studies of soil fungi. In this technique, once organisms have been isolated and cultivated, they can be identified through morphological indicators, such as the type of resting structures they exhibit. The limitations of these approaches have been consistently underscored, with the data they yield offering only a partial and inevitably skewed perspective on diversity (Zak and Visser, 1996; Bridge and Spooner, 2001; Anderson and Cairney, 2004; Bevivino and Dalmastri, 2017). To address the challenges linked to culture-based techniques, diverse methods have been devised (Kirk et al., 2004). In recent years, the detection of alterations in soil microbiota has been achieved through

genetic fingerprinting methods. These methods generate a profile of microbial communities by directly analyzing PCR-amplified products derived from soil DNA (Rastogi and Sani 2011). Direct nucleic acid extraction methods have gained broader utilization in the study of soil fungal ecology. These approaches are starting to yield notable progress in enhancing our comprehension of genetic diversity within soil fungal communities.

2.6.1 Molecular Approaches

The early molecular identification studies of the 1990s revealed a variety of fungal species that were often uncultivable. These species were previously undiscovered through fruiting bodies and other structures, and their nutritional behaviors and ecological relationships frequently posed challenges for characterization (Tedersoo and Nilsson, 2016; Nilsson et al., 2019). Various methods have been employed for extracting fungal genomic DNA from soil. These include the utilization of commercially available kits specifically designed for extracting microbial DNA from soil, as well as techniques that have been fine-tuned for extracting nucleic acids from particular soil types (Yeates and Gillings, 1998; Dickie et al., 2002; Guidot et al., 2002; Ranjard et al., 2003; Anderson et al., 2003).

With the advancement of DNA sequencing technologies, moving from sequencing individual specimens to parallel Sanger sequencing in the early 2000s, it became evident that the mycobiota that remains hidden, and possibly beyond observation, surpasses the diversity that can be discerned through fruiting bodies and traditional cultivation methods (O'Brien et al., 2005). Therefore, the combined impact of Sanger sequencing (Sanger et al., 1977) and the polymerase chain reaction (Mullis and Faloona, 1987) led to a dual revolution that reshaped the landscape of fungal ecology and systematics, commencing in the early 1990s (Vilgalys and Hester, 1990; White et al., 1990; Bruns et al., 1991, 1992). In addition, the emergence of second-generation sequencing techniques occurred in the latter half of the 2000s, marking the beginning of HTS analyses for studying fungal communities (Hibbett et al., 2016).

2.6.1.1 Massively Parallel Sequencing

Recent technological breakthroughs have opened doors for high-throughput analysis of fungal genetic material obtained directly from the environment, a process commonly termed metagenomics. Metagenomics studies employ massively parallel sequencing techniques to

comprehensively analyze communities within the soil microbiome, minimizing inherent biases. Metagenomics sequencing can be categorized into two primary types: amplicon sequencing and shotgun sequencing (Escobar-Zepeda et al., 2015).

Amplicon sequencing is employed to specifically target and identify a subset of microorganisms within a provided environmental DNA sample. In this method, the process entails amplifying a barcoding gene from all organisms within the environmental DNA sample, sequencing these amplicons, and subsequently aligning the sequences with a suitable database to discern and identify the organisms present (Lundberg et al., 2013; Escobar-Zepeda et al., 2015). Distinguishing individual organisms is achievable by assessing variations in sequence within hypervariable regions (Lundberg et al., 2013). Diversity indices can be employed to assess and quantify microbial community diversity in various environments. Alpha diversity is defined as the number of distinct organisms within a sample (richness) and how evenly their abundance is distributed (evenness). Beta diversity, in contrast, measures the dissimilarity or variation in species composition between different samples or locations, revealing the degree of turnover in species between those environments (Whittaker, 1960). Utilizing amplicon sequencing results is an effective tool for conducting functional and network analyses of microbial communities in a given environment (Escobar-Zepeda et al., 2015). Amplicon sequencing allows for the identification and quantification of microbial taxa present in ecosystems. This rich taxonomic information serves as the foundation for conducting functional analyses, providing insights into the potential roles of the microbial community. Several sequencing platforms have been established for metagenomics sequencing, including technologies like the MiSeq (Illumina Inc., California, USA) and Sequel (Pacific Biosciences of California Inc., California, USA) DNA sequencing instruments.

2.6.1.2 MiSeq

Among the second-generation HTS platforms, the MiSeq holds a predominant position. The MiSeq platform employs sequencing-by-synthesis technology utilizing fluorescently labeled deoxynucleotide triphosphates. The platform employs a solid-phase bridge amplification technique in which the template is initially immobilized on a flow cell through attachment to an adapter sequence. During each step, a single nucleotide is added with a 3’ reversible terminator,

and the fluorophores in the imaged sequences indicate the specific dNTP that was added. Subsequently, the 3' terminator is removed, and the cycle is repeated (Werner et al. 2012).

Although MiSeq provides relatively short single-end read lengths, reaching up to 300 bases, its exceptional read quality, substantial throughput, and the capability to employ a paired-end strategy result in the creation of superior assemblies for complete internal transcribed spacer subregion 1 (ITS1) or full ITS2 fungal sequences across most fungal taxa. Currently, MiSeq sequencing stands as the primary selection for metabarcoding investigations involving fungi and other organisms. The paired-end strategy accommodates amplicons of approximately 550 bases in length (MiSeq 2x300), establishing it as the standard choice for such studies (Nilsson et al., 2019).

2.6.1.3 Selection of Marker and Primer; Soil Fungal Communities

The careful selection of genetic markers, primers, and amplification conditions constitutes a pivotal stage in HTS-based studies. Sequencing the ITS region within the nuclear rDNA operon holds a central position in species identification based on HTS-driven metabarcoding methods (Begerow, et al., 2010; Sommerman et al., 2018; Yang et al., 2018; Lofgren et al., 2019). Due to occurrence in multiple copies within fungal genomes, varying from just a few to well over 1000 copies, the rDNA operon emerged as an initial focal point in phylogenetic investigations of fungi (Bruns et al., 1990, 1991, 1992), primarily due to its ability to be amplified easily, even from minute quantities of material (Kauserud 2023).

Studies addressing aquatic fungi and AMF frequently employ the small subunit (SSU) (18S) and large subunit (LSU) (28S) nuclear rRNA genes. However, for ascomycetes and basidiomycetes, these markers often yield informative results only at taxonomic levels higher than species (and occasionally genera). This is due to the potential absence or minimal variation in SSU and LSU sequences between species within these fungal groups, making it challenging to confidently determine species distinctions (Větrovský, et al., 2016; Kauserud, 2023).

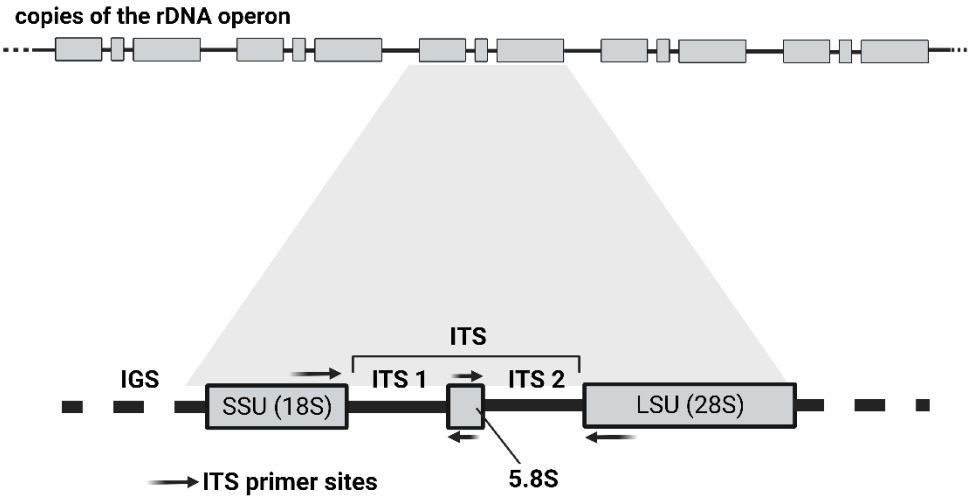

Figure 2. 5. Sketch of the rDNA operon, highlighting the ITS region (the relative sizes depicted do not accurately represent actual distances.) (Modified from Kauserud 2023 by BioRender.com 2023). Although many primers have been created for the amplification of various segments within the ITS region, forward primer gITS7ngs in combination with reverse primer ITS4ngs resulted in the best coverage for fungal kingdom (Tedersoo and Lindahl 2016).

Because the ITS region usually covers a range of 500 to 700 bases, the majority of HTS-based studies concentrate on either the ITS1 or ITS2 subregions, which typically span 250 to 400 bases (Figure 2.5). Advantages associated with the ITS2 subregion encompass reduced length variation and broader applicability of primer sites. Consequently, the ITS2 subregion exhibits fewer taxonomic biases compared to ITS1 (Sommerman et al., 2018; Kauserud, 2023).

The selection of primers determines which fungi will be extracted from the sample. In many literature reviews, it is strongly recommended that, when considering the ITS region, the targeting of the ITS2 subregion or the complete ITS region is best achieved by utilizing the degenerate forward primers gITS7ngs and ITS9MUNngs, respectively, along with the reverse primer ITS4ngs. This selection is preferred due to their extensive coverage across the fungal kingdom (Tedersoo and Lindahl, 2016; Sommerman et al., 2018; Nilsson et al., 2019). It is also important to

acknowledge that the ITS1 primers recommended by various extensive microbiome projects (namely, ITS1F and ITS2) exhibit notable primer biases and are affected by the presence of a common intron found in multiple fungal groups. This situation can result in biased amplification (Tedersoo and Lindahl, 2016).

In HTS-based studies, samples are typically subjected to amplification using primer variants that incorporate distinctive identifier indices (commonly referred to as tags, molecular identifiers, or barcodes). These indices enable the bioinformatic recognition of individual samples. These indices are recommended to exceed a length of six bases and possess at least three nucleotide differences from one another. To mitigate index-switching artifacts, it's advised to employ distinct indices for each sample on both the forward and reverse primers. The primers may also contain a spacer of 1 to 7 bases preventing index ends from acting as primers. Furthermore, sequencing adaptors tailored for specific platforms can also be integrated into the primers. The inclusion of platform-specific adaptors in the primers increases their cost and can lead to a decrease in PCR efficiency. However, this adaptation facilitates the determination of the sequencing direction (Lindahl et al., 2013; Wu et al., 2015; Nilsson et al., 2019).

2.7 Reference databases; Soil Fungal Communities

In the 1990s and 2000s, active research in fungal evolution and systematics led to a substantial accumulation of ITS sequences in the international nucleotide sequence databases (INSD), such as EMBL and NCBI, as reported by (Schoch et al., 2012). The count of ITS sequences within the INSD databases gradually increased during this period. However, it became evident fairly quickly that a substantial portion of the reference sequences, which were employed for the indirect taxonomic annotation of environmental sequences, had been assigned an incorrect taxon name or were lacking accurate metadata or contained errors (Nilsson et al., 2006; Bidartondo et al., 2008). This circumstance acted as a driving force for the creation of more effectively managed reference sequence databases. In response to this requirement, the UNITE database was established, initially focusing solely on Ectomycorrhizal fungi. However, its scope was subsequently expanded to encompass the entirety of the fungal kingdom (Koljalg et al., 2005; Abarenkov et al., 2010). Through systematic curation and the careful removal of errors from ITS sequence accessions within the INSD, UNITE offers enhanced reference sequence datasets to the research community (Nilsson

et al., 2019). UNITE is an accessible publicly available database housing around 1 million fungal sequences that can be grouped into Operational Taxonomic Units (OTUs) with 97-100% similarity (Nilsson et al., 2019). These OTUs receive taxonomic classifications through comparisons with reference sequences in databases that share 97% similarity. This taxonomic information enables subsequent statistical analyses in downstream applications (Edgar, 2018).

In addition to UNITE, other fungal reference databases have been created with a focus on particular fungal groups and genetic markers. For instance, MaarjAM was tailored for AMF (Opik et al., 2010). A more recent example is the GlobalFungi database, which presents an extensive compilation of HTS data for fungal ITS from around the world (Vetrovsky et al., 2020).

3 Materials and Methods

3.1 Study Site and Experimental Design

The present field site was located in Portage la Prairie at a research and development centre of Agriculture and Agri-Food Canada (AAFC), Manitoba, Canada (49.9589° N and 98.2691° W) (Figure 3.1A). The research field site, characterized by a temperate and moderately dry climate (with average annual temperature of 3.8°C), was at an elevation of 261 meters and featured clay-loam soil with a pH level of 7.8 (Table A1). Soil samples were collected from the site of a long-term trial that commenced in 2017. The sampling effort encompassed three fields, with each field representing a distinct phase of the crop rotation system in each year, as outlined in Table 4. To assess the impact of different tillage treatments and crop rotations on the soil microbiome, the experiment was arranged using a split plot design. The plots had dimensions of 1.5 m × 6 m and were replicated four times (Figure 3.1B).

For this study, a subset of the parent experiment was chosen, encompassing four different tillage practices [conventional tillage (CT), deep tillage (DT), raised bed (RB), and vertical tillage (VT)] in 2021 and 2022 (details of tillage systems is presented in Table 4). In both years, a rotation of corn, canola, and soybean was practiced across all tillage systems. The seedbed preparation in the spring was done by disking, with a spacing of 30.5 cm between rows for soybean and canola field, while for corn field, the spacing between rows was set at 76.2 cm. After seeding, herbicide (Roundup Max at a rate of 1.65 l/ha) was used to control broadleaf weeds and millet in all tillage treatments. Nitrogen (N), phosphate (P), potassium (K), and sulfur (S) fertilizers were applied at farmer standard rates. The harvesting of each field took place using a plot combine on three different dates: September 15th for soybean, October 15th for corn, and November 2nd for canola (same dates of harvest for both 2021 and 2022).

A

B

AAFC - Portage		15 m	6 m	2.0 m	6 m	10 m	6 m	2.0 m	6 m
		4.0 m							SM3
		0.75 m							
	4.5 m	1.5 m	101 (2021)		102 (2022)		105 (2021)		106 (2022)
		1.5 m	103		104		107		108
Rep 1		0.75 m	SM1	Spray row		SM2		Spray row	
		0.75 m							
	4.5 m	1.5 m	109 (2021)		110 (2022)		113(2021)		114(2022)
		1.5 m	111		112		115		116
		0.75 m							

Conventional tillage
Deep tillage
Raised bed
Vertical tillage
Soil moisture sensor

Figure 3. 1. A. Map of field site, Portage la Prairie, research and development centre. **B.** Schematic figure representing one replicate of the corn field that illustrates the arrangement of plots within the blocks. Within each block, the plots were split and assigned to different tillage practices. Soil samples were obtained from each plot in accordance with the respective sampling years of 2021 and 2022. The application of tillage practices was randomized across all four blocks within each field.

Table 3. 1. Experimental setup including crop rotation and tillage implemented at the Portage la Prairie site. The following four distinct tillage systems were employed: conventional tillage (CT), deep tillage (DT), raised bed (RB), and vertical tillage (VT).

Crop in rotation															
	2017 – 2018		2018 – 2019			2019 – 2020			2020- 2021			2021 – 2022			
Crops	Canola	Barley and Wheat	Canola	Soybean	Spring wheat	Soybean	Corn	Canola	Corn	Canola	Soybean	Canola	Soybean	Corn	
Field 2	*		*			*			*			*			
Field 5		*		*			*			*			*		
Field 7	*				*			*			*			*	
Tillage systems															
	CT		**VT**			**RB**			**DT**						
Instrument	Highspeed disc		Rotary disc			Raised bed maker			Deep tiller						
Depth	5 cm depth		15 cm depth			20 cm depth			40 cm depth						

3.2 Soil Sampling

Two separate soil sampling events were carried out. The first soil sampling was carried out on May 11, 2021, resulting in a total of 96 soil samples (3 fields × 4 tillage treatments × 8 plot level replicates = 96). The decision to include 8 plot levels during the first year of soil sampling was initially based on the inclusion of an irrigation treatment in the experiment. However, this treatment was later removed from the study due to drought conditions. The second sampling occurred on October 21, 2022, yielding a collection of 48 soil samples (3 fields × 4 tillage treatments × 4 plot level replicates = 48). Soil sampling was performed using a soil core sampler (4 cm diameter) in an S-shaped pattern, and involved obtaining 10 soil cores per plot to a depth of 5 cm. Soil cores were collected from the areas between the inner rows in order to prevent any confounding effects from nearby plots. The collected soil cores from each plot were pooled into a single plastic bag, and hand mixed to obtain a representative composite sample for each plot. To prevent cross contamination, soil core samplers were cleaned using 70% ethanol solution before proceeding to the next plot.

In the second soil sampling, soil samples from soybean and canola field were collected after the plants were harvested, whereas corn was not harvested from the field when the sampling took place. All soil samples were brought to the laboratory on dry ice and were stored at -20 °C until further processing.

3.3 DNA Extraction

Each soil sample was split into three fractions: large aggregates (retained on a 2 mm sieve), small aggregates (passed through a 2 mm sieve), and particulate organic matter (picked by hand from each soil sample) (Figure 3.2). To avoid cross-contamination among soil samples, we ensured thorough disinfection of all tools by immersing them in 70% ethanol for a duration of 2 minutes before processing new soil samples.

The total community DNA was extracted utilizing a QIAcube robotic system, which is designed for fully automated DNA purification with the DNeasy PowerSoil Pro Kit (Qiagen) for soil aggregate size fractions and the DNeasy Plant Pro Kit (Qiagen) for particulate organic matter. The manufacturer's instructions were followed, with slight modifications. To process soil fractions, approximately 250 mg of soil was placed in a 2 ml sterile bead beating tube and resuspended in 800 µl of lysis buffer.

For the extraction of DNA from particulate organic matter, 30-50 mg of plant materials were added to a sterile bead beating tube, followed by the addition of 500 µl of lysis buffer.

Mechanical disruption used the bead beating tubes provided by DNA extraction kits. The speed was maintained at 4 m/s for a duration of 2-minutes, followed by a 30-second rest period, and then repeating the 2-minute bead beating cycle (Bead Ruptor Elite, Omni International). After this point, DNA extraction was specified by the manufacturer. The extracted DNA was stored at-20 °C until use.

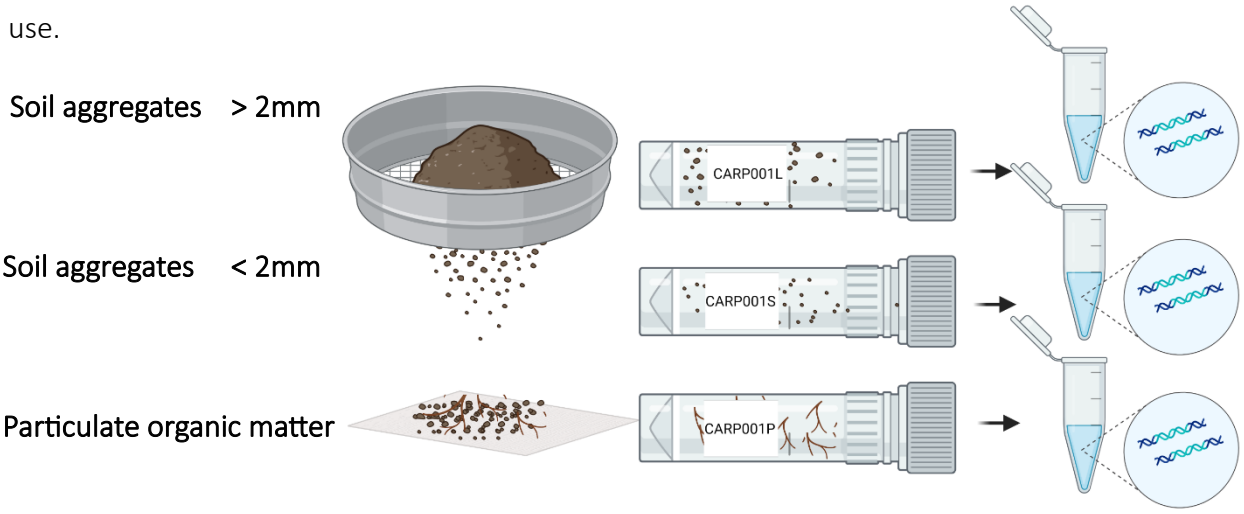

Figure 3. 2. Schematic of soil fractionation into large aggregates (retained on a 2 mm sieve), small aggregates (passed through a 2 mm sieve), and particulate organic matter, followed by DNA extraction from each fraction (Figure generated by BioRender.com 2023).

The quality and purity of extracted DNA were measured in 260/280 nm and 260/230 nm absorbance ratios using a spectrophotometer (NanoDrop One, Thermo Fisher Scientific). All DNA extracts were adjusted to a uniform 20 ng ml^{-1} by dilution for downstream applications. This promotes consistency during the DNA library preparation step, and minimizing the potential for bias or variations that can arise during this step.

3.4 Library Preparation

For soil samples from 2021, DNA extracts were shipped on dry ice to the Génome Québec Innovation Centre (Montreal, QC, Canada) for library preparation and amplicon sequencing using the MiSeq platform, generating 250 bp paired-end reads. For the soil samples collected in 2022, we performed library preparation and amplicon sequencing (MiSeq, 250 bp paired-end reads) at the University of Manitoba.

The DNA library preparation for both years involved multiple steps to amplify and prepare DNA fragments for sequencing with slight differences due to preparation at Genome Quebec vs. at the University of Manitoba. We conducted two common PCR stages during our DNA library preparation: the amplification of target DNA fragments and the indexing PCR.

3.4.1 Amplicon PCR to Enrich for Target DNA Fragment

This step uses PCR to selectively amplify specific DNA templates from a given sample. This is achieved by using primers designed to match the regions of interest, with attached overhang adapters. To prepare the samples for amplicon sequencing, we selected the ITS2 locus for PCR amplification. The ITS2 subregion offers several advantages, including reduced length variation and a greater number of flanking universal primer sites. As a result, it exhibits less taxonomic bias compared to the ITS1 region (Nilsson et al., 2019; Tedersoo et al., 2015).

For the amplification, we utilized the forward primer gITS7ngs (GTGARTCATCRARTYTTTG) and the reverse primer ITS4ngsUni (GTGARTCATCRARTYTTTG) (Tedersoo and Lindahl 2016), both of which incorporated 5' overhanging adapter sequences for compatibility with subsequent indexing steps and variable length spacers to enhance the diversity of signals during sequencing (Table 3.2). This primer set offers comprehensive coverage and achieves superior taxonomic accuracy for the kingdom Fungi, as they account for nearly all mismatches in fungi and most eukaryotes (Nilsson et al., 2019).

Table 3. 2. Sequences of the primer sets (in black) containing the Illumina sequence adapters (in red) and variable length spacers (in green) that were used for the amplification of the regions ITS2 in this study (primer portion from Tedersoo and Lindahl 2016).

Primer name	Sequences (5'-3')	Target
gITS7ngs	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGARTCATCRARTYTTTG TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAGTGARTCATCRARTYTTTG TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTAGTGARTCATCRARTYTTTG TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCTAGTGARTCATCRARTYTTTG	Fungi, plants, many protists
ITS4ngsUni	CGTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTSCSCTTANTDATATGC CGTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGACTSCSCTTANTDATATGC CGTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTACTSCSCTTANTDATATGC CGTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTACTSCSCTTANTDATATGC	Eukaryotes (except Foraminifera, Microsporidia)

For libraries prepared at the University of Manitoba, PCR reactions were conducted in 20 µl volumes consisting of 10 µl of Kapa HiFi HotStart ReadyMix (Roche), forward and reverse primers

(at 0.5 µM each), nuclease-free water, and template DNA at a final concentration of 0.8 ng/µl. The thermal cycling conditions used for the amplicon PCR included an initial denaturation step at 95 °C for 3 minutes, followed by 33 cycles of denaturation at 98 °C for 20 seconds, annealing at 52 °C for 30 seconds, and elongation at 72 °C for 20 seconds. There was a final extension step at 72 °C for 20 seconds. The PCR product was examined using a FlashGel cassette (Lonza, Switzerland) to validate the amplification process (Figure 3.3). For details of the PCR and thermal cycling conditions used in the first year of sampling, as performed by Genome Quebec, see Table 3.3.

Table 3. 3. Details of PCR Master Mix component and thermal cycling conditions for each PCR reaction performed during amplicon generation for first year of soil sampling, by Genome Quebec.

PCR Master Mix						
Components	10X Buffer with $MgCl_2$	Final concentration	1 X			
	DMSO (Roche)		5%			
	dNTP mix 10 mM (NEB)		0.2 mM			
	HotStarTaq 5U-ul (Qiagen)		0.02 U/ul			
	gITS7ngs		0.8 uM			
	ITS4ngsUni		0.8 uM			
	PCR Grade Water					
Thermal Cycling Conditions						
Steps	Initial denaturation	Temperature	96°C	Time	15 min	
	Denaturation		96°C		30 sec	X 33 cycles
	Annealing		52°C		30 sec	
	Elongation		72°C		60 sec	
	Extension		72°C		10 min	

3.4.2. Amplicon Library Clean- up

KAPA HyperPure Beads were used to purify amplified ITS fragments away from free primers, primer dimer species, and longer pieces of remaining genomic DNA. The size range of the ITS fragments recovered during a bead-based cleanup is dependent on the ratio of KAPA HyperPure Beads added

to the DNA sample (Table 3.4). To purify amplified ITS fragments, 18 µl of KAPA HyperPure Beads were added into each well of the PCR 96-well plates, which contained 20 µl of PCR product in spent PCR reaction mix. After thorough mixing by vortexing, each plate was subsequently positioned on the magnetic plate to capture the beads until the supernatant became clear. The cleared supernatant was carefully discarded, and an 80% ethanol rinse was applied. Finally, beads were resuspended with 10 mM Tris-HCl (pH 8.0 – 8.5) and the clear supernatant was transferred to a new PCR 96-well plate for the next step.

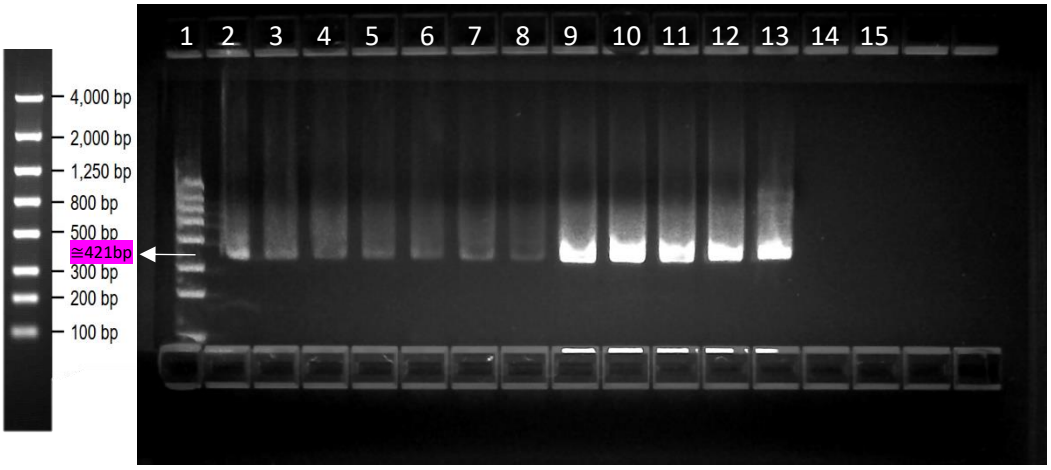

Figure 3. 3. PCR amplification results from a set of random soil samples on FlashGel electrophoresis. 1 = Ladder, 2-12 = soil samples, 13 = mock community as a positive control, 14-15 = negative controls.

Table 3. 4. Recommendations for the purification of fragmented DNA and amplicons using KAPA HyperPure Beads (technical data sheet for KAPA HyperPure Beads).

Fragments to be preserved	Suggested volumetric ratio of KAPA HyperPure Beads to the sample.
≥1 kb	0.5X
≥450 bp	0.6X
≥350 bp	0.7X
≥300 bp	0.8X
≥250 bp	0.9X
≥150 bp	1.5X
≥100 bp	2.2X- 3X

3.4.3. Indexing PCR

Indexing PCR reactions were carried out to facilitate the multiplexing of prepared samples for sequencing. By integrating these indices, it becomes possible to pool and sequence multiple samples concurrently, thereby optimizing the efficiency and capacity of the sequencing workflow. The attachment of a unique per sample index was accomplished through a second PCR step using the Nextera XT Index Kit v2 (Illumina Inc). PCR reactions in this step were performed in a final volume of 30 µl, consisting of 15 µl of Kapa HiFi HotStart ReadyMix (Roche), 3 µl of Nextera XT Index primer1 (i7), 3 µl of Nextera XT Index primer2 (i5), 6 µl of PCR nuclease-free water, and 3 µl of purified amplicon. The indexing PCR program consisted of the following steps: Initial denaturation at 95°C for 3 minutes, 8 cycles of denaturation at 98 °C for 20 seconds, annealing at 55 °C for 30 seconds, and elongation at 72 °C for 30 seconds, and one final extension at 72 °C for 30 seconds. Finally, the products were purified again using KAPA HyperPure Beads Kit as described in section 3.5. Moreover, detail of indexing PCR reactions for first year of sampling is described in Table 3.5. To validate the amplification process, a random of 32 samples were examined using a FlashGel cassette (Lonza, Switzerland) (Figure 3.4).

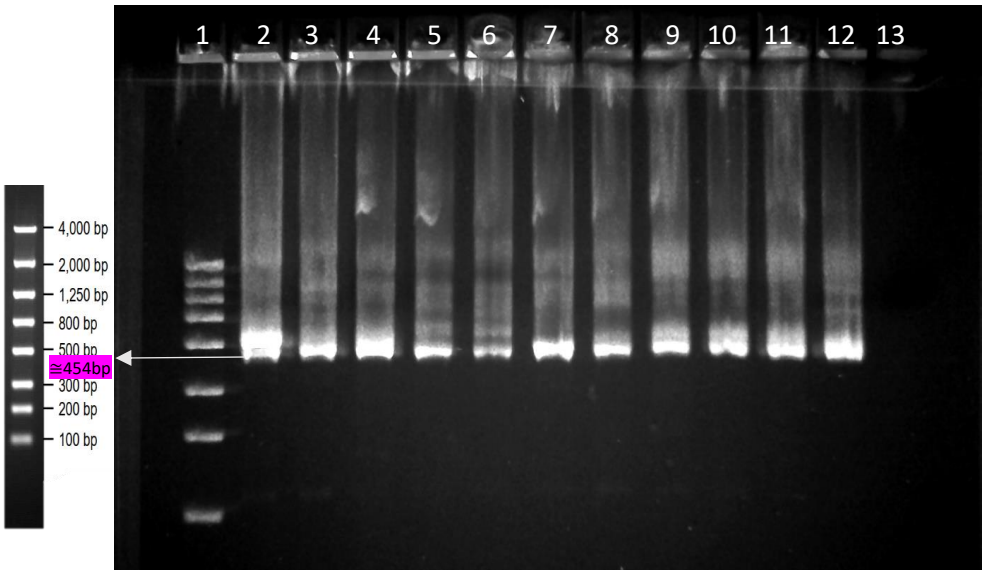

Figure 3. 4. Results of indexing PCR amplification from a random subset of soil samples on FlashGel electrophoresis. 1 = Ladder, 2-11 = indexed amplicon PCR, 12 = mock community as a positive control, 13 = negative control. Sizes depicted by the ladder are shown at the left. The observed increase in band size after indexing PCR is attributed to the addition of index sequences plus a new 5' overhanging sequence during the PCR amplification step.

Table 3. 5. Details of indexing PCR master mix components and thermal cycling conditions for each PCR reaction performed during indexing PCR step for the first year of soil sampling (Nextera XT Index Kit v2 was used in order to attachment of a unique per sample index).

Index PCR Master Mix						
Components	Roche 10X Buffer no $MgCl_2$	Final concentration	1X			
	Roche DMSO		5%			
	dNTP mix 10 mM (NEB)		0.2 mM			
	Roche FastStart High Fi5U-ul		0.025 U/ul			
	Roche $MgCl_2$ 25mM		1.8 mM			
	PCR Grade Water					
Thermal Cycling Conditions						
Steps	Initial denaturation	Temperature	95°C	Time	10 min	
	Denaturation		95°C		15 sec	X 8 cycles
	Annealing		60°C		30 sec	
	Elongation		72°C		60 sec	
	Extension		72°C		3 min	

3.4.4. Quantification of Indexed Amplicon Concentrations

The Quant-iT dsDNA assay Kit, High Sensitivity (Invitrogen by Thermo Fisher Scientific) was employed to measure DNA concentration. In a 96-well plate, each reaction consists of 95 µl of working solution and 5 µl of PCR product obtained from the Indexing PCR step. A set of 6 λ DNA standards were incorporated in each plate. The fluorescence was measured using a qPCR machine (excitation/emission maxima ~502/523 nm). The equation of the line of best fit from standard curve was calculated and used to quantify indexed DNA concentration per each sample (Figure 3.5). Finally, all purified indexed DNA samples were diluted using 10 mM Tris pH 8.5 to normalize their concentrations ahead of pooling.

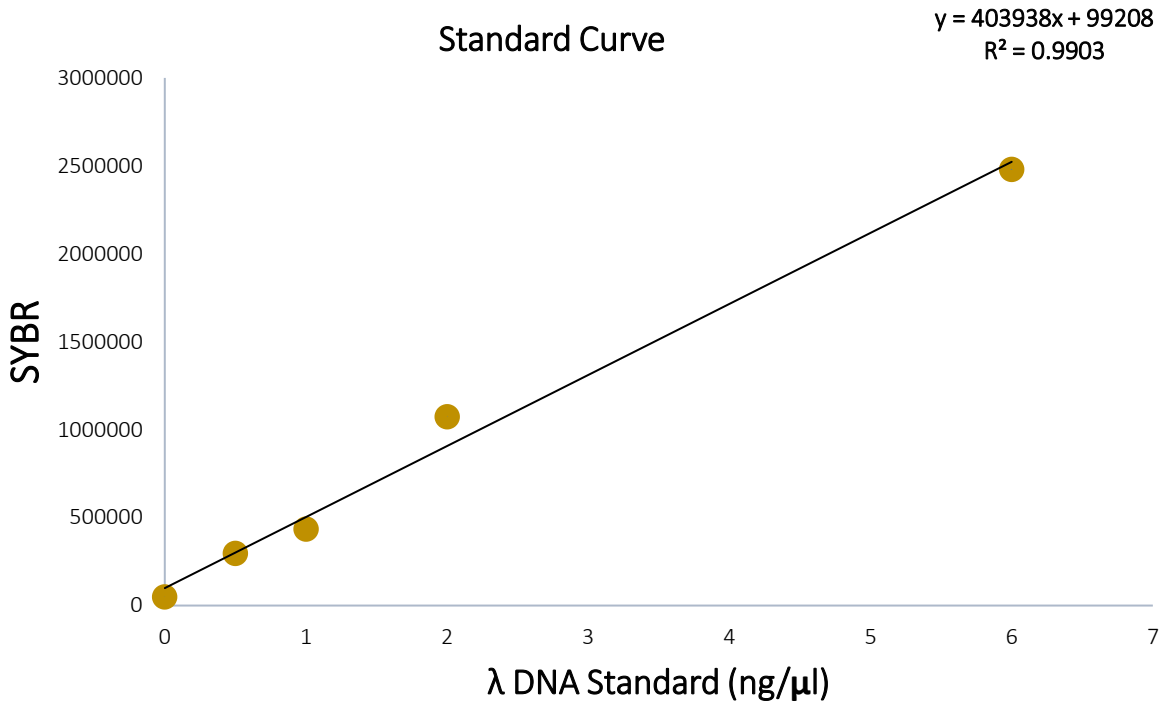

Figure 3. 5. The standard curve results from the fluorometric concentration analysis were used to determine the indexed DNA concentration. Based on the line equation and observed SYBR signal for λ DNA standards we measured the concentration of indexed PCR samples.

3.4.5. Pooling, Size Distribution, and Purification

Following the quantification step, pools were made for a maximum of 278 and 152 DNA samples for the first and second year of soil sampling, respectively. The quality and the size distribution of amplicons within the pooled libraries were observed using the TapeStation system (4150 Agilent) (Figure 3.6). Due to the presence of larger-than-expected fragments (likely contaminations) in the library, the pooled library was size-selected using a FlashGel recovery cassette (Lonza) and purified again with Zymo DNA Clean & Concentrator Kit (Figure 3.6).

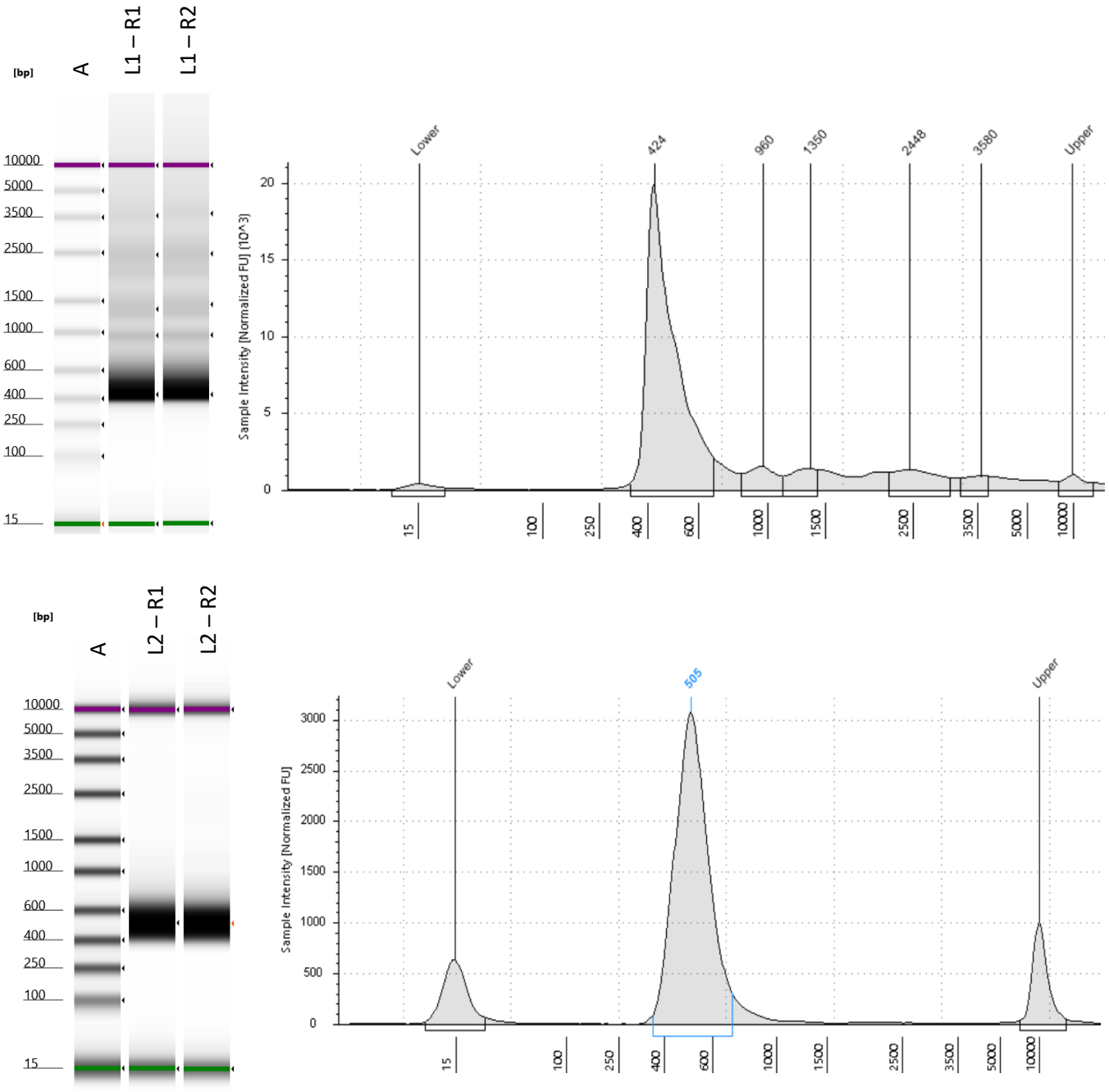

Figure 3. 6. The size distribution and quality of pooled library were check using TapeStation 4150 system (Agilent). Since larger fragments were also present in the pooled library, we used a FlashGel recovery cassette to remove the contaminations. In addition, Zymo DNA Clean & Concentrator Kit was also used to concentrate the amount of pooled library. A= ladder, L1 – R1 = first replicate of pooled library before purification, L1 – R2 = second replicate of pooled library before purification, L2 – R1 = first replicate of pooled library after purification, L2 – R2 = second replicate of pooled library after purification.

The concentration of appropriately prepared indexed amplicons in each pooled library was measured using the KAPA Library Quantification Kit designed for Illumina Platforms (Roche). A total

of 6 DNA standards in this step were used to generate a standard curve for pooled library quantification.

The q-PCR mixture was comprised of 121 µl of KAPA SYBR FAST qPCR Master Mix (2X) with 24.2 µl of Primer Premix (10X), 4.8 µl of ROX Low (50X), and 46 µl of PCR-grade water. A 16 µl of q-PCR mixture (including 12 µl of KAPA SYBR FAST qPCR Master Mix, 2 µl of Primer Premix, 0.4 µl of ROX Low, and 3.6 µl of PCR-grade water) was mixed with 4 µl of template DNA (i.e., standards or pooled & diluted library). Details of DNA standards and q-PCR conditions are outlined in Table 3.6. The standard curve was generated using the instrument software (Figure 3.7) and we removed any library dilutions that were outside the dynamic range of the assay. Finally, the standard curve was used to convert the average Cq score for each dilution of every library and internal control to a concentration (in pM). The average size-adjusted concentration (in pM) was calculated by following formula:

$$\text{Average size} - \text{adjusted concentration} = \frac{\text{Size of DNA standard in bp (452)}}{\text{Average fragment length of library in bp}}$$

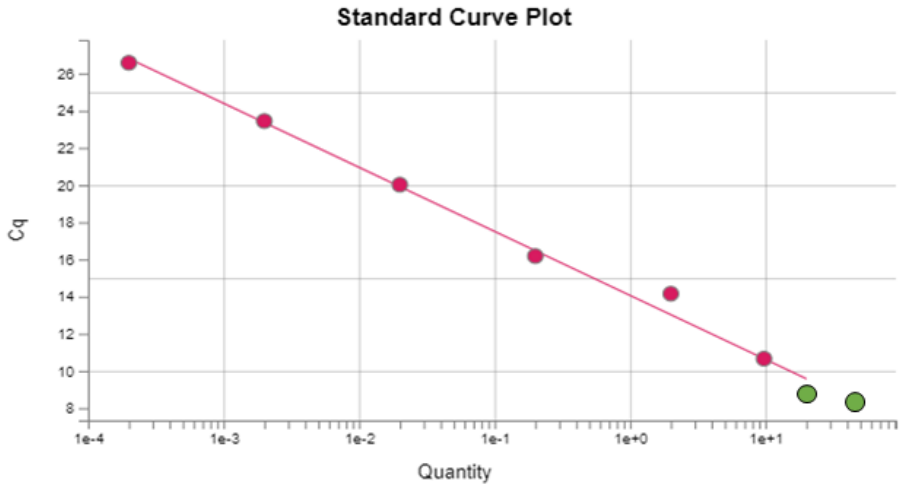

Figure 3. 7. The standard curve was generated using DNA standards of KAPA library quantification kit (red points represent standard DNA, whereas green points indicate a pooled and diluted library). The reliability of standard curve was checked using these criteria: The calculated reaction efficiency is in the range of 90 – 110%; the slope of the standard curve is between-3.1 and-3.6, and it has an $R^2 \geq 0.99$.

Table 3. 6. Details of DNA standards and the q-PCR conditions that were employed to measure the concentration of the pooled library using the KAPA Library Quantification Kit.

Quantification of Pooled Library						
DNA Standards	DNA Standard 1	Final concentration	20 pM			
	DNA Standard 2		2 pM			
	DNA Standard 3		0.2 pM			
	DNA Standard 4		0.02 pM			
	DNA Standard 5		0.002 pM			
	DNA Standard 6		0.0002 pM			
Thermal Cycling Conditions						
Steps	Initial denaturation	Temperature	95°C	Time	5 min	
	Denaturation		95°C		30 sec	X 35 cycles
	Annealing		60°C		45 sec	
	Melt curve analysis		65- 95°C			

3.5. Sequencing of Indexed Amplicons

The pooled libraries were denatured with NaOH, followed by dilution with hybridization buffer, and were subsequently heat-denatured at 96°C for 2 minutes before starting the sequencing process. PhiX was spiked into each library at varying concentrations for low diversity libraries. In each sequencing run, negative controls (PCR reactions without template DNA) and mock communities were included (Table 3.7). Sequencing was performed over three runs for the first year of soil sampling and over two runs for the second year. This was accomplished using a MiSeq instrument with v2 paired-end 2×250-bp flow cells, following the manufacturer's instructions. The raw sequences for all samples have been archived in the NCBI Sequence Read Archive (SRA) database under the accession number PRJNA882805.

Table 3. 7. Detail of sequencing runs for both first and second year of soil sampling using a MiSeq instrument.

Sampling Year	MiSeq Run	Input Concentration	PhiX Concentration	Cluster Density	Q30 Pass Rate	Mock Communities	Reference
2021	1	10 pM	15%	364 K/mm2	78.7%	Staggered B	Bakker 2018
	2	11 pM	15%	421 K/mm2	73%	Staggered B	Bakker 2018
	3	12 pM	15%	453 K/mm2	76.6%	Staggered B	Bakker 2018
2022	1	10 pM	5%	1000 K/mm2	71.8%	MSA 1010	American Type Culture Collection
	2	9 pM	15%	814 K/mm2	81.3%	MSA 1010	ATCC

3.6. Bioinformatics and Statistical Analyses

The raw paired-end sequence reads were processed using the DADA2 (version 1.14) (Callahan et al., 2016) designed for ITS Pipeline Workflow within RStudio (version 4.3.1). The paired-end reads were quality trimmed with Cutadapt (version.4.1) (Martin, 2011) and in order to avoid primer bias in the sequenced fragments, primer sequences were trimmed off from the 5′-end of both forward and reverse sequences using Cutadapt. Reads were filtered to allow a maximum of 2 anticipated errors, and the low-quality sequence reads (below the length of 50 or having ambiguous nucleotides) were trimmed at the 3′ ends (as DADA2 requires no Ns). The DADA2 algorithm was used to correct likely mis-called bases. The forward and reverse reads were combined, allowing for a maximum of one discrepancy in the overlapping region. Chimeras of sequences were identified and removed by the consensus-based chimera removal algorithm implemented in the DADA2 package. For improving the taxonomic resolution, ITSx (version 1.1b1) (Bengtsson-Palme et al., 2013) was utilized to eliminate flanking genes and extract the ITS2 sequences. The construction of an amplicon sequence variant (ASV) table was performed in DADA2 pipeline and taxonomy of ITS reads was assigned using the modified UNITE database (version 8.3, 99% with singletons, developer).

Negative control samples were used to identify contaminants; in the second year of soil sampling 34 ASVs were removed from the biological samples as probable contaminants. The mock community controls were processed in parallel with the biological samples to monitor the accuracy and reliability of the sequencing and data analysis pipelines. These controls contained known compositions of microorganisms, which allowed for the assessment of any potential biases or errors in the entire workflow.

Diversity statistics including alpha and beta diversity analyses were used to characterize the variation in fungal communities both within and between samples. Alpha diversity was evaluated through rarefaction curves, observed richness, and calculated Shannon and Simpson diversity indices using the Vegan, DECIPHER, and Phyloseq R packages. Meanwhile, the visualization of distances between fungal communities was achieved through a Non-Metric Multidimensional Scaling ordination, which was generated based on a Bray-Curtis dissimilarity matrix (using the “vegan” package in the R, version 4.3.1). The principal coordinate analysis (PCoA) was performed

based on the Bray Curtis dissimilarity. Statistical significance of community distance was tested with permutational multivariate analysis of variance (PERMANOVA). Alpha diversity indices within the tillage and crop rotation treatments, and among soil fractions, were analyzed using ANOVA. Tukey's honest significant difference (HSD) test was employed to distinguish between means, with a significance level set at 0.05.

We also conducted Canonical Analysis of Principal coordinates (CAP) to statistically confirm the observed differences among the treatments implemented in vegan package through *capscale* function. Taxonomic compositions were determined based on the relative abundances of dominant phyla. The visualization of the results was carried out using the Phyloseq, ggplot2 and Microbiome R packages.

A differential abundance analysis using the DESeq2 package in R (Love et al., 2014) was used to identify specific microbial taxa that responded to the treatments. Prior to analysis, the infrequent and low abundance taxa were removed from ASV abundance table (i.e., ASVs not observed at least 2 times in more than 3 samples were removed prior to analysis). We employed Wald Tests to calculate the $\log_2$ fold-change in abundance for every individual ASV when comparing all possible pairwise contrasts within the treatment variables (for example, between any given two tillage systems, or between soil aggregates within the soil fractions). The criteria for statistical significance were established as an adjusted *P-value* of ≤ 0.05 and an absolute $\log_2$ fold-change exceeding 1 (i.e., at least a 2-fold difference in relative abundance between treatments).

In addition, we utilized functional annotation packages, FUNGuild (https://github.com/UMNFuN/FUNGuild), to further classify the observed amplicon sequences into ecologically relevant functional groups. This approach allowed us to gain a deeper understanding of the impact of treatments on potential agroecosystem functions.

4 Results

4.1 Analysis of Sequence Data; Read Distribution; and Community Abundance

Analysis of soil microbiomes using high-throughput amplicon sequencing of the fungal ITS2 region revealed substantial fungal diversity in the agricultural soil samples. Across both years of conducting ITS sequencing, a total of 24,500,255 reads were generated across 423 samples. Chimeras and other spurious sequences (including sequences with ambiguous bases, flanking regions, sequences of likely non-fungal origin, and etc.) were eliminated, resulting in 1,376,865 reads for the initial year of soil sampling and 3,859,254 reads for the subsequent year. The total sequences for the first year were clustered into a total of 1842 fungal ASVs whereas a total of 4423 ASVs were observed for the second year.

The rarefaction curves for both years using the observed ASVs from each sample reached a plateau, (Figure 4.1A and 4.2A). For both years, all the curves eventually reached a plateau, indicating that further sequencing beyond a certain point does not significantly increase the number of observed ASVs. This plateau suggests that we have effectively captured the majority of fungal diversity present in our soil samples, ensuring that our dataset is comprehensive and suitable for subsequent analyses. The rarefaction curves for both years provide confidence in the robustness of our sequencing approach, highlighting that we have achieved a balance between capturing a diverse range of fungal species and avoiding oversampling, thus facilitating meaningful comparisons and ecological assessments within our soil fungal communities.

Before looking at the fungal diversity and community abundance, we generated a plot illustrating the correlation between observed ASVs richness and total read count (Figure 4.1B and 4.2B). Notably, the plot for each year of soil sampling revealed intriguing patterns in the correlation between sequencing depth and fungal diversity for each sample type. For both large and small soil aggregates, the correlation lines exhibit steep inclines, suggesting that as sequencing coverage increases, the observed ASV richness also increases significantly. This steep slope indicates that these sample types contain a diverse fungal community, and deeper sequencing effectively uncovers additional fungal species. In contrast, the correlation line for particulate organic matter (POM) samples exhibited a less steep slope. This finding suggests that POM samples may have a relatively lower fungal diversity or a more even representation of fungal species at the sequencing

depths achieved in this study. While the sequencing depth is still positively associated with ASVs richness in POM samples, the rate of increase in richness is less pronounced compared to the soil aggregate samples. This analyses not only provided insights into the fungal diversity within these distinct sample types but also offered a valuable assessment of the sequencing depth conducted. It underscored the importance of tailoring sequencing efforts to the specific characteristics of each sample type to maximize the capture of fungal diversity while optimizing resource allocation.

The relative abundance of fungal phyla for both years of soil sampling was evaluated using the ASV tables. The high-throughput amplicon sequencing results for 2021 showed a comparatively different microbial community composition compared to the 2022 (Figure 4.3).

The total of 1842 ASVs for 2021 were assigned to 13 phyla, 80 orders, 153 families, and 244 genera. In addition, these results highlighted that the 2021 field-trial was dominated by Ascomycota (42.50% of sequences, 783 ASVs), followed by Basidiomycota (22.42% of sequences, 413 ASVs), Chytridiomycota (5.21% of sequences, 96 ASVs) Glomeromycota (3.80% of sequences, 70 ASVs), Mortierellomycota (2.28% of sequences, 42 ASVs), and with 18.62% of ASVs were attributed to unidentified fungal Phyla (343 ASVs). The details of rare Phyla with low abundance (less than 2% of sequences) in all samples from 2021 is also demonstrated in Figure 4.3A. In 2022, a total of 4423 ASVs were assigned to 16 phyla, 88 orders, 181 families, and 296 genera. In addition, the high-throughput amplicon sequencing results for 2022 revealed that Ascomycota (18.56% of sequences, 821 ASVs), Basidiomycota (18.72% of sequences, 828 ASVs), Glomeromycota (6.69% of sequences, 296 ASVs), Chytridiomycota (4.90% of sequences, 217 ASVs), and Mortierellomycota (2.84 of sequences, 126 ASVs) were among the five dominant fungal Phyla in the field-trial. Notably, 43.74% of the sequences for the second year of soil sampling were attributed to unidentified fungal Phyla (Figure 4.3B).

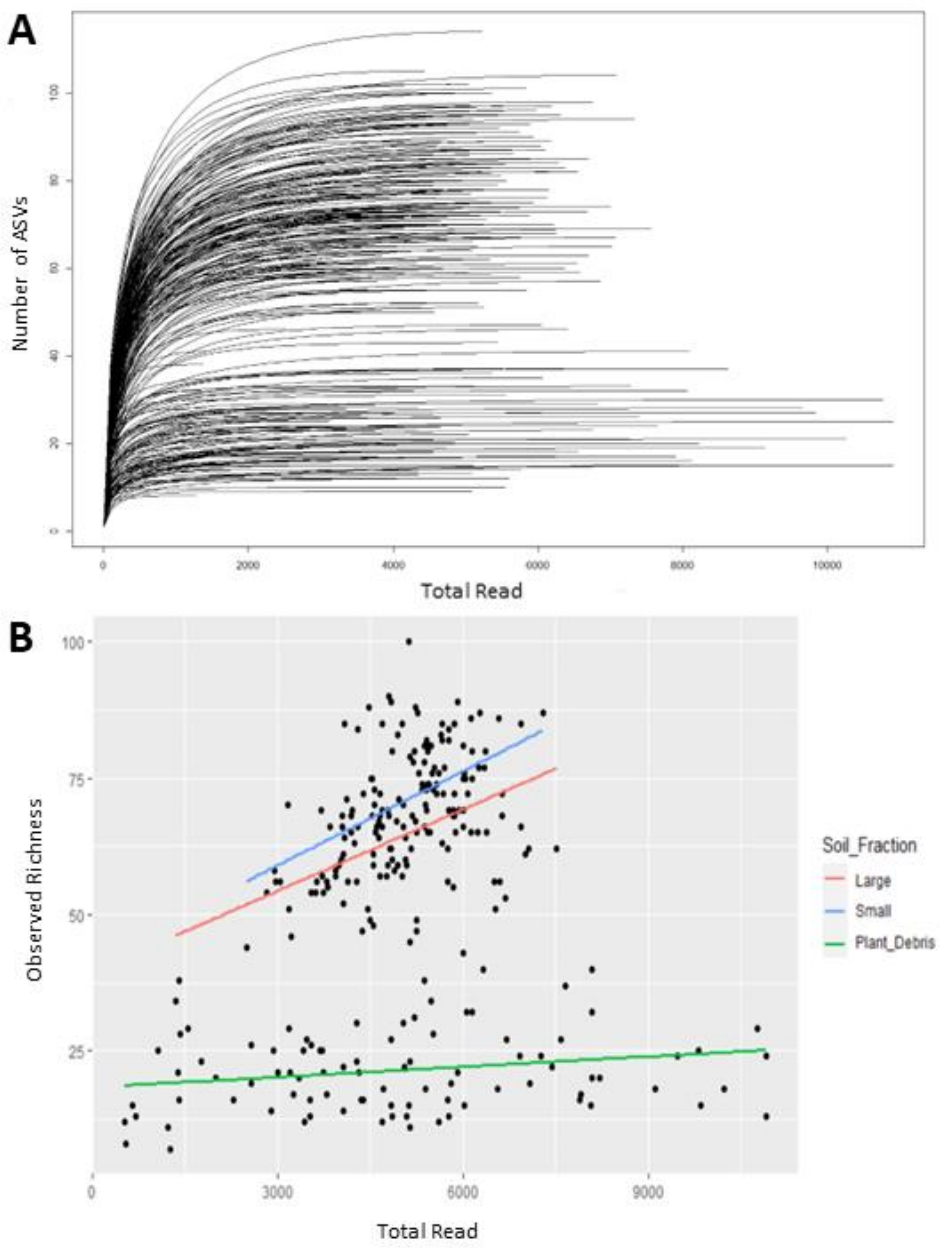

Figure 4. 1.A. Rarefaction curves for soil samples collected in 2021. Each curve represents a specific soil sample from our study, and the x-axis represents the depth of sequencing coverage, while the y-axis indicates the number of observed amplicon sequence variants (ASVs), which serve as proxies for fungal species richness. **B.** Correlation between observed ASV richness and total read count in three soil sample types (large: soil aggregates > 2 mm in diameter, small: soil aggregates < 2 mm in diameter, plant debris: particulate organic matter) for the first year of sampling**.** Each point on the graph represents a specific sample, and the x-axis denotes the total read count, while the y-axis represents the number of observed ASVs.

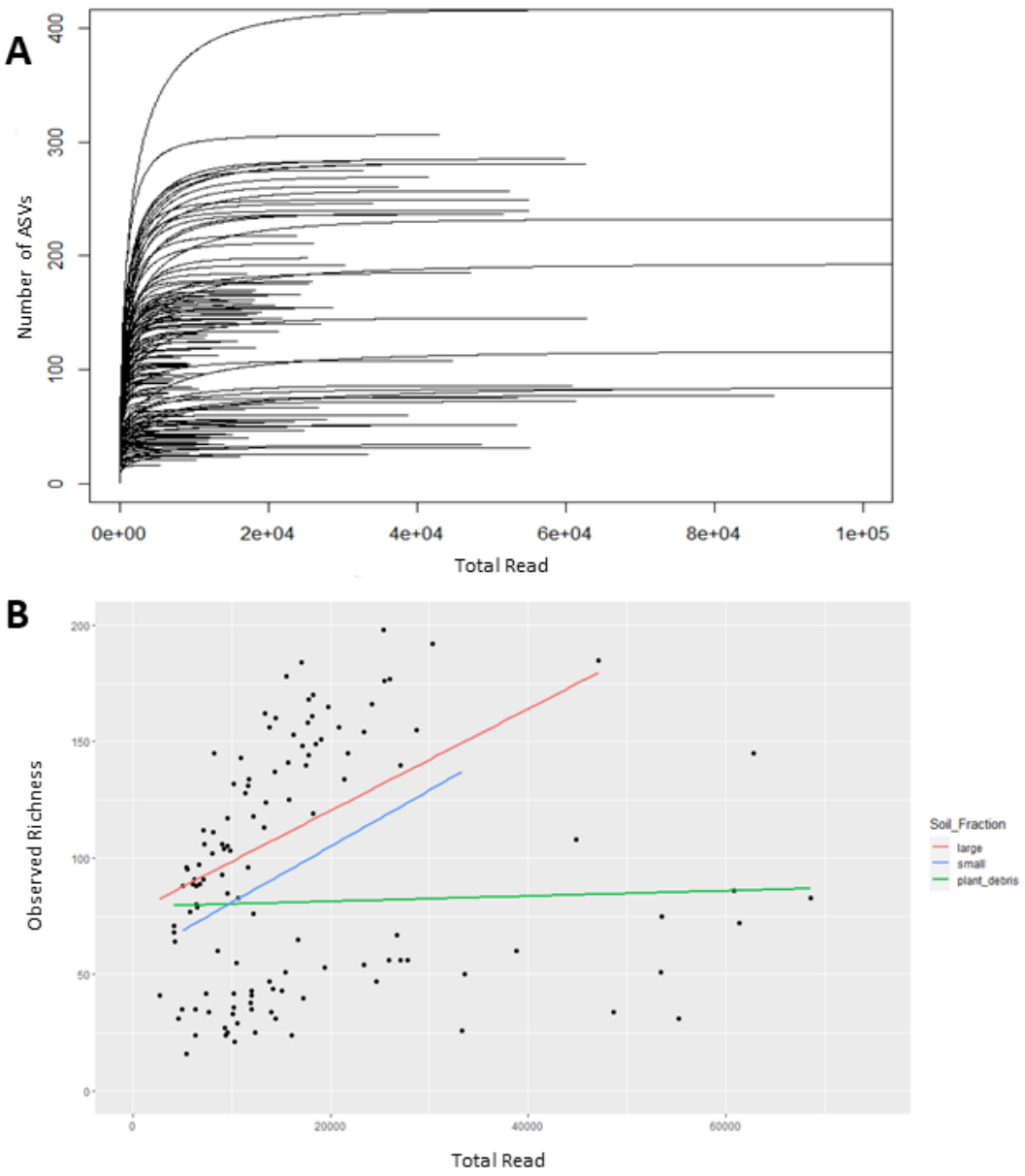

Figure 4. 2. A. Rarefaction curves for soil samples collected in 2022. Each curve represents a specific soil sample from our study, and the x-axis represents the depth of sequencing coverage, while the y-axis indicates the number of observed amplicon sequence variants (ASVs), which serve as proxies for fungal species richness. **B**. Correlation between observed ASV richness and total read count in three soil sample types (large: soil aggregates > 2 mm diameter, small: soil aggregates < 2 mm diameter, plant debris: particulate organic matter) for the second year of sampling. Each point on the graph represents a specific sample, and the x-axis denotes the total read count, while the y-axis represents the number of observed ASVs.

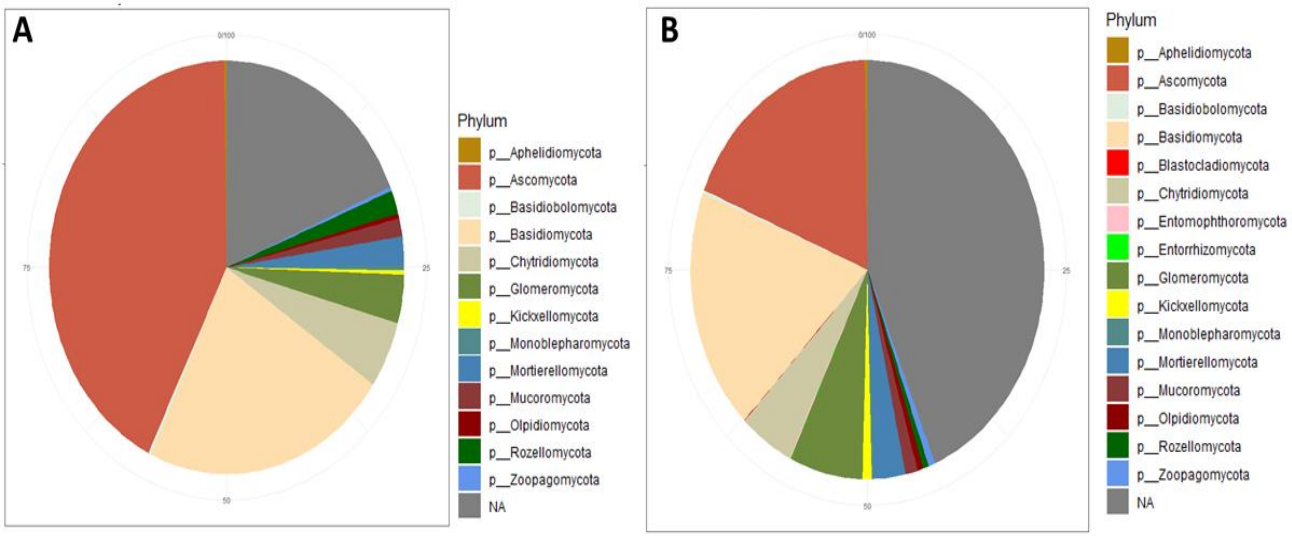

Figure 4. 3. A. Pie chart showing the average relative abundance of fungal phyla across all soil samples (n= 271) from Portage la Prairie (2021). B. Pie chart showing the average relative abundance of fungal phyla across all soil samples (n= 144) from Portage la Prairie (2022). Each segment of the graph corresponds to a specific fungal phylum, and the size of each segment represents the proportion or relative abundance of that phylum within the overall fungal community.

In 2021, three dominant fungal phyla emerged as prominent players across all four tillage systems: Ascomycota (ranging from 77.38% to 82.44% of the observed amplicon sequences), Basidiomycota (ranging from 12.28% to 18.19%), and Mortierellomycota (ranging from 1.82% to 2.19%). In 2022, however, five dominant fungal phyla emerged as main players across all four tillage systems: Ascomycota (ranging from 59.67 to 66.24%), Basidiomycota (ranging from 13.52 to 17.32%), Mortierellomycota (ranging from 9.43 to 11.68%), Chytridiomycota ranging from (ranging from 1.22% to 2.24%), and Glomeromycota (ranging from 1.21% to 2.6%) (Table 4.1). Details of relative abundance of other fungal phyla (fungi with low abundance) for both 2021 and 2022 are presented in Figure 4.4.

The relative abundance (proportion of observed amplicon sequences) for the top 5 most abundant phyla in each tillage system in 2021 is presented in Table 4.1. In both years, the RB tillage system demonstrated a higher relative abundance of Ascomycota. Additionally, in 2021, Basidiomycota exhibited greater relative abundance in the DT system, however, in 2022, Basidiomycota showed a higher relative abundance in the CT system. Furthermore, in 2022, Glomeromycota displayed a higher relative abundance in both CT and VT systems.

Taxonomical classification at the class level indicated that the predominant fungal classes in 2021 were Sordariomycetes and Dothideomycetes (together accounting for 70.54% of the observed amplicon sequences). In 2022, the presence of high levels of fungi belonged to the other class followed by Sordariomycetes and Dothideomycetes (together comprising 49.96% of the observed amplicon sequences). Details of relative abundance of dominant classes for both 2021 and 2022 are presented in Table 4.1.

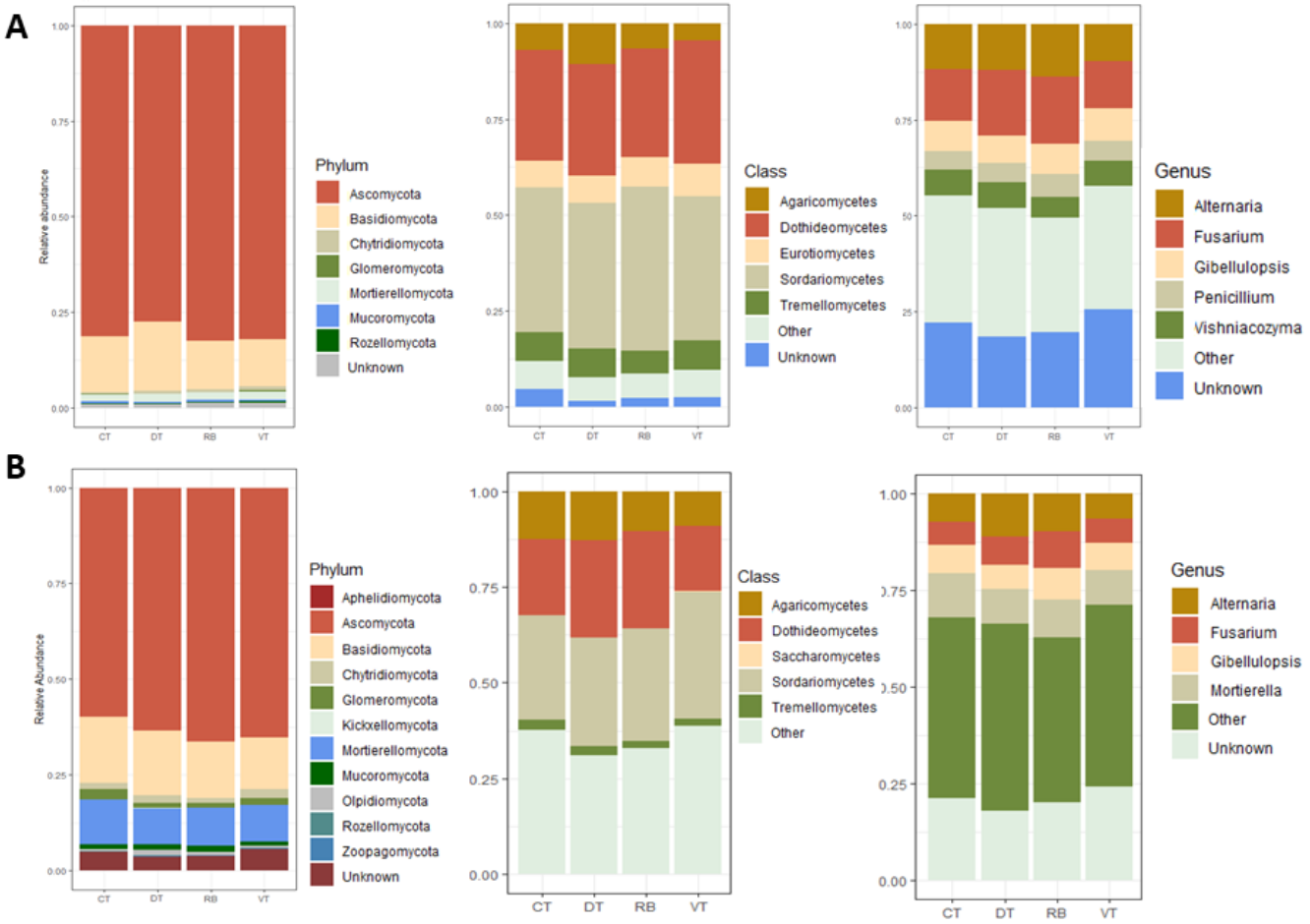

Figure 4. 4. Stacked bar-plot representation of the relative abundances of the main fungal taxa (at the rank of phylum, class, and genus) in the tillage systems based on metagenomic ITS2 sequences in the first year of soil sampling (**A**) and second year (**B**) [CT: conventional tillage, DT: deep tillage, RB: raised bed, VT: vertical tillage].

Further taxonomical classification of both years revealed that across all four tillage systems the most abundant fungal genera were unidentified taxa within the phylum Ascomycota. Additionally, across all four tillage systems, other genera collectively constituted 29.82% - 33.43% of the

observed amplicon sequences in 2021. In contrast, in 2022, the contribution of other genera increased to range from 42.73%- 48.66% across the tillage systems. Details of relative abundances for the top 5 most abundant genera are presented in Table 4.1. In 2021, *Fusarium*, *Alternaria*, *Gibellulopsis, Vishniacozyma*, and *Penicillium* represented 47.58% of the amplicon sequences observed. In 2022, *Alternaria, Mortierella*, *Fusarium*, and *Gibellulopsis* represented 32.53% of the amplicon sequences observed (Figure 4.4B).

4.1.1 Community Abundance; Soil Aggregate Fractions

The relative abundance of fungal taxa represented obvious variations among different soil fractions. In both years of the study, the most predominant fungal phyla within the three different soil fractions were identified as Ascomycota, Basidiomycota, and Mortierellomycota.

In 2021, the relative abundance of Ascomycota ranged from 78.41% to 83.68%, with the higher relative abundance found in large soil aggregates. Basidiomycota accounted for 8.49% to 20.62% of the fungal community in the same year, with higher relative abundance observed in particulate organic matter. Mortierellomycota made up a smaller proportion of observed amplicon sequences, ranging from 0.03% to 3.48%, with the higher representation in large soil aggregates (Figure 4.5A). In 2022, the distribution of the predominant fungal phyla also varied among soil aggregates. Ascomycota ranged from 60.15% to 67.72%, with the higher relative abundance belonging to particulate organic matter. Basidiomycota accounted for 13.48% to 18.83%, with higher relative abundance found in particulate organic matter. Mortierellomycota ranged from 6.35% to 12.81%, with the relative abundance observed in large soil aggregates.

Taxonomical classification at the class level across three soil fractions indicated the presence of high levels of fungi in both years belonging to the classes Sordariomycetes and Dothideomycetes. Across both years of the study, we observed a greater relative abundance of Dothideomycetes in particulate organic matter, accounting for 55.48% in 2021 and 30.99% in 2022. Conversely, a higher relative abundance of Sordariomycetes in both years was observed in large soil aggregates, representing 52.48% in 2021 and 33.17% in 2022 (Figure 4.5).

Further taxonomical classification revealed that in 2021, *Fusarium*, *Gibellulopsis, Alternaria, Vishniacozyma*, and *Penicillium* were among the most predominant identified genera in all three soil fractions (Figure 4.5A). In 2021, *Fusarium* was the most abundant fungal genus in both large and small soil aggregates, ranging from 22.10% to 22.30%, while in particulate organic matter, *Fusarium* was found at low relative abundance (less than 1%). In 2022, *Mortierella*, *Gibellulopsis, Alternaria, and Fusarium* were among the most predominant identified genera in all three soil fractions. Additionally, the most abundant fungi across all three soil fractions were unidentified taxa within the phylum Ascomycota. *Mortierella* and *Fusarium* showed higher relative abundance in large and small soil aggregates compared to the particulate organic matter (Figure 4.5B). More details of shifts in relative abundance of genera within the soil fractions in both years are presented in Figure 4.5.

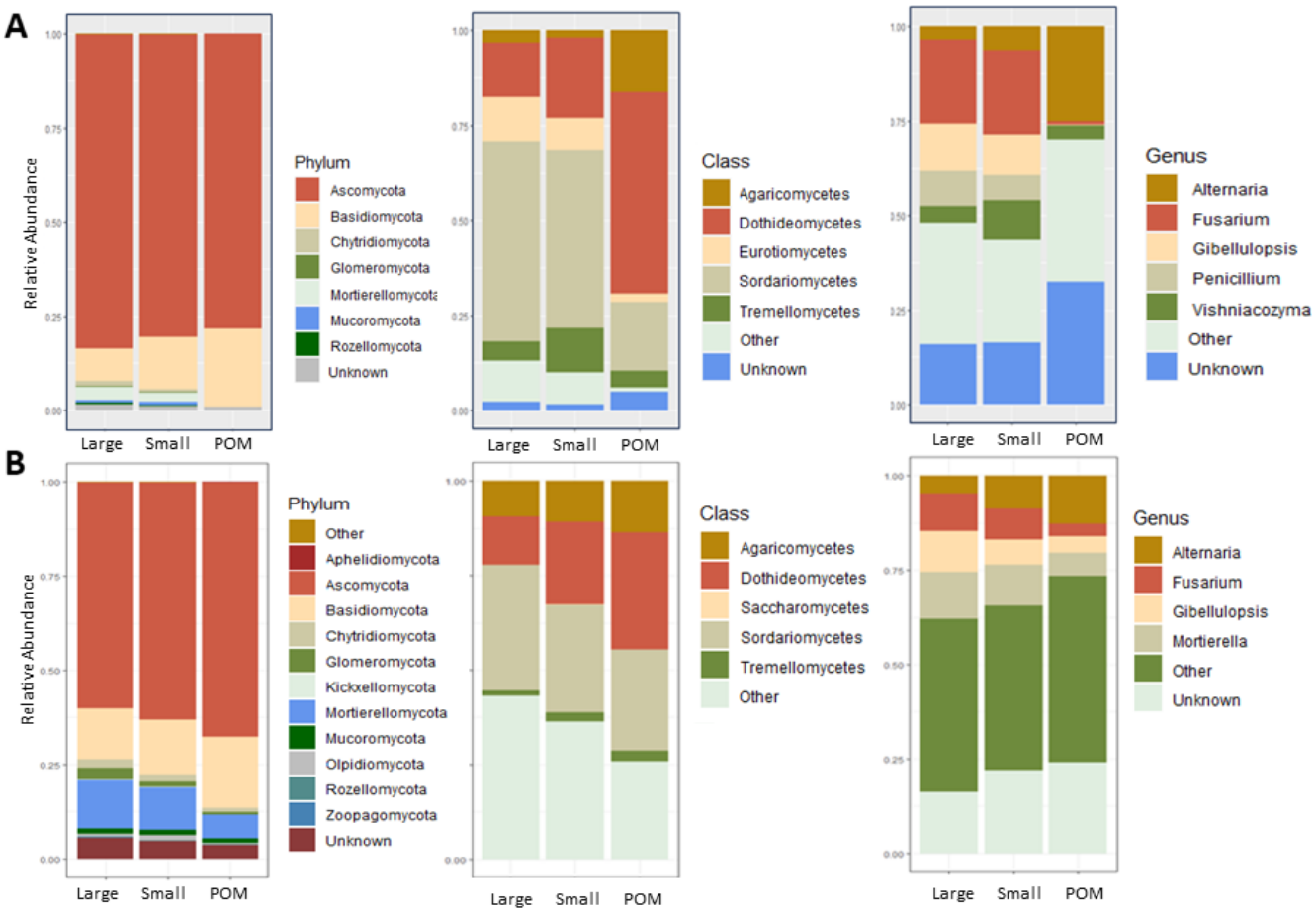

Figure 4. 5. Stacked bar-plot representation of the relative abundances of the main fungal taxa (at the rank of phylum, class, and genus) in the soil fractions based on ITS2 sequences in the first year of soil sampling (**A**) and second year (**B**) [Large: large soil aggregates (>2 mm), Small: small soil aggregates (<2 mm), POM: particulate organic matter].

4.1.2 Community Abundance; Crops in Rotation

The composition of the fungal community also exhibited variations among the plots within the three different fields: corn, canola, and soybean. In both years of the study, the most predominant fungal phyla in plots within these fields were identified as Ascomycota, Basidiomycota, and Mortierellomycota.

In 2021, the relative abundance of Ascomycota ranged from 72.06% to 88.72%, with the higher proportion found among plots in the corn field. Basidiomycota accounted for 7.13% to 21.97% of the fungal community in the same year, with the higher relative abundance observed in plots within the soybean field. Mortierellomycota made up a smaller proportion of observed amplicon sequences, ranging from 1.94% to 2.09%, with the higher representation also in plots of soybean field (Figure 4.6A). In 2022, Ascomycota ranged from 60.05% to 67.38%, with the higher proportion belonging to plots within the soybean field. Basidiomycota accounted for 14.02% to 17.03%, with the higher relative abundance found in plots within the canola field. Mortierellomycota exhibited a relative abundance ranging from 8.66% to 11.53% across all three field, with the highest relative abundance recorded in plots within the corn field (Figure 4.6B).

Taxonomical classification at the class level across all three fields indicated the presence of high levels of fungi in both years belonging to the classes Sordariomycetes and Dothideomycetes. Throughout both years of the study, Sordariomycetes emerged as the most predominant class in plots within both corn and canola field. In 2022, plots in the field planted to soybean showed a distinct pattern, where Dothideomycetes was identified as the predominant class. Details of relative abundances for the top 5 most abundant class for both years are presented in Figure 4.6B.

Further taxonomical classification revealed that in 2021, unidentified genera, *Fusarium*, *Alternaria, Gibellulopsis, Vishniacozyma*, and *Penicillium* were among the most predominant genera in all three fields (Figure 4.6A). The relative abundances for the top 5 most abundant genera in each field are presented in Table 4.1. In 2021, the most abundant fungi in plots within the canola and soybean field were unidentified taxa within the phylum Ascomycota. In addition, *Fusarium* emerged as the most predominant fungi in plots within the corn field. In 2022, *Mortierella*, *Alternaria, Fusarium,* and *Gibellulopsis* were among the most predominant identified genera in plots within the fields. Additionally, the most abundant fungi across all three field were unidentified

taxa within the phylum Ascomycota. Moreover, compared to the plots within the canola and soybean field, *Fusarium* was identified at a higher relative abundance in plots within the corn field. Consequently, in both years of the study, *Fusarium* consistently exhibited a higher relative abundance in plots within the corn field, indicating its prevalence and potential significance within the field plots associated with corn (Figure 4.6B).

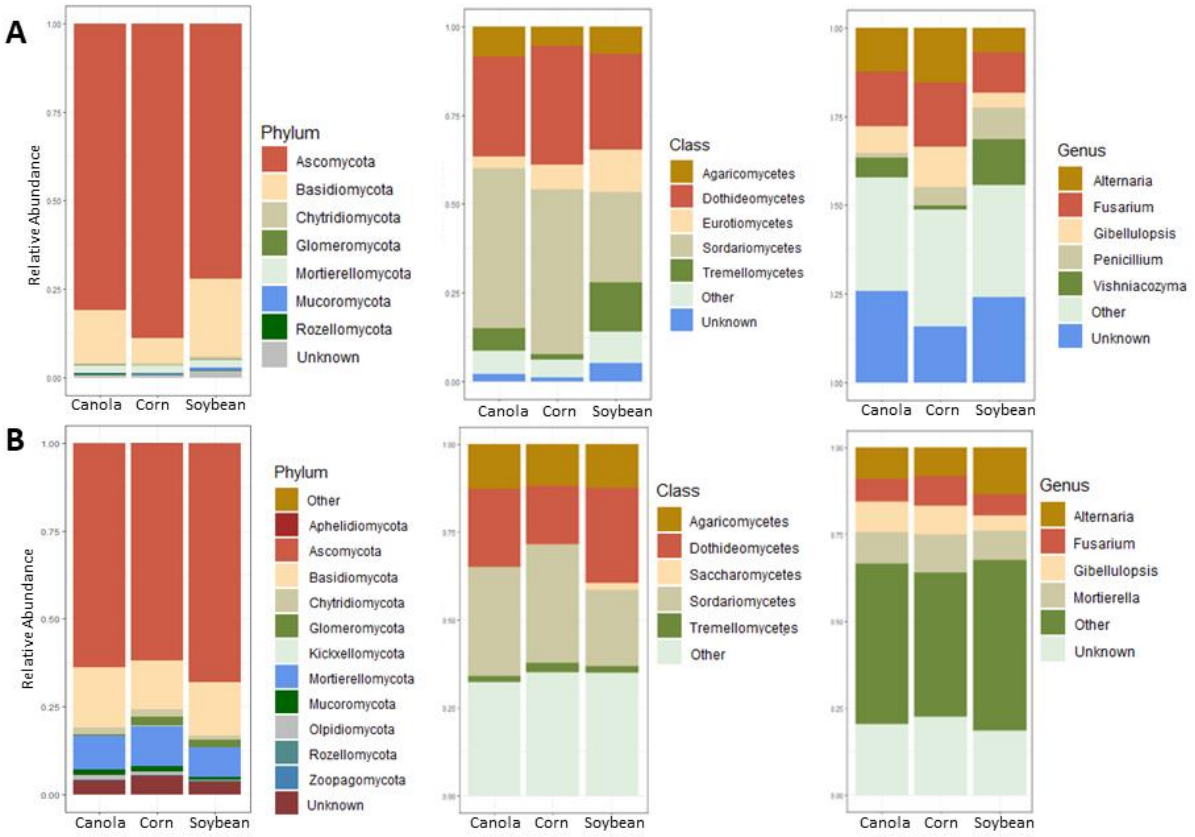

Figure 4. 6. Stacked bar-plot representation of the relative abundances of the main fungal taxa (at the rank of phylum, class, and genus) in the different fields (canola, corn, and soybean) based on ITS2 sequences in the first year of soil sampling (A) and second year (B).

Table 4. 1. Relative abundances (proportion of observed amplicon sequences) for the top 5 most abundant taxa, at the rank of phylum, class, or genus. Counts were summed across all ASVs included within each given taxonomic group, and proportions were cumulative proportion across all samples within a given experimental category [CT: conventional tillage, VT: vertical tillage, DT: deep tillage, RB: raised bed, LA: large aggregates, SM: small aggregates, POM: particulate organic matter, CO: corn, CA: canola, SO: soybean]

Taxonomic level	Top Taxa	First Year									
		Tillage				Soil Fraction			Crop Rotation		
		CT	DT	VT	RB	LA	SM	POM	CO	CA	SO
Phylum	Ascomycota	0.812	0.773	0.820	0.824	0.836	0.804	0.784	0.887	0.808	0.720
	Basidiomycota	0.146	0.181	0.122	0.127	0.084	0.139	0.206	0.071	0.150	0.219
	Mortierellomycota	0.018	0.020	0.021	0.019	0.034	0.025	0.000	0.019	0.019	0.020
	Chytridiomycota	0.005	0.006	0.009	0.005	0.012	0.008	0.000	0.008	0.004	0.007
	Unknown	0.009	0.009	0.013	0.013	0.015	0.010	0.008	0.007	0.008	0.018
Class	Sordariomycetes	0.377	0.380	0.375	0.427	0.524	0.499	0.190	0.463	0.449	0.253
	Dothideomycetes	0.289	0.290	0.321	0.282	0.144	0.225	0.554	0.334	0.281	0.268
	Tremellomycetes	0.075	0.074	0.075	0.000	0.051	0.126	0.044	0.015	0.641	0.139
	Eurotiomycetes	0.000	0.070	0.085	0.077	0.118	0.093	0.023	0.069	0.338	0.120
	Agaricomycetes	0.070	0.106	0.000	0.066	0.031	0.020	0.170	0.054	0.084	0.077
Genera	Unknown	0.221	0.185	0.256	0.196	0.158	0.163	0.325	0.157	0.258	0.241
	Fusarium	0.134	0.171	0.124	0.175	0.223	0.221	0.009	0.180	0.153	0.114
	Alternaria	0.117	0.119	0.095	0.136	0.033	0.065	0.249	0.154	0.123	0.069
	Gibellulopsis	0.079	0.071	0.083	0.079	0.127	0.106	0.003	0.114	0.751	0.042
	Vishniacozyma	0.067	0.066	0.066	0.053	0.043	0.105	0.039	0.011	0.056	0.127
	Penicillium	0.048	0.051	0.051	0.059	0.092	0.066	0.000	0.052	0.013	0.087

Table 4.1. continued. Relative abundances (proportion of observed amplicon sequences) for the top 5 most abundant taxa, at the rank of phylum, class, or genus. Counts were summed across all ASVs included within each given taxonomic group, and proportions were cumulative proportion across all samples within a given experimental category [CT: conventional tillage, VT: vertical tillage, DT: deep tillage, RB: raised bed, LA: large aggregates, SM: small aggregates, POM: particulate organic matter, CO: corn, CA: canola, SO: soybean]

Taxonomic level	Top Taxa	Second Year									
		Tillage				Soil Fraction			Crop Rotation		
		CT	DT	VT	RB	LA	SM	POM	CO	CA	SO
Phylum	Ascomycota	0.596	0.633	0.652	0.662	0.601	0.630	0.677	0.610	0.637	0.673
	Basidiomycota	0.173	0.169	0.135	0.146	0.134	0.144	0.188	0.140	0.170	0.155
	Mortierellomycota	0.116	0.094	0.094	0.098	0.128	0.111	0.063	0.115	0.096	0.086
	Chytridiomycota	0.016	0.019	0.022	0.012	0.021	0.020	0011	0.022	0.018	0.010
	Glomeromycota	0.026	0.012	0.017	0.012	0.031	0.014	0.005	0.025	0.004	0.020
	Unknown	0.041	0.027	0.030	0.044	0.037	0.045	0.035	0.055	0.041	0.037
Class	Sordariomycetes	0.271	0.282	0.330	0.291	0.331	0.283	0.268	0.344	0.308	0.221
	Dothideomycetes	0.197	0.252	0.170	0.255	0.126	0.219	0.309	0.169	0.223	0.276
	Tremellomycetes	0.025	0.023	0.019	0.048	0.013	0.024	0.028	0.026	0.018	0.020
	Agaricomycetes	0.126	0.128	0.091	0.020	0.094	0.107	0.135	0.100	0.126	0.105
	Other	0.377	0.311	0.386	0.328	0.431	0.363	0.257	0.357	0.322	0.355
Genera	*Alternaria*	0.074	0.112	0.063	0.097	0.46	0.087	0.126	0.063	0.090	0.117
	Fusarium	0.058	0.072	0.062	0.095	0.100	0.081	0.034	0.087	0.064	0.060
	Gibellulopsis	0.073	0.062	0.070	0.082	0.107	0.066	0.042	0.082	0.087	0.046
	Mortierella	0.113	0.088	0.091	0.096	0.124	0.108	0.060	0.112	0.090	0.117
	Other	0.468	0.486	0.469	0.427	0.458	0.437	0.493	0.425	0.461	0.502
	Unknown	0.211	0.177	0.241	0.200	0.162	0.219	0.241	0.228	0.204	0.187

4.2 Fungal Community Diversity; Compositional Structure; and Community Similarity

In both years, the fungal alpha diversity indices in all treatments were calculated including observed ASV richness, Shannon, Simpson and Inverse-Simpson diversity indices. The Shannon index is a measure of alpha diversity that measures both the richness and evenness of fungal species in a community, while the Simpson index is another alpha diversity metric that focuses on the dominance of fungal species in a community.

In our analysis, we performed an analysis of variance (ANOVA) to examine the differences in Shannon and Simpson alpha diversity indices among different tillage treatments. The alpha-diversity indices of the soil fungal community were not significantly affected by tillage practice in the first year of soil sampling (Shannon; F=1.83 P=0.142, Simpson; F=1.107 P=0.347) (Table 4.2, Figure 4.7A). Although no significant (P > 0.05) differences were detected among the different tillage practices, the highest average fungal alpha diversity (based on Observed, Shannon, and Simpson alpha diversity) was predicted for VT in the first year of soil sampling.

Table 4. 2. Results from separate analysis of variance (ANOVA) models for each alpha diversity measure (Observed, Shannon, Simpson) across different treatment variables in 2021. Each column represents the outcomes of individual ANOVA tests, where the factors examined include Tillage Practices (T), Soil Fraction, Crop in Rotation (R), and the interaction of Tillage Practices and Crop in Rotation (T × R). Degrees of freedom (DF), F-values, and P-values are provided for each factor, indicating the statistical significance of their effects on alpha diversity indices in the first year of soil sampling.

	First Year						
		Observed		Shannon		Simpson	
Variables	DF	F-value	*P-value*	F-value	*P-value*	F-value	*P-value*
Tillage Practice (T)	3	0.997	0.395	1.83	0.142	1.107	0.347
Soil Fraction	2	483.9	<2e-16***	208.7	<2e-16***	79.38	<2e-16***
Crop in Rotation (R)	2	1.925	0.148	6.991	0.001**	3.578	0.0293*
T × R	6	0.218	0.971	0.555	0.765	1.207	0.303

The ANOVA results showed that soil fungal alpha diversity differed significantly among soil fractions in 2021 (Shannon; F=208.7 P=2e-16, Simpson; F=79.38 P=2e-16). Specifically, the particulate organic matter fraction exhibited significantly lower fungal diversity compared to the small (SSA) and large soil aggregates (LSA) (Shannon and Simpson; P<0.0001). Additionally, the SSA showed slightly higher fungal diversity compared to the LSA (Shannon P=0.01, Simpson P=0.3), with a difference of 0.17 for Shannon index (95% CI: Shannon; 0.02 to 0.3) (Figure 4.7B).

The results also revealed significant differences in both Shannon and Simpson alpha diversity indices among the plots in different fields with their associated crops in 2021. For the Shannon index, the ANOVA test indicated a statistically significant difference among the plots in different fields (F=6.991, P=0.001). Tukey's HSD tests for pairwise comparisons further elucidated the differences: the plots in the corn field exhibited significantly lower Shannon diversity compared to the plots in the canola field (P=0.016) and significantly higher diversity compared to the plots in the soybean field (P=0.001). However, there was no significant difference in the Shannon diversity between plots in the canola and soybean field (P=.804). Similarly, for the Simpson index, the ANOVA test revealed a significant difference among plots in different fields (F= 3.578, P=0.029). Tukey's HSD tests for pairwise comparisons showed that plots in the corn field had a slightly lower Simpson diversity compared to plots in the canola field, although this difference was not statistically significant (P=0.219). Additionally, there was no significant difference in Simpson diversity between plots in the canola and soybean fields (P=0.659). However, Simpson diversity of plots in the soybean field was significantly higher compared to plots in the corn field (P=0.024) (Table 4.2, Figure 4.7C). Thus, according to both Shannon and Simpson indices, plots in the soybean field were predicted to have the highest average fungal richness in the first year of soil sampling.

In addition, both Shannon and Simpson diversity were not significantly affected by the interaction of tillage and crop rotation in 2021 (Shannon; F=0.555 P=0.765, Simpson; F=1.207 P=0.303).

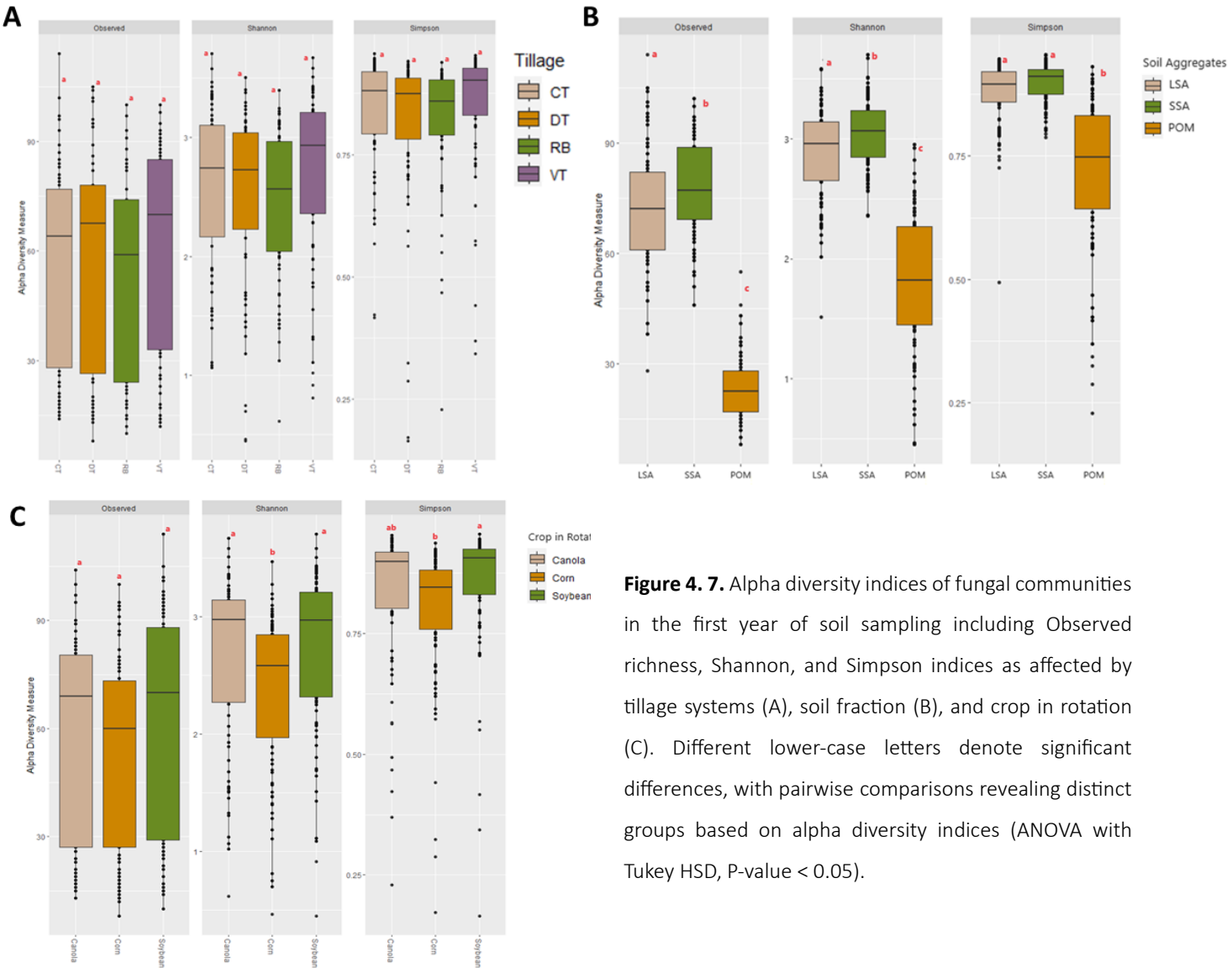

Figure 4. 7. Alpha diversity indices of fungal communities in the first year of soil sampling including Observed richness, Shannon, and Simpson indices as affected by tillage systems (A), soil fraction (B), and crop in rotation (C). Different lower-case letters denote significant differences, with pairwise comparisons revealing distinct groups based on alpha diversity indices (ANOVA with Tukey HSD, P-value < 0.05).

In 2022, we also evaluated fungal alpha diversity among different tillage systems including observed richness, Shannon, Simpson, and Inverse-Simpson diversity indices (Table 4.3). The alpha diversity indices of the soil fungal community revealed there was an increase in soil fungal richness in 2022 when compared 2021. Although fungal alpha diversity indices varied across all tillage systems in the second year, however, soil fungal community was not significantly affected by all four tillage systems (Shannon; F=0.791 P=0.501, Simpson; F=0.509 P=0.677). In addition, the highest average fungal richness (based on all evaluated alpha diversity indices) was predicted for CT in the second year of soil sampling (Figure 4.8A).

Furthermore, the results of the ANOVA test revealed significant differences in all diversity indices among the soil fractions in the second year (Shannon *F*=7.699, *P*=0.0006; Simpson *F*=5.362, *P*=0.0057) (Table 4.3). The Tukey's post-hoc tests results also showed that particulate organic matter exhibited significantly lower fungal diversity compared to the large and small soil aggregates (Shannon; *p*= 0.01, Simpson; *P*=0.01). Additionally, there was a small difference (Shannon; *diff*=0.1 *P*=0.69, Simpson; *diff*=0.001 *P*=0.99) between large and small soil aggregates, supporting that both Shannon and Simpson alpha diversity indices were not significantly affected in large soil aggregates and small soil aggregates (Figure 4.8B).

Table 4. 3. The table presents results from separate analysis of variance (ANOVA) models for each alpha diversity measure (Observed, Shannon, Simpson) across different treatment variables in 2022. Each column represents the outcomes of individual ANOVA tests, where the factors examined include Tillage Practices (T), Soil Fraction, Crop in Rotation (R), and the interaction of Tillage Practices and Crop in Rotation (T × R). Degrees of freedom (DF), F-values, and *P-values* are provided for each factor, indicating the statistical significance of their effects on alpha diversity indices in the second year of soil sampling.

	Second Year						
		Observed		Shannon		Simpson	
Variables	DF	F-value	*P-value*	F-value	*P-value*	F-value	*P-value*
Tillage (T)	3	1.327	0.268	0.791	0.501	0.509	0.677
Soil Fraction	2	3.682	0.0276*	7.699	0.000***	5.362	0.0057**
Crop in Rotation (R)	2	4.547	0.122*	1.191	0.307	0.027	0.973
T × R	6	0.138	0.9910	0.340	0.914	0.621	0.714

Furthermore, the ANOVA results revealed that alpha diversity measured using both Shannon and Simpson index did not show significant differences among the plots in different fields with their associated crops in 2022 (Shannon *F*=1.19, *P*=0.307; Simpson *F*=0.027, *P*=0.973) (Figure 4.8C). In addition, Shannon and Simpson diversity was not significantly affected by interaction of tillage and crop rotation in 2022 (Shannon; *F*=0.340 *P*=0.914, Simpson; *F*=0.621 *P=0.*714).

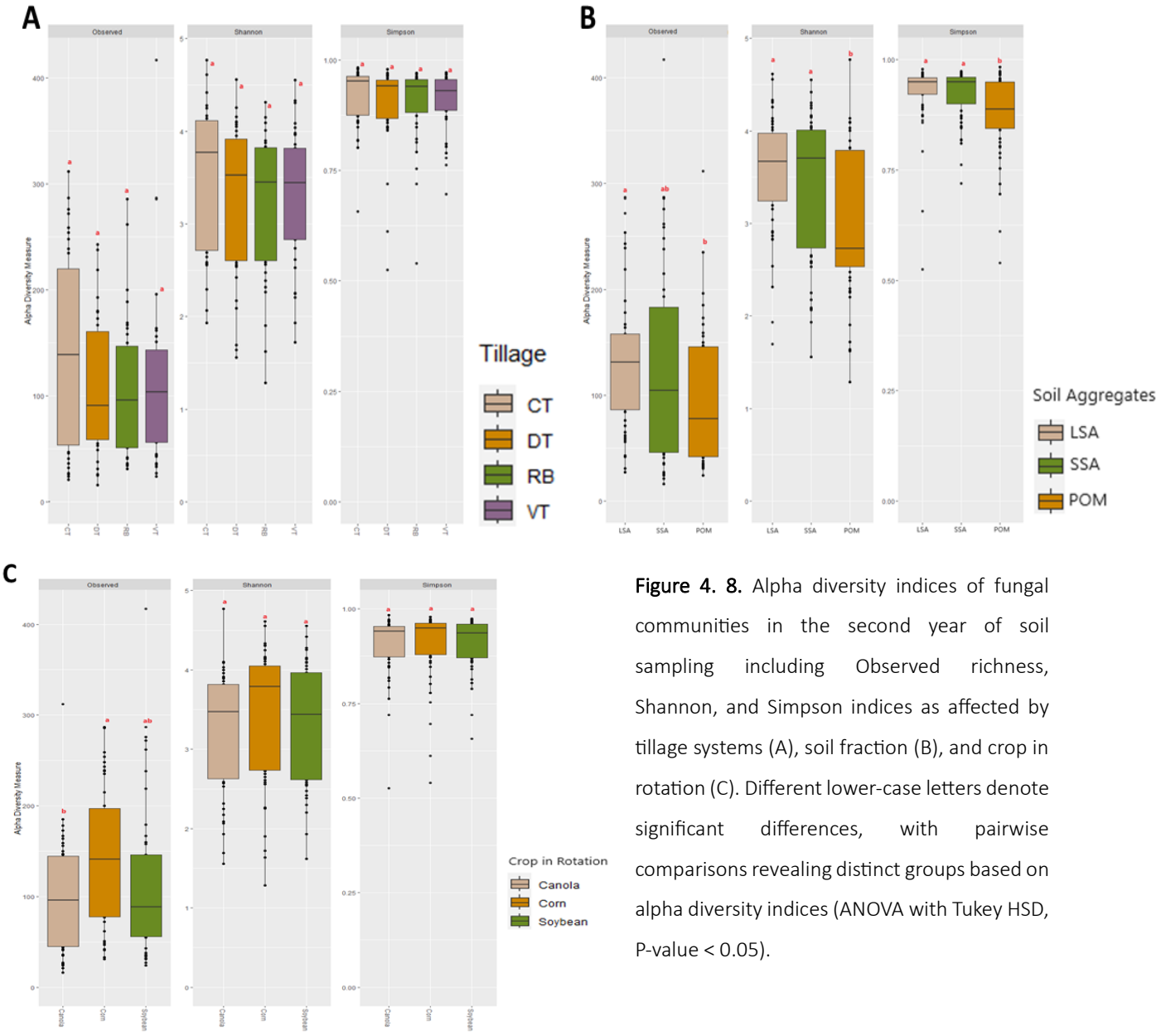

Figure 4. 8. Alpha diversity indices of fungal communities in the second year of soil sampling including Observed richness, Shannon, and Simpson indices as affected by tillage systems (A), soil fraction (B), and crop in rotation (C). Different lower-case letters denote significant differences, with pairwise comparisons revealing distinct groups based on alpha diversity indices (ANOVA with Tukey HSD, P-value < 0.05).

Non-metric multi-dimensional scaling (NMDS) was used to illustrate the clustering of fungal community composition (β diversity) for all crop rotation, tillage systems, and soil fractions in both years of soil sampling. Although the NMDS results indicated distinct groupings of soil fungal community composition among plots within the field and soil fractions, they exhibited clustering among different tillage treatments in the first year of soil sampling. The PERMANOVA results showed that soil fungal community similarities were not significantly affected by tillage in 2021 (F=0.97, P=0.476; Figure 4.9A). However, soil fractions (Figure 4.9C, F=36.65, P=0.001) and crop

rotation (Figure 4.9E, F=12.51, P=0.001) in 2021 showed a significant effect on fungal community dissimilarities in the first year of soil sampling. Samples from LSA and SSA are clustered close to each other on the NMDS plot indicating high fungal community similarity, while the POM samples showed a higher dispersion on the NMDS bi-dimensional space (Figure 4.9B).

Furthermore, we conducted a Canonical Analysis of Principal coordinates (CAP) to explore the compositional differences among three distinct soil fractions: LSA, SSA, and POM. The CAP analysis in 2021 revealed that distinct soil fractions exhibited dissimilar compositional patterns, as indicated by the direction of arrows in the CAP plot (Figure 4.10A). SSA and LSA displayed arrows pointing in different directions, suggesting a dissimilarity between these two soil aggregates. Meanwhile, POM exhibited an arrow with opposite direction that was distinct from both SSA and LSA further emphasizing its unique compositional profile. Expanding the CAP analysis to crop rotation for the year 2021 indicated that the fungal community compositions associated with plots within corn, canola, and soybean field are indeed significantly distinct (Figure 4.10C F=11.54, P=0.001). The CAP results for all tillage systems also showed that the fungal community composition did not exhibit statistically significant differences among tillage systems (Figure 4.10E F=0.97, P=0.466).

The results of NMDS based on the Bray-Curtis dissimilarity matrix in 2022 showed that soil fractions clustered in significantly different groups (PERMANOVA F=3.91, P=0.001). Samples from LSA and SSA were clustered more closely to each other on NMDS plot indicating high fungal community similarity in these soil aggregates, while the POM samples showed a higher dispersion on the NMDS bi-dimensional space (Figure 4.9D). In addition, The NMDS plot showed a separate grouping of the fungal community composition for crop in rotation in 2022. In this year, samples from plots within the corn, canola, and soybean fields were clustered in three significant groups (Figure 4.9F, PERMANOVA F=4.37, P=0.001). Soil samples from plots within the corn and canola field were clustered closely compared to the soybean on the NMDS plot indicating a higher fungal community similarity.

Furthermore, the results PERMANOVA showed that soil fungal community structure was not significantly affected by tillage (F=1.10, P=0.238). The NMDS based on the Bray-Curtis dissimilarity

matrix showed that samples from different tillage systems were clustered closely and not significantly differentiated (Figure 4.9B).

The ANOVA results for CAP analysis showed that in the second year, SSA and LSA displayed arrows pointing in different directions, suggesting a dissimilarity between these two soil aggregates. In addition, POM exhibited a distinct opposite direction from both SSA and LSA further emphasizing its unique compositional profile. These results confirmed how the different soil fractions impact the fungal community structure and indicated the significant dissimilarity among the two soil aggregate types (LSA and SSA) and particulate organic matter (Figure 4.10B, F=3.8979, p<0.001). Expanding the CAP analysis to crop rotation for year 2022 indicated that the fungal community compositions associated with each crop, including corn, canola, and soybean, were also significantly distinct (Figure 4.10D, F=4.345, P<0.001). The CAP plot for all four tillage systems illustrated their influence on fungal community composition (Figure 4.10F) The CAP results for all tillage systems also showed that in 2022 no statistically significant difference in the fungal community composition across all tillage systems (F=0.974, P=0.458).

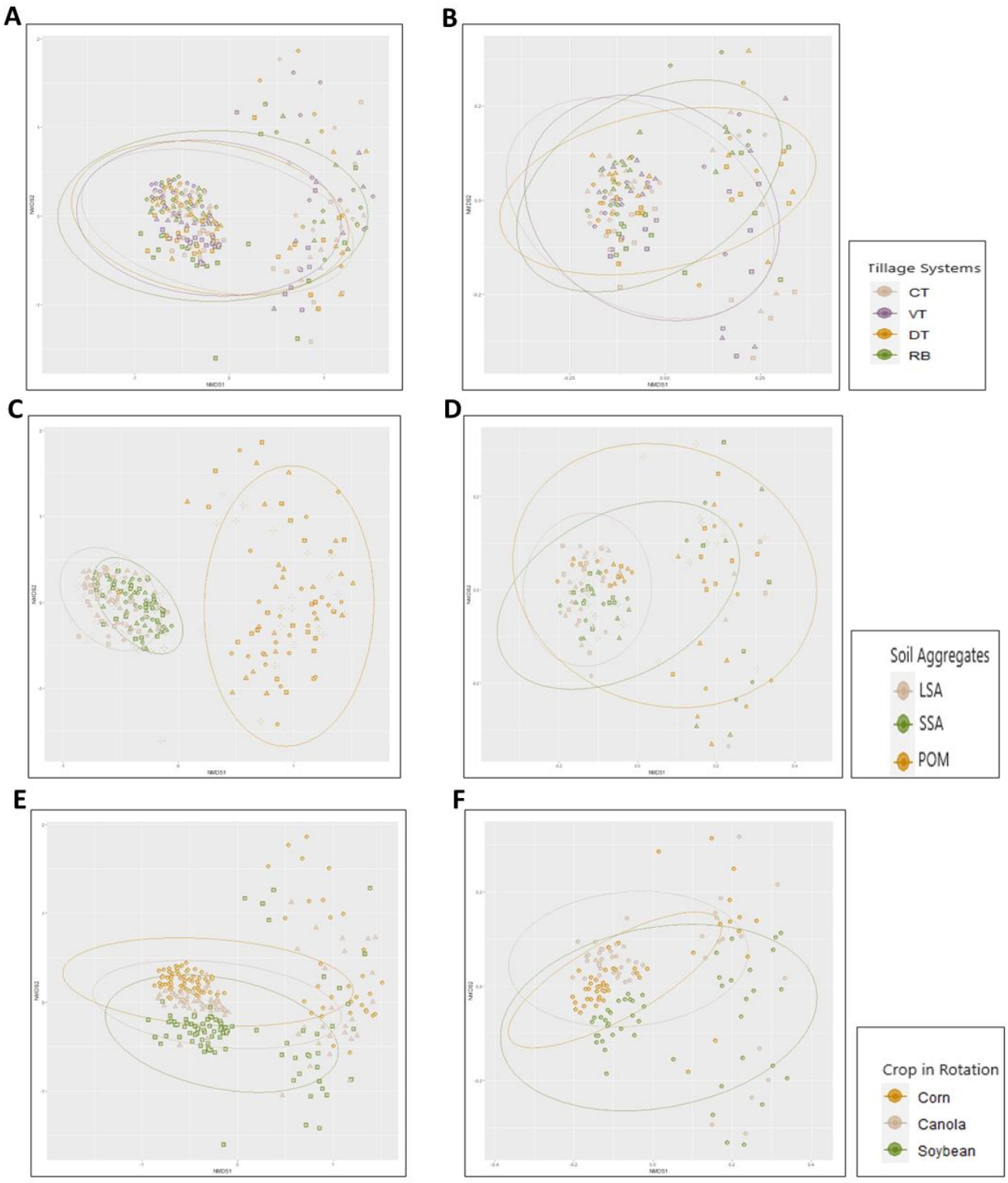

Figure 4. 9. NMDS plots indicating microbiome structural differences by treatment for **(A,B)** tillage practices (**C,D**) soil aggregates, and (E,F) crop in rotation in 2021 and 2022, respectively. Each point represents a single sample, and the ellipses encompass the centroid of each treatment group with a standard error margin. Colors denote tillage practice and shapes denote crop types in panel A and B. In panels C and D colors denote soil aggregates and shapes denote tillage practices, and in E and F both colors and shapes denote crop types.

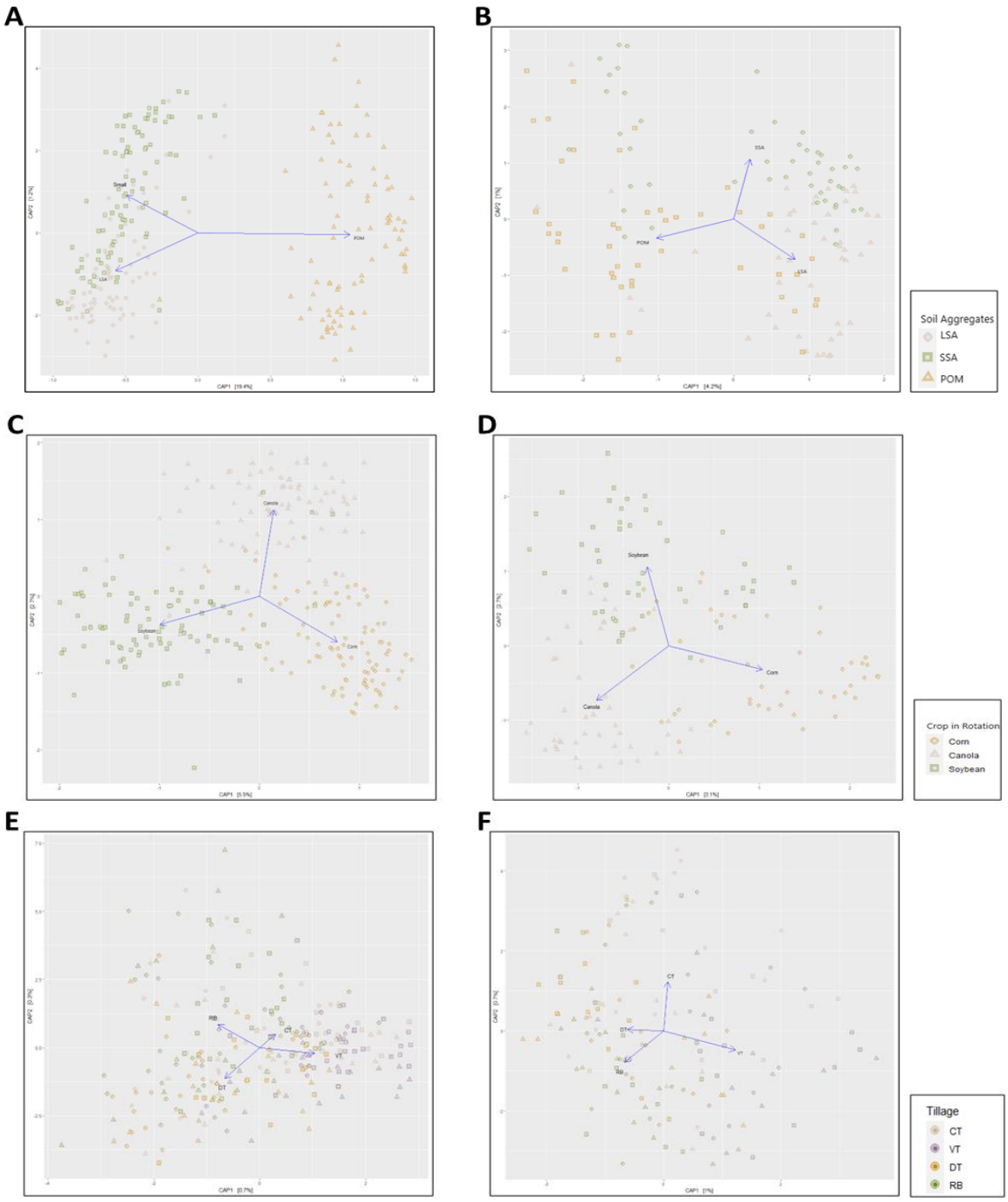

Figure 4. 10. CAP analysis of fungal community composition in different soil aggregates, crop types, and tillage systems in soil sampling in 2021 (A,C,E) and 2022 (B,D,F). Arrows in the CAP plot represent sample variations in the fungal community composition associated with four distinct tillage systems (E,F) crop types in rotation (C,D) and soil aggregates (E,F). The length and direction of the arrows reflect the extent and direction of impact each treatment has on the fungal community structure. Each point represents a single sample. For each treatment group a unique fungal community composition is exhibited (tillage; $P > 0.05$, crop types in rotation; $P < 0.05$, soil aggregates $P < 0.05$). Colors and shapes denote soil aggregates (A,B) and crop types and (C,D). Colors denote tillage systems and shapes denote crop types in rotations (E,F).

4.3 Fungal Functional Grouping

FUNGuild (https://github.com/UMNFuN/FUNGuild) was used to taxonomically parse fungal ASVs into functional groups based on their anticipated roles within ecosystems. In 2021, a total of 1062 fungal ASVs were classified into three functional trophic groups in FUNGuild; 427 ASVs were classified as saprotrophs, 94 ASVs as pathotrophs, and 100 ASVs as symbiotrophs. The Venn-analysis of these predictions revealed that some ASVs exhibited multiple functional traits, blurring the lines between these trophic modes. For instance, a set of 188 ASVs were classified as both pathotrophs and saprotrophs, highlighting their potential dual roles in the ecosystem. Additionally, 138 ASVs (approximately 7.5% of the total) exhibited all three trophic functions, indicating their capacity to play diverse roles in the ecosystem (Figure 4.11A).

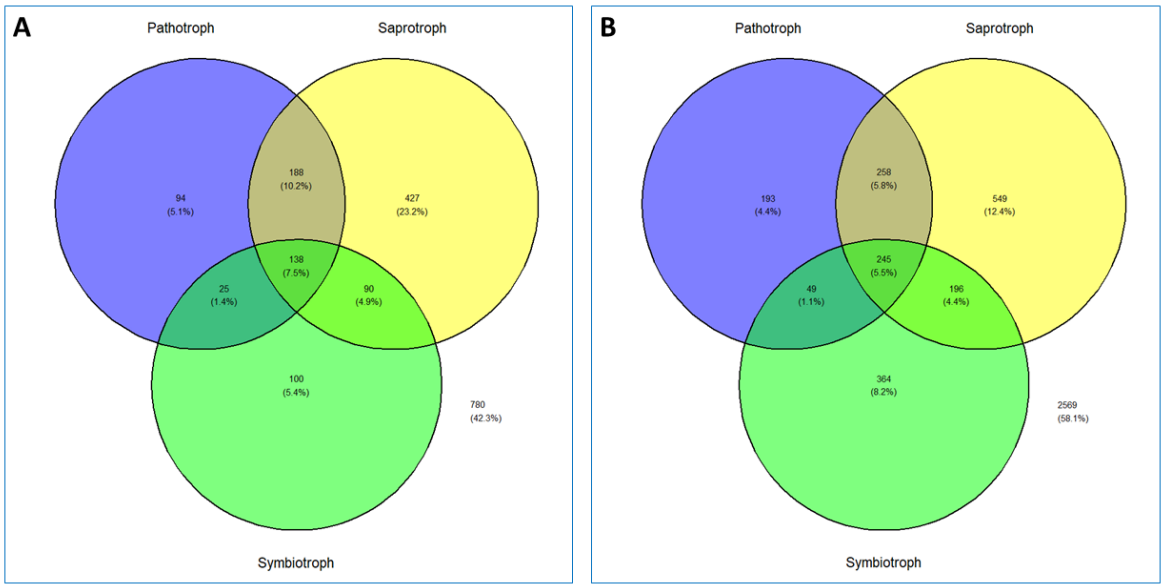

Figure 4. 11. Venn Diagram illustrates the fungal ASVs functional groups in 2021 (A) and 2022 (B) based on their anticipated trophic roles within ecosystems.

The results of functional guild groups in FUNGuild in 2021 revealed that in plots within the field planted to canola had a higher number of pathotrophic ASVs (28% of total ASVs), symbiotrophic ASVs (20.6% of total ASVs) and saprotrophic ASVs compared to the plots within corn and soybean. More details of the number of ASVs corresponding to each functional trophic mode are presented in Table 4.4.

Among the four implemented tillage systems in 2021, CT and VT systems had a higher number of pathotrophic ASVs (CT accounted for 27.9% and VT accounted for 26.9% of total ASVs). A higher number of saprotroph-ASVs was also recorded in the soils under the CT and VT systems. In addition, VT systems had the higher number of symbiotrophic ASVs (20.9% of total ASVs) compared to the other three tillage systems (Table 4.4). Fungal pathotrophic ASVs were lower in POM compared soil aggregates. The higher number of symbiotrophic ASVs were recorded in LSA (21.6% of total ASVs) compared to other soil fractions (Table 4.4).

Table 4. 4. Distribution of amplicon sequence variants across trophic modes among the treatments in 2021. The table presents the counts of ASVs classified into pathotroph (P), saprotroph (S), and symbiotroph (M) categories, as well as the counts of shared ASVs among these modes for each treatment (crop in rotation, tillage, and soil aggregates).

Treatments	Variables	Pathotroph (P)	Saprotroph (S)	Symbiotroph (M)	P+S	P+M	S+M	P+S+M
Rotation	Corn	42	210	44	92	7	37	69
	Canola	62	208	63	102	16	46	79
	Soybean	45	206	13	102	16	46	73
Tillage	CT	49	199	21	91	15	46	69
	DT	41	198	27	91	14	39	70
	VT	47	204	54	106	14	45	77
	RB	49	177	35	88	10	40	69
Aggregates	LSA	65	251	74	96	19	62	91
	SSA	66	259	45	127	16	56	79
	POM	11	131	6	83	6	14	49

The Venn diagram provided a visual representation of the distribution of ASVs in plots within the three distinct crop fields in 2021: corn, canola, and soybean (Figure 4.12A). A total of 348 ASVs were identified in plots within the corn field, 513 ASVs in plots within the canola field, and 398 ASVs were identified in plots within the soybean field. Additionally, the Venn-diagram of distribution of ASVs in plots within the field showed the abundance of the shared ASVs, highlighting the microbial communities common to more than one crop. Notably, a substantial intersection of 271 ASVs was observed in all three fields. Moreover, a total of 124 ASVs were detected in plots within both corn and canola, 84 ASVs common to plots within canola and soybean field, and 89 ASVs shared to plots within corn and soybean field.

The functional guild groups in FUNGuild results showed the presence of well-known plant pathogens within this shared microbial pool including *Fusarium* spp., *Alternaria* spp., *Plectosphaerella* spp., *Verticillium* spp., and *Bipolaris* spp. We also identified the presence of fungi

with biocontrol potential in the shared ASVs microbial pool such as *Funneliformis* spp., *Dominikia* spp., and *Trichoderma* spp. In plots within the canola field, 259 were identified as pathotrophic using functional trophic grouping in FUNGuild (Figure 4.12A). These ASVs included several well-known plant pathogens such as *Fusarium* spp., *Alternaria* spp. and *Verticillium* spp. Furthermore, in plots within the canola field, 47 ASVs also encompassed notable arbuscular mycorrhizal fungi (AMF) belonging to the Glomeraceae family, such as *Funneliformis* spp. (Schübler and Walker, 2010), *Glomus* spp. (Mathimaran et al., 2005), *Rhizophagus* spp. (Rashad, et al., 2020), *Dominikia* spp. (Błaszkowski et al., 2016), and *Kamienskia* spp. (Błaszkowski et al., 2016).

In plots within the corn field, out of a total of 832 identified ASVs, 210 ASVs were identified as pathotrophic. These ASVs included several well-known plant pathogens such as *Ustilago* spp., *Penicillium* spp., *Fusarium* spp. Furthermore, there were 29 ASVs classified as mycorrhizal fungi, with some of the most well-known ones being *Funneliformis* spp., *Rhizoglomus* spp., and *Kamienskia* spp. In plots within the soybean field, from a total 842 identified ASVs, 236 ASVs were classified as pathotrophic (Figure 4.12A). Notably, among these ASVs, *Fusarium* spp., *Bipolaris* spp., and *Septoria* spp. stood out as well-known plant pathogens in plots within the soybean field. Additionally, *Funneliformis* spp., *Dominikia* spp., and *Kamienskia* spp. were the well-known AMFs in plots within the soybean field.

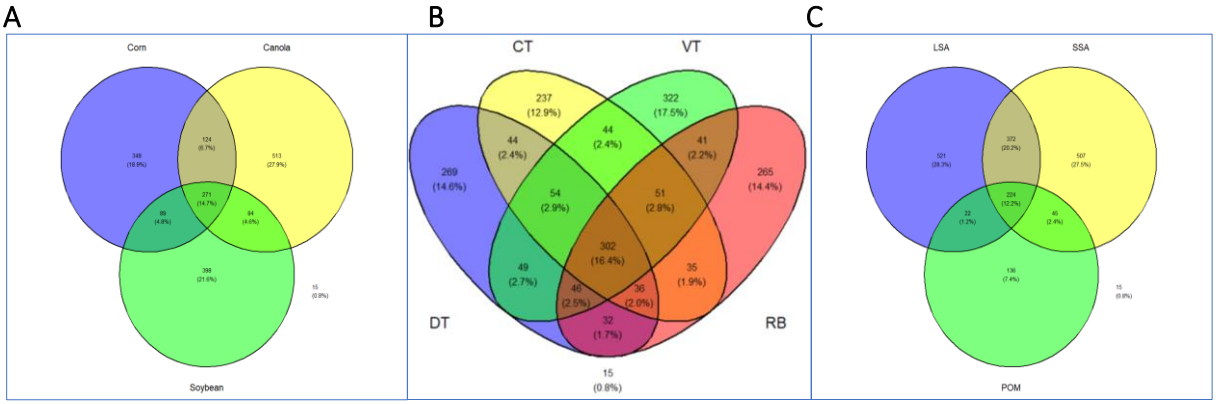

Figure 4. 12. Venn diagrams illustrating the distribution of ASVs within the crop involved in rotation (A), tillage treatment (B) and soil fractions (C) in the first year of soil sampling.

The Venn diagram presented a clear depiction of the distribution of ASVs among functional guilds within distinct tillage systems in 2021: DT, CT, VT, and RB (Figure 4.12B). Each tillage system harbors a different number of ASVs representing their fungal microbial community. The Venn diagram also

demonstrates the presence of shared ASVs among these tillage systems. Notably, there were 302 shared ASVs among all four tillage systems. Using the functional trophic grouping in FUNGuild showed the presence of well-known plant pathogens within the tillage shared ASVs including *Fusarium* spp., *Alternaria* spp., and *Ustilago* spp.

In VT systems, the identified pathotrophic ASVs included several well-known plant pathogens such as *Fusarium* spp., *Alternaria* spp. and *Verticillium* spp. and *Corynespora* spp. Additionally, 36 ASVs also encompassed notable AMF belonging to the Glomeraceae family including, *Funneliformis* spp., *Dominikia* spp., and *Kamienskia* spp. and *Rhizoglomus* spp. In soils under the CT system, the pathotrophic ASVs included several well-known plant pathogens such as *Fusarium* spp., *Plectosphaerella* spp., and *Verticillium* spp. In addition, *Funneliformis* spp., and *Kamienskia* spp. were the only two AMFs in the soils under the CT system. In soils under the DT system, the well-known plant pathogens included *Fusarium* spp., *Alternaria* spp. *Bipolaris* spp., *Ustilago* spp., and *Paraphoma* spp. In addition, 20 ASVs also encompassed AMF including *Glomus* spp., *Funneliformis* spp., and *Kamienskia* spp. In soils under the RB system, the well-known plant pathogens included *Fusarium* spp., *Alternaria* spp. and *Bipolaris* spp. Additionally, the functional trophic grouping FUNGuild revealed the presence of 23 ASVs identified as AMF, which included genera such as *Dominikia* spp., *Claroideoglomus* spp. and *Funneliformis* spp.

In addition, the Venn diagram presented the distribution of identified ASVs within each of the three soil fractions: LSA (521 ASVs), SSA (507 ASVs), and POM (136 ASVs) in 2021 (Figure 4.12C). The Venn diagram also reveals the presence of shared ASVs among these soil aggregates. Notably, a total of 224 shared ASVs were identified, comprising ASVs common to all three soil aggregates. Moreover, a total of 372 ASVs were detected in both LSA and SSA, with 45 ASVs common to SSA and POM, and 22 ASVs shared between LSA and POM.

The analysis of trophic modes using FUNGuild in 2021 revealed there were several well-known plant pathogens including *Fusarium* spp., *Alternaria* spp., *Verticillium* spp., *Ustilago* spp., *Bipolaris* spp., *Penicillium* spp., *Diaporthe* spp., and *Fusicolla* spp. Additionally, AMF in LSA included *Funneliformis* spp., *Glomus* spp., *Rhizophagus* spp., *Dominikia* spp., and *Kamienskia* spp. Among these ASVs within the SSA, we also identified several important plant pathogenic ASVs included

Fusarium spp., *Alternaria* spp., *Verticillium* spp., *Ustilago* spp., *Bipolaris* spp., *Penicillium* spp., and *Corynespora* spp. Furthermore, mycorrhizal fungi were also identified within SSA with a total of 111 ASVs related to this group. Notably, among these mycorrhizal fungi, prominent members include *Funneliformis* spp., and *Kamienskia* spp. FUNGuild analysis revealed among the ASVs identified in POM plant pathogenic ASVs included *Fusarium* spp., *Alternaria* spp., *Verticillium* spp., *Gaeumannomyces* spp., *Phaeosphaeria* spp., *Penicillium* spp., and *Corynespora* spp. Additionally, among these mycorrhizal fungi, *Rhizophagus* spp. was the only AMF detected in POM in 2021.

We also employed the FUNGuild tool to classify fungal ASVs obtained from soil samples collected in 2022 into functional groups, according to their expected ecological roles (Figure 4.11B). In 2022, 1854 fungal ASVs were sorted into three distinct functional trophic categories using FUNGuild. The Venn-analysis of these predictions showed that certain ASVs displayed multiple functional traits, blurring the lines between these trophic modes. Interestingly, 364 ASVs exhibited all three trophic functions, indicating their capacity to play diverse roles in the ecosystem (Figure 4.11B).

In 2022, plots within the corn field had a higher number of pathotroph-specific ASVs (19.3% of total ASVs) compared to the plots within canola and soybean. In plots within the corn field, we also observed a higher number of symbiotrophs-specific ASVs compared to the plots within canola and soybean.

Among the four implemented tillage systems in 2022, DT and VT systems had a higher number of pathotrophic ASVs (DT accounted for 20.6% and VT accounted for 19.3% of total ASVs). A higher number of saprotroph-specific ASVs was also recorded in the soils under the DT (34.7% of total ASVs) and CT (32.3% of total ASVs) systems. In addition, DT and CT systems had a greater number of symbiotrophs-specific ASVs. Furthermore, a lower number of pathotroph, saprotroph and symbiotroph-specific ASVs was found in POM compared to the soil aggregates. In 2022, LSA showed a higher number of these specific ASVs compared to the SSA (Table 4.5).

The Venn diagram provided a visual representation of the distribution of ASVs in plots within the three distinct crop fields in 2022: corn, canola, and soybean (Figure 4.13A). The Venn-diagram of distribution of ASVs in plots within all field showed their shared ASVs, highlighting the microbial communities common to more than one crop. Notably, a substantial intersection of 428 ASVs

(approximately 6.5% of total ASVs in 2022) was observed in plots of all three fields. The functional analysis in FUNGuild showed the presence of well-known plant pathogens within these shared ASVs including *Fusarium* spp., *Alternaria* spp., *Rhizoctonia* spp., *Verticillium* spp., and *Bipolaris* spp. We also identified the presence of fungi with biocontrol potential within the shared ASVs such as *Rhizophagus* spp., *Dominikia* spp., and *Kamienskia* spp.

Table 4. 5. Distribution of amplicon sequence variants across trophic modes among the treatments in 2022. The table presents the counts of ASVs classified into pathotroph (P), saprotroph (S), and symbiotroph (M) categories, as well as the counts of shared ASVs among these modes for each treatment (crop in rotation, tillage, and soil fraction).

Treatments	Variables	Pathotroph (P)	Saprotroph (S)	Symbiotroph (M)	P+S	P+M	S+M	P+S+M
Rotation	Corn	110	300	223	135	29	124	149
	Canola	73	233	73	98	23	92	112
	Soybean	77	225	172	149	28	85	117
Tillage	CT	71	249	136	126	23	90	106
	DT	95	265	171	123	28	115	124
	VT	93	222	124	119	29	96	108
	RB	79	226	123	111	24	82	96
Soil Fraction	LSA	110	285	234	127	27	114	133
	SSA	102	278	173	154	25	110	129
	POM	71	272	103	132	27	106	129

Out of 1622 ASVs in plots within the canola field, 306 were identified as pathotrophic using the functional guild groups in FUNGuild. Among these ASVs several well-known plant pathogens were identified including *Fusarium* spp., *Alternaria* spp. and *Rhizoctonia* spp. Furthermore, among the ASVs identified in plots within the canola field, 54 ASVs also encompassed notable AMF belonging to the Glomeraceae family, such as *Funneliformis* spp., *Microdominikia* spp., *Rhizophagus* spp., *Dominikia* spp., and *Kamienskia* spp.

In plots within the corn field, out of a total of 2354 ASVs, 423 ASVs were identified as pathotrophic. These ASVs included several well-known plant pathogens such as *Ustilago* spp, *Diaporthe* spp., *Fusarium* spp., *Alternaria* spp., and *Verticillium* spp. Furthermore, there were 192 ASVs classified as AMF, with some of the most well-known ones being *Atractiella* spp. (Bonito et al., 2016), and *Kamienskia* spp. In plots within the soybean field, from a total of 1930 ASVs, 236 ASVs were classified as pathotrophic. Notably, among these ASVs, *Fusarium* spp., *Bipolaris* spp., *Diaporthe* spp., and *Stemphylium* spp. stood out as well-known plant pathogens in the soybean field.

Additionally, 149 ASVs were classified as AMF including *Funneliformis* spp., *Dominikia* spp., *Glomus* spp., *Rhizoglomus* spp., *Septoglomus* spp., *Rhizophagus* spp., and *Kamienskia* spp.

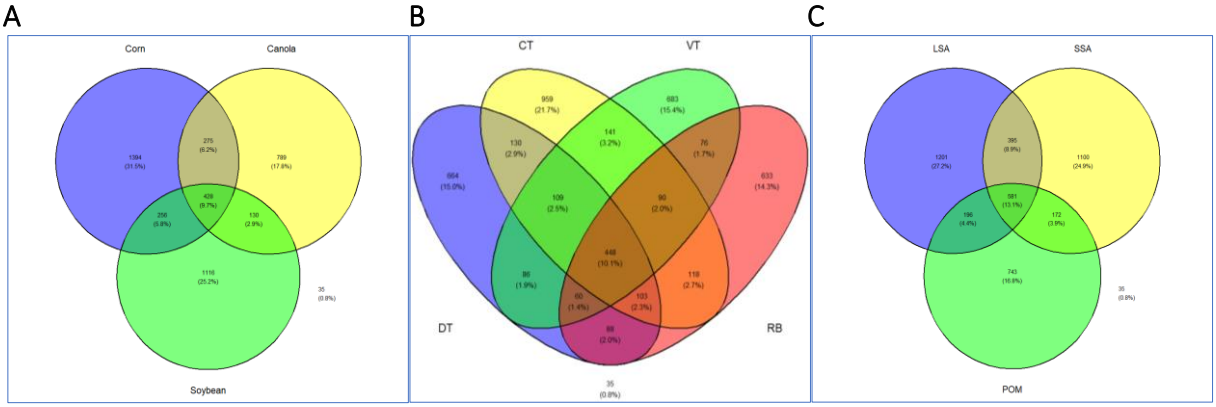

Figure 4. 13. Venn Diagram illustrates the distribution of ASVs within the crop involved in rotation (A), tillage treatment (B) and soil fraction (C) in the second year of soil sampling.

The Venn diagram presented a clear depiction of ASVs distribution within distinct tillage systems in 2022: DT, CT, VT, and RB (Figure 4.13B). Each tillage system harbors some ASVS that were not found in plots under the other tillage systems. The Venn diagram also demonstrates the presence of shared ASVs among these tillage systems. Notably, there were 448 shared ASVs among all four tillage systems. The functional trophic grouping in FUNGuild showed the presence of well-known plant pathogens within the tillage shared fungal ASVs including *Fusarium* spp., *Alternaria* spp., *Rhizoctonia* spp., *Stemphylium* spp., *Bipolaris* spp., and *Ustilago* spp.

In VT systems (1688 ASVs in total), the identified pathotrophic ASVs included several well-known plant pathogens such as *Fusarium* spp., *Alternaria* spp., *Bipolaris* spp., *Diaporthe* spp., and *Stemphylium* spp. Additionally, 102 ASVs also encompassed notable AMF belonging to the Glomeraceae family including, *Glomus* spp., *Dominikia* spp., and *Kamienskia* spp. and *Rhizophagus* spp. In the soil under the CT system (1694 ASVs in total), the pathotrophic ASVs included several well-known plant pathogens such as *Fusarium* spp., *Alternaria* spp., *Diaporthe* spp., and *Stemphylium* spp. In addition, *Dominikia* spp., *Rhizophagus* spp., and *Kamienskia* spp. were the well-known AMFs in the soils under the CT system. In DT systems (2100 ASVs in total), the well-known plant pathogens included *Fusarium* spp., *Alternaria* spp. *Cladosporium* spp., and *Ustilago* spp. In addition, out of the 178 ASVs, some also included AMF, with notable examples such as *Dominikia* spp. In RB tillage systems (1616 ASVs), several well-known plant pathogens were

identified such as *Fusarium* spp., *Alternaria* spp. *Diaporthe* spp., and *Bipolaris* spp. Additionally, the functional trophic grouping in FUNGuild revealed the presence of 105 ASVs identified as AMF, which some included genera such as *Dominikia* spp., *Glomus* spp. *Kamienskia* spp., and *Funneliformis* spp.

The Venn diagram also reveals the presence of shared ASVs among the soil fractions. Notably, there were 395 ASVs shared between LSA and SSA, 172 ASVs shared between SSA and POM, and 196 ASVs shared between LSA and POM. Moreover, a total of 581 shared ASVs were identified, comprising ASVs common to all three soil fractions. Additionally, using the functional trophic grouping in FUNGuild showed the presence of well-known plant pathogens that were shared among soil fractions, including *Fusarium* spp., *Alternaria* spp., and *Rhizoctonia* spp. In addition, there were 41 ASVs assigned to AMF in soil aggregates microbial pool including *Dominikia* spp., *Glomus* spp. and *Kamienskia* spp. Among the 2373 ASVs identified within LSA, an exploration of their ecological functions through FUNGuild revealed that 407 ASVs were ascribed pathotrophic functions, with some of the most well-known ones being *Fusarium* spp., *Alternaria* spp., *Rhizoctonia* spp., *Diaporthe* spp., *Ustilago* spp., *Bipolaris* spp., and *Verticillium* spp. Additionally, 361 ASVs were categorized as mycorrhizal fungi including, *Funneliformis* spp., *Glomus* spp., *Rhizophagus* spp., *Dominikia* spp., and *Kamienskia* spp.

Additionally, the analysis of trophic modes using FUNGuild revealed that within the SSA, out of a total of 2248 ASVs, 410 ASVs were associated with pathotrophic functions. Among these pathotrophic ASVs *Fusarium* spp., *Alternaria* spp., *Rhizoctonia* spp., *Diaporthe* spp., *Ustilago* spp., *Bipolaris* spp., and *Verticillium* spp. were the well-known plant pathogenic microorganisms identified within the SSA. Furthermore, mycorrhizal fungi were also identified within SSA with a total of 306 ASVs related to this group. Notably, among these mycorrhizal fungi, prominent members include *Glomus* spp., *Dominikia* spp., *Funneliformis* spp., and *Kamienskia* spp.

Furthermore, in POM, FUNGuild analysis revealed that out of a total of 1692 ASVs, 359 ASVs were associated with pathotrophic functions. Among these pathotrophic ASVs, *Penicillium* spp., *Alternaria* spp., *Cladosporium* spp., *Verticillium* spp., and *Fusarium* spp. were among the well-

known plant pathogenic microorganisms in POM. Additionally, among the 204 ASVs ascribed to the mycorrhizal fungi in POM, *Rhizophagus* spp. and *Glomus* spp. were the well-known AMF.

4.4 Differential Abundance of Significant Individual Fungal Taxa Across Treatments

The dataset was next analyzed for differences in abundance between individual fungal taxa using a negative binominal model (DESeq2). This analysis enhanced our understanding of how individual ASVs respond to experimental factors and offered a more comprehensive insight into the observed changes in the overall community structure within our dataset. To assess the quality and dispersion of the transformed count data, we first checked the relationship between mean expression patterns of ASVs and standard deviation in both years of soil sampling. There was a positive association between mean expression and standard deviation in both years of soil sampling showing that as mean expression levels increase, the variability in expression levels also tends to increase (Figure 4.14). In addition, the information from the mean-standard deviation plot was utilized to assess the stability of dispersion in the relative abundance of microbial taxa and the read counts of specific ASVs in the DNA sequencing data.

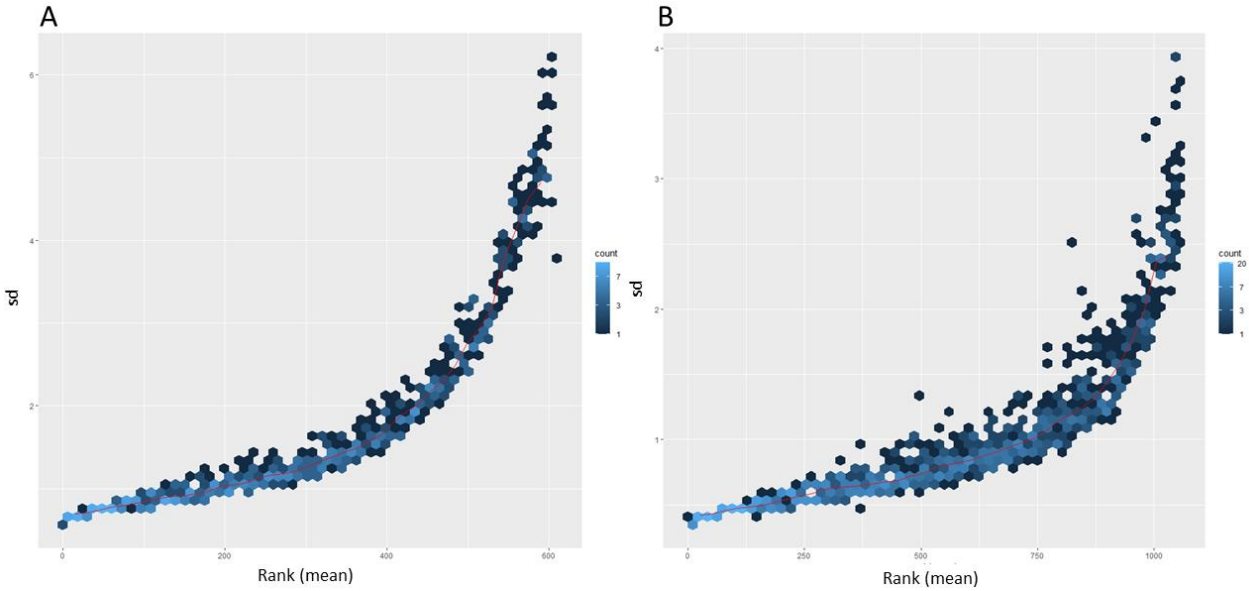

Figure 4. 14. Scatter plots showing the expression patterns of ASVs and their standard deviation in 2021 (A) and 2022 (B). Points represent taxa, and their position is determined by the average expression (x-axis) and the standard deviation (y-axis). The red line represents a positive relationship between the mean and standard deviation, the hexagons surrounding the red line donate different data points (taxa) and their color indicates the count of ASVs. The darker shades indicate a higher count of ASVs, while the lighter shades suggest a lower count.

4.4.1 Differential Abundances among Tillage Practices; 2021

Fungal taxa that exhibit differential abundance among the tillage systems could have significant implications for agriculture and soil fertility. The top five fungal ASVs whose relative abundance differed more significantly are presented in Table 4.6. In 2021, the abundances of 54 fungal ASVs spanning four phyla were significantly impacted by tillage systems. We employed a likelihood ratio test using DESeq2 for each pairwise contrast, and features with an adjusted p-value less than 0.05 were considered statistically significant, indicating differential abundance between the two tillage practices. The majority of affected ASVs by tillage systems belonged to the phylum Ascomycota and Basidiomycota, Chytridiomycota, and Rozellomycota

Table 4. 6. Results of differential abundance of the five top-most strongly affected taxa in 2021 among the tillage treatments (based on the Adjusted *P*). Statistical significance was established at a threshold of p<0.05, following adjustments for comparisons utilizing the Benjamini-Hochberg false discovery rate (BH-FDR). The threshold for log2 fold change (log2FC) was defined as 1.0, with lfcSE representing the standard error of log2FC.

Tillage Comparison; DT vs CT						
Base Mean	Log2FC	lfcSE	Adjusted *P*	Level 1; Phylum	Level 2; Family	Level 3; Genus
0.77	-29.60	3.90	1.50E-10	Ascomycota	Melanommataceae	*Herpotrichia*
10.42	-30	4.09	2.35E-10	Ascomycota	NA	NA
8.55	-29.95	4.09	2.35E-10	Ascomycota	Lasiosphaeriaceae	*Podospora*
0.11	-29.97	4.09	2.35E-10	Ascomycota	Phaeosphaeriaceae	*Phaeosphaeria*
8.96	-27.51	4.09	1.10E-08	Ascomycota	Pleosporaceae	*Alternaria*
Tillage Comparison; VT vs CT						
10.42	-30	4.01	2.89E-10	Ascomycota	NA	NA
7.30	-29.68	4.02	2.89E-10	Basidiomycota	Bolbitiaceae	*Panaeolus*
8.55	-29.31	4.01	3.73E-10	Ascomycota	Lasiosphaeriaceae	*Podospora*
1.03	-28.79	4.02	7.08E-10	Basidiomycota	Psathyrellaceae	*Coprinellus*
0.70	28.36	4.01	1.16E-09	Ascomycota	Melanommataceae	*Herpotrichia*
Tillage Comparison; RB vs CT						
0.50	-23.05	2.70	2.02E-13	Rozellomycota	NA	NA
8.55	-29.50	4.13	1.54E-09	Ascomycota	Lasiosphaeriaceae	*Podospora*
11.97	-24.52	3.51	4.68E-09	Ascomycota	Hypocreales_fam_Incertae_sedis	*Fusariella*
0.33	-26.75	4.12	6.75E-08	Ascomycota	Sporormiaceae	*Sporormiella*
2.66	-20.38	3.53	4.92E-06	Basidiomycota	Bulleribasidiaceae	*Vishniacozyma*

Table 4.6. continued. Results of differential abundance of the five top-most strongly affected taxa in 2021 among the tillage treatments (based on the Adjusted *P*). Statistical significance was established at a threshold of p<0.05, following adjustments for comparisons utilizing the Benjamini-Hochberg false discovery rate (BH-FDR). The threshold for log2 fold change (log2FC) was defined as 1.0, with lfcSE representing the standard error of log2FC.

Tillage Comparison; VT vs DT						
Base Mean	Log2FC	lfcSE	Adjusted *P*	Level 1; Phylum	Level 2; Family	Level 3; Genus
0.82	-45.18	4.06	1.03E-24	Basidiomycota	Psathyrellaceae	*Coprinellus*
0.147	33.25	4.06	6.70E-13	Ascomycota	Herpotrichiellaceae	*Exophiala*
2.74	30.91	4.067	3.79E-11	Ascomycota	Pezizaceae	NA
0.14	-29.79	4.06	2.20E-10	Ascomycota	Sporormiaceae	*Preussia*
0.44	27.80	4.06	5.32E-09	Ascomycota	Herpotrichiellaceae	*Exophiala*
Tillage Comparison; RB vs DT						
0.70	-41.93	4.17	6.93E-20	Ascomycota	Melanommataceae	*Herpotrichia*
0.45	-38.61	4.17	4.40E-17	Ascomycota	Phaeosphaeriaceae	*Neosetophoma*
0.41	-38.64	4.17	4.40E-17	Chytridiomycota	Rhizophlyctidaceae	*Rhizophlyctis*
1.03	36.59	4.17	2.42E-15	Basidiomycota	Psathyrellaceae	*Coprinellus*
0.14	36.15	4.17	4.68E-15	Ascomycota	Herpotrichiellaceae	*Exophiala*
Tillage Comparison; RB vs VT						
0.70	-47.78	4.10	2.12E-27	Ascomycota	Melanommataceae	*Herpotrichia*
0.82	44.12	4.10	2.07E-23	Basidiomycota	Psathyrellaceae	*Coprinellus*
0.41	-40.58	4.10	9.00E-20	Chytridiomycota	Rhizophlyctidaceae	*Rhizophlyctis*
1.032	38.09	4.10	2.10E-17	Basidiomycota	Psathyrellaceae	*Coprinellus*
11.97	-31.42	3.49	3.43E-16	Ascomycota	Hypocreales_fam_Incertae_sedis	*Fusariella*

4.4.1.1 Differential Abundance by Tillage; DT vs. CT in 2021

In total, the relative abundances of 28 ASVs were strongly affected between DT vs. CT. Details of the top five fungi whose relative abundance differed most strongly in soils between DT vs. CT in 2021 are presented in Table 4.6 Out of a total of 28 ASVs strongly affected in soils under DT vs. CT, the relative abundance of 13 ASVs categorized within the phylum Ascomycota, 14 ASVs within Basidiomycota, and 1 ASV within phylum Chytridiomycota.

Among ASVs identified as Ascomycota, *Alternaria* (fungal pathogen of cereal crops; Dettman et al., 2023) (LFC=-29.95, *P*=1.56E-12), *Phaeosphaeria* (fungal wheat pathogen; Stukenbrock et al., 2006) (LFC=-29.97, *P*=1.51E-12), *Herpotrichia* , (a fungal plant pathogen; Schneider et al., 2009) (LFC=-29.60, *P*=2.49E-13), and *Exophiala*, agents of human and animal mycoses (Zeng et al., 2007) (LFC=-19.87, *P*=4.11E-06) had significantly lower relative abundance in samples under DT vs. CT.

Among fungal ASVs identified in phylum Basidiomyota, *Exobasidium,* blister blight disease (Chaliha et al., 2020), (LFC=-22.65, *P*=1.26E-7), *Dioszegia*, basidiomycetous yeasts, (LFC=-17.62, *P*=4.94E-05), *Conocybe* (LFC=-23.47, *P*=4.08E-08) *Coprinellus* (LFC=-27.28, *P*=1.40E-10) and *Coprinopsis* (LFC=-25.87, *P*=1.28E-9) had significantly lower relative abundance in samples under the DT vs. CT in 2021.

Although the majority of the strongly differed ASVs in soils between DT vs. CT systems belonged to the phylum Ascomycota and Basidiomycota, one ASV belonging to phylum Chytridiomycota (Rhizophlyctidaceae: *Rhizophlyctis*) showed significant lower abundance in soil samples under the DT vs. CT in 2021 (LFC=21.43, *P*=6.06E-07) (Figure 4.15A, Table 4.6). Details of abundances of individual microbial taxa respond to soils under the DT vs. CT systems in 2021 are presented in Figure 4.15A.

4.4.1.2 Differential Abundance by Tillage; VT vs. CT in 2021

The abundances of 25 ASVs were significantly impacted by VT vs. CT practices (Figure 4.15B), including 11 ASVs categorized within the phylum Ascomycota, 13 ASVs within Basidiomycota, and 1 ASV within Chytridiomycota.

Among ASVs identified as Ascomycota, *Herpotrichia* (LFC=28.36, *P*=9.82E-12), *Exophiala* (LFC=14.09, *P*=0.001), and *Neosetophoma* (LFC=14.81, *P*=0.0005) had higher relative abundances

in soils under VT vs. CT in 2021. However, *Podospora* a saprobic filamentous ascomycete (Espagne et al., 2008) (LFC=-29.31, *P*=9.76E-13) and *Preussia* (LFC=-20.33, *P*=1.53E-06) had lower relative abundance under VT.

Among fungal ASVs identified in phylum Basidiomyota, the relative abundance of *Panaeolus* (LFC=-29.68, *P*=9.76E-13), *Coprinellus* (LFC=-28.79, *P*=4.78-E12), *Exobasidium* (LFC=-22.95, *P*=4.75E-08), and *Sphaerobolus* (LFC=-13.60, *P*=0.001) had lower relative abundance in the soil samples under the VT vs. CT in 2021.

In addition, *Rhizophlyctis* was also the only affected ASV from phylum Chytridiomycota detected in this contrast, with higher relative abundance in the soil samples under the VT vs. CT in 2021 (LFC=23.37, *P*=2.61E-08). Details of significantly affected ASVs in the soils under the VT practices compared to the CT practices in 2021 are presented in Figure 4.15B.

4.4.1.3 Differential Abundance by Tillage; VT vs. DT in 2021

Moreover, a total of 21 fungal ASVs were significantly affected in the soils between the VT vs. DT practices, including 11 ASVs affiliated with Ascomycota and 10 ASVs associated with Basidiomycota.

Within the Ascomycotan ASVs, the relative abundance of *Exophiala* (LFC=33.25, *P*=2.26E-15), *Herpotrichia* (LFC=23.76, *P*=4.59E-09), and *Phaeosphaeria* (LFC=22.05, *P*=2.31E-07) were significantly higher in soils under the VT than in soils under the DT practices. However, the relative abundance of *Preussia,* a dark septate endophyte (He et al., 2021), (LFC=-29.79, *P*=1.48E-12), and *Alternaria* (LFC=-22.67, *P*=1.00E-07), were significantly lower in VT soils compared to DT soils.

In addition, *Coprinellus* (LFC=-45.18, *P*=1.75E-27) and *Sphaerobolus* (LFC=-25.89, *P*=9.46E-10) were the most significant ASVs within the phylum Basidiomycota that showed a significantly lower relative abundance in VT compared to DT soils. However, the relative abundance of *Dioszegia* (LFC=25.49, *P*=1.72E-09) and *Coprinopsis* (LFC=23.72, *P*=2.35E-08) were significantly higher in soils under the VT vs. DT practices. Details of significantly affected ASVs in the soils under the VT vs. DT practices in 2021 are presented in Figure 4.15C.

4.4.1.4 Differential Abundance by Tillage; RB vs. CT in 2021

Comparing the soils under the RB practice with CT practices showed that 22 ASVs were significantly affected (Figure 4.15D). Interestingly, the fungus whose relative abundance differed most strongly between RB vs. CT was an unclassified taxon in phylum Rozellomycota (LFC=23.05, *P*=3.35E-16).

Within the Ascomycotan ASVs, the relative abundance of *Podospora* (LFC=-29.50, *P*=5.11E-12), *Fusariella* (LFC=-24.52, *P*=2.32E-11), *Sporormiella* (LFC=-26.75, *P*=4.47E-10), and *Neosetophoma* (LFC=-20.32, *P*=2.90E-06) was significantly lower in the soils under the RB practice vs CT. However, the relative abundance of *Exophiala (*LFC=21.10, *P*=1.13E-06), *Tetrapisispora* (LFC=19.34, *P*=8.93E-06), and *Pyxidiophora (*LFC=17.19, *P*=8.83E-05), an arthropod associated fungus (Blackwell and Malloch 1989), were significantly higher in RB compared to CT soils.

Within the Basidiomycotan ASVs, the relative abundance of *Coprinellus* (LFC=23.16, *P-value*=7.99E-08) was higher in soil samples under the RB vs. CT. However, the relative abundance of *Vishniacozyma* (LFC=-20.38, *P*=4.07E-08), and *Dioszegia* (LFC=-17.35, *P*=7.54E-5), psychrophilic basidiomycetous yeasts (Connell et al., 2010) were significantly lower in the soils under the RB vs. CT practice. Details of significantly affected ASVs in the soils under the RB practices vs. CT practices in 2021 are presented in Figure 4.15D.

4.4.1.5 Differential Abundance by Tillage; RB vs. DT in 2021

Furthermore, the fungi whose relative abundance differed significantly between RB vs. DT practices were 30 ASVs (Figure 4.15E). We also identified *Rhizophlyctis* as the third ASV that strongly affected soils between RB vs DT (details are presented in Table 4.6). In soil samples under the RB vs. DT in 2021, there were 15 ASVs categorized within the phylum Ascomycota, 13 ASVs within Basidiomycota, 1 ASV within phylum Chytridiomycota, and 1 ASV within Rozellomycota.

Within the Ascomycotan ASVs, *Herpotrichia* (LFC=-41.93, *P*=1.15E-22), *Neosetophoma* (LFC=-38.61, *P*=2.19E-19), a plant pathogen causing leaf spots (Phookamsak et al. 2014), and *Fusariella* (LFC=-23.41, *P*=3.05E-10) had lower relative abundance in the soils under the RB vs. DT in 2021. In addition, *Arthrobotrys*, a nematode-trapping fungus (Tunlid et al., 1994) also had lower relative abundance in RB vs. DT (LFC=-17.48, *P*=8.03E-05).

Within the Basidiomycotan ASVs, the relative abundance of *Coprinellus* (LFC=36.59, *P*=1.60E-17), *Coprinopsis* (LFC=35.96, *P*=5.45E-17), and Exobasidium (LFC=22.14, *P*=4.16E-07) was higher in soil samples under the RB vs. DT. However, *Vishniacozyma* (LFC=-27.26, *P*=1.89E-13) had a lower relative abundance in RB vs. DT.

Furthermore, the only ASV belonging to Chytridiomycota was identified as *Rhizophlyctis* (LFC=-38.64, *P*=2.01E-19) and exhibited a lower relative abundance in soil samples under RB vs. DT. A non-identified taxon within the Rozellomycota also had lower relative abundance in RB vs. DT (LFC=-20.66, *P*=6.66E-13). Details of significantly affected ASVs in the soils under the RB vs. DT practices in 2021 are presented in Figure 4.15E.

4.4.1.6 Differential Abundance by Tillage; RB vs. VT in 2021

Finally, comparing soils between RB vs. VT practices showed that a total 33 ASVs differed significantly in this contrast (Figure 4.15F), including 15 ASVs categorized within the phylum Ascomycota, 15 ASVs within Basidiomycota, 1 ASV within phylum Chytridiomycota, and 1 ASV within Rozellomycota.

Within the Ascomycotan ASVs, the relative abundance of *Alternaria* (LFC=24.69, *P*=2.39E-13) and *Preussia* (LFC=31.05, *P*=7.75E-09) were significantly higher under RB vs. VT, while *Herpotrichia* (LFC=-47.78, *P*=3.98E-30) and *Neosetophoma* (LFC=-35.14, *P*=8.82E-17) showed a significant lower relative abundance in soil samples under RB vs VT practice.

Among the ASVs identified as belonging to Basidiomycota, *Exobasidium* (LFC=22.45, *P*=1.73E-07), *Coprinellus* (LFC=-44.12, *P*=7.78E-26), and *Clitopilus* (LFC=29.06, *P*=7.91E-12) had significantly higher relative abundance under RB vs. VT. However, a lower relative abundance of *Vishniacozyma* (LFC=-28.35, *P*=6.07E-15) and *Dioszegia* (LFC=-25.21, *P*=3.57E-09) was recorded in RB vs. VT practice.

In addition, *Rhizophlyctis* (LFC=-40.58, *P*=5.07E-22) from phylum Chytridiomycota showed a significantly lower relative abundance in soils under RB vs. VT practice (Figure 4.15F). The only affected ASV belonging to phylum *Rozellomycota* was an unclassified taxon and was present as lower abundance in RB vs. VT (LFC=-18.23, *P*=1.48E-10). Details of significantly affected ASVs in the soils under the RB vs. VT practice in 2021 are presented in Figure 4.15F.

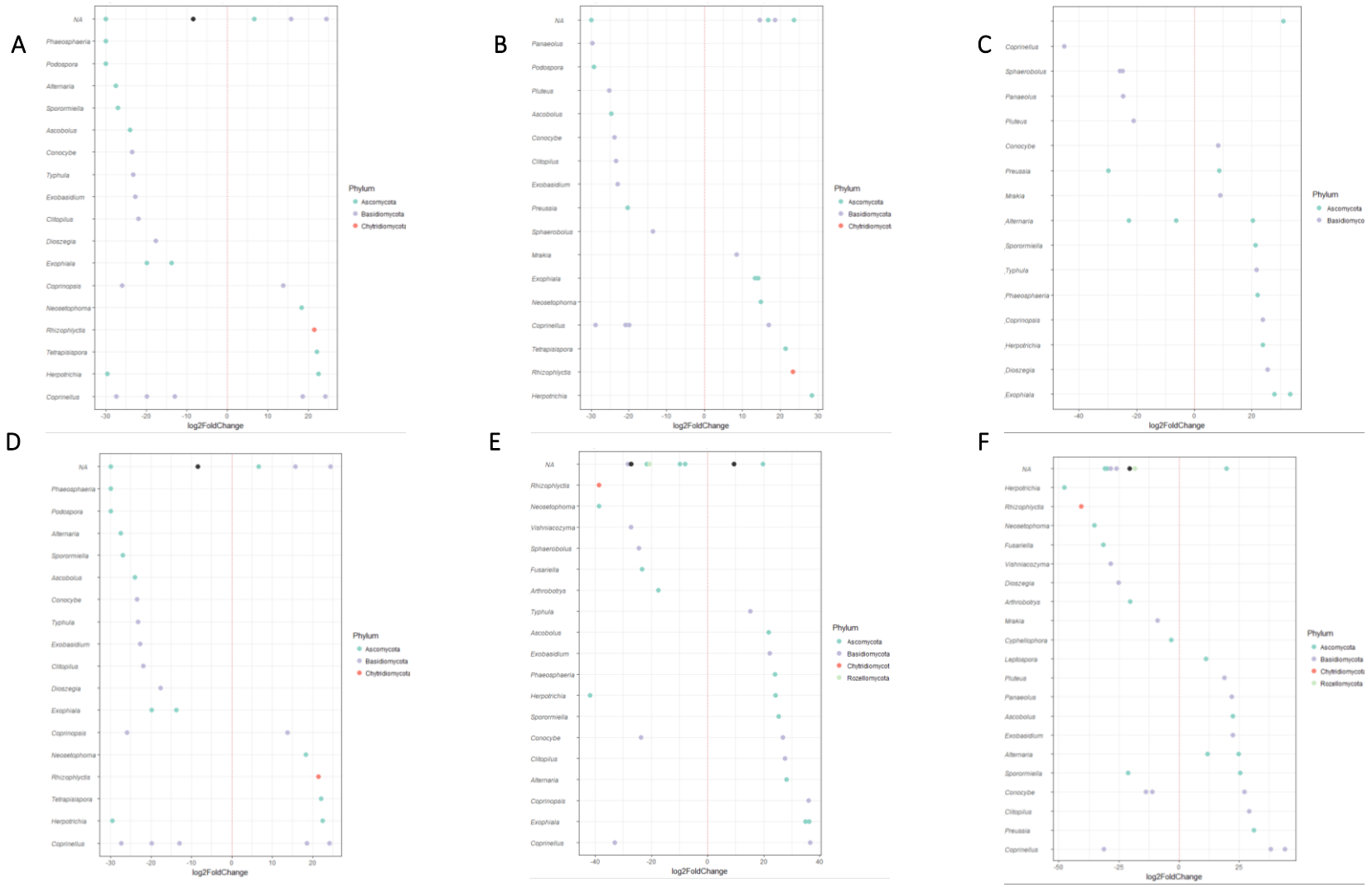

Figure 4. 15. Log_2 fold changes in the relative abundances of amplicon sequence variants (ASVs), in pairwise contrasts of the influence of tillage practices in the first year of soil sampling. ASVs are colour coded by phylum. ASVs that were significantly differentially abundant between A) deep tillage compared to conventional tillage, B) vertical tillage compared to conventional tillage, C) vertical tillage compared to deep tillage, D) raised-bed compared to conventional tillage, E) raised-bed compared to deep tillage, and F) raised-bed compared to vertical tillage. Adjusted P for significance < 0.05 and threshold for the log2FC was set at 1.0.

4.4.2 Differential Abundance among Crop Species in Different Fields; 2021

Differential abundance analysis clarified the responsiveness of individual ASVs to plots among the fields planted to corn, canola, or soybean. In 2021, the abundances of 107 fungal ASVs spanning four phyla were significantly impacted by crop rotation. The majority of ASVs affected by crop rotation belonged to the phyla Ascomycota, Basidiomycota, Chytridiomycota, and Mucoromycota. Table 4.7 shows the result of differential abundance of the five top-most strongly affected taxa in 2021 among plots within the different fields.

Table 4. 7. Five top-most strongly affected taxa in 2021 among plots within the different fields (based on the Adjusted *P*). Statistical significance was established at a threshold of p<0.05, following adjustments for comparisons utilizing the Benjamini-Hochberg false discovery rate (BH-FDR). The threshold for log2 fold change (log2FC) was defined as 1.0, with lfcSE representing the standard error of log2FC.

Field Comparison; Corn vs Canola						
Base Mean	Log2FC	lfcSE	Adjusted *P*	Level 1; Phylum	Level 2; Family	Level 3; Genus
11.32	25.22	1.34	3.67E-70	Ascomycota	Sporormiaceae	*Sporormiella*
24.25	-30	3.36	1.86E-15	Basidiomycota	Psathyrellaceae	*Coprinellus*
4.15	26.59	3.20	2.20E-13	Basidiomycota	Psathyrellaceae	*Coprinopsis*
1.03	29.93	3.64	2.20E-13	Basidiomycota	Psathyrellaceae	*Coprinellus*
0.57	-29.94	3.64	2.20E-13	Ascomycota	Pyxidiophoraceae	*Pyxidiophora*
Field Comparison; Canola vs Soybean						
5.46	24.65	1.16	4.26E-88	Ascomycota	Hyaloscyphaceae	*Cistella*
11.32	-22.66	1.28	1.24E-61	Ascomycota	Sporormiaceae	*Sporormiella*
1.93	26.35	1.55	4.01E-57	Basidiomycota	NA	NA
52.08	30	1.94	3.86E-48	Ascomycota	NA	NA
9.79	-25.31	1.86	8.77E-37	Ascomycota	Lasiosphaeriaceae	*Cercophora*
Field Comparison; Soybean vs Corn						
9.79	33.89	1.91	2.13E-63	Ascomycota	Lasiosphaeriaceae	*Cercophora*
5.46	-21.54	1.21	3.56E-62	Ascomycota	Hyaloscyphaceae	*Cistella*
2.56	-27.44	1.94	1.26E-39	Ascomycota	Helotiaceae	*Tetracladium*
10.42	43.91	3.56	2.98E-31	Ascomycota	NA	NA
26.57	-30.01	2.56	1.12E-27	Ascomycota	NA	NA

4.4.2.1 Differential Abundance by Crop Rotation; Corn vs. Canola in 2021

The comparison of fungal ASVs in plots between the corn field vs. canola field revealed that the relative abundances of 54 fungal ASVs differed significantly, including 31 ASVs affiliated with the phylum Ascomycota, 19 ASVs with the phylum Basidiomycota, and 2 ASVs with the phylum Chytridiomycota (Figure 4.16A).

Among the Ascomycotan ASVs, the relative abundance of plant pathogenic fungi such as *Herpotrichia* (LFC=27.65, *P*=1.71E-14), *Sporormiella*, causing leaf spot in corn (Do-Amaral et al., 2005) (LFC=25.22, *P*=6.74E-73), *Neosetophoma* (LFC=18.36, *P*=1.87E-6), *Preussia* (LFC=15.63, *P*=4.65E-12), and *Alternaria* (LFC=11.86, *P*=0.002) were significantly higher in plots from the corn field vs. plots from the canola field. However, the relative abundance of well-known plant pathogens in corn like *Fusarium,* causing ear rot disease (Thangaraj et al., 2023) (LFC=-3.27, *P*<0.001), *Paraphoma*, causing root rot (Cao et al., 2020; Liu et al., 2022) (LFC=-6.01, *P*<0.001), *Aspergillus*, causing ear rot disease (Masiello et al., 2023) (LFC=-8.09, *P*=5.78E-06), and *Penicillium*, causing mold in corn kernels (LFC=-3.05, *P*=5.80E-10) were significantly lower in plots from the corn field vs. plots from the canola field.

Among the Basidiomycotan ASVs, the relative abundance of *Coprinellus*, a lignin-degrading fungus in corn stalks (Yuchun et al., 2023) (LFC=29.93, *P*=2.02E-15), *Coprinopsis*, a litter-degrading fungus (Liu et all., 2022) (LFC=26.59, *P*=1.37E-15), *Dioszegia* (LFC=24.65, *P*=8.37E-11), and *Exobasidium* (LFC=14.88, *P*<0.001), were significantly higher in plots from the corn field vs. plots from the canola field. Importantly, ASVs with antagonistic ability against plant pathogens such as *Trichoderma*, a biocontrol agent of plant pathogens (Pollard-Flamand et al., 2022) (LFC=3.95, *P*<0.001), *Paraphaeosphaeria*, an endophytic biocontrol agent (Baroncelli et al., 2020) (LFC=3.36, *P*=7.87E-06), and *Talaromyces*, an antagonist of plant pathogens (Kazerooni et al., 2019) (LFC=3.53, *P*=1.06E-09) had significantly higher relative abundances among corn plots than among canola plots. *Rhizophlyctis*, the only ASV affiliated with Chytridiomycota, showed a significantly lower relative abundance (LFC=-4.51, *P*=4.18E-05) within the plots from the corn field vs. plots from the canola field.

4.4.2.2 Differential Abundance by Crop Rotation; Soybean vs. Corn in 2021

Comparison of the relative abundances of fungal ASVs between the plots in the soybean vs. in the corn field revealed significant differences for 63 fungal ASVs, including 37 ASVs affiliated with the phylum Ascomycota, 24 ASVs with the phylum Basidiomycota, and 2 fungal ASVs with the phylum Chytridiomycota (Figure 4.16B).

Within the fungal ASVs belonging to Ascomycota, the relative abundance of *Penicillium* (LFC=2.24, *P*=0.0001), *Preussia* (LFC=4.44, *P*=0.0001), *Bipolaris* (LFC=2.69, *P*=0.0019), and *Alternaria* (LFC=11.44, *P*=0.0010) were significantly higher in plots from the soybean field vs. the corn field. We also found that the relative abundance of *Neosetophoma* (LFC=-3.11, *P*=0.0002), *Sporormiella* (LFC=-25.98, *P*=2.35E-12), and *Exophiala* (LFC=-30.59, *P*=9.77E-17), all within the phylum Ascomycota, were significantly lower in plots from the soybean field vs. the corn field.

Among the Basidiomycotan fungal ASVs, the relative abundance of *Vishniacozyma*, an endophytic antagonist yeast (Nian et al., 2023) (LFC=-2.11, *P*=0.002) and *Coprinopsis* (LFC=-10.13, *P*=0.003) were significantly lower in plots from the soybean field vs. the corn field. In addition, *Coprinellus* (LFC=17.27, *P*=7.66E-07) and *Panaeolus* (LFC=39.29, *P*=5.92E-27) were the Basidiomycotan fungal ASVs that exhibited a significantly higher relative abundance in plots from the soybean field vs. the corn field in 2021.

Rhizophlyctis, the only ASV affiliated with Chytridiomycota, showed a significantly higher relative abundance (LFC=21.71, *P*=6.11E-09) within the plots from the soybean field vs. the corn field. Details of significantly affected fungal ASVs in plots of soybean vs corn field are presented in Figure 4.16B.

4.4.2.3 Differential Abundance by Crop Rotation; Canola vs. Soybean in 2021

The comparison of fungal ASV relative abundances in plots from the canola field vs. soybean field in 2021 revealed significant differences for 76 fungal ASVs, including 40 ASVs affiliated with the phylum Ascomycota, 29 ASVs with the Basidiomycota phylum, 2 fungal ASVs with the phylum Chytridiomycota and 1 fungal ASV with the Mucoromycota (Figure 4.16C).

Within the fungal ASVs belonging to Ascomycota, *Geosmithia* (LFC=-4.99, *P*=1.24E-18), *Verticillium* (LFC=-4.34, *P*=4.35E-16), *Preussia* (LFC=-5.23, *P*=1.06E-06), and *Alternaria* (LFC=-13.06, *P*=0.0001)

were among those plant pathogenic fungal ASVs that displayed significantly lower relative abundance among plots in the canola field vs. the soybean field. However, the relative abundance of other well-known plant pathogenic fungal ASVs displayed the opposite pattern, being present at higher relative abundances in the canola field than in the soybean field; these ASVs included *Herpotrichia* (LFC=12.00, *P*=4.93E-11), *Paraphoma* (LFC=4.94, *P*=3.91E-08), *Neosetophoma* (LFC=4.05, *P*=5.39E-08), *Phaeosphaeria* (LFC=19.85, *P*=5.58E-08), *Sporormiella* (LFC=13.03, *P*=0.0005), and *Aspergillus* (LFC=5.90, *P*=0.0009).

Among the Basidiomycotan ASVs, *Vishniacozyma* (LFC=29.07, *P*=2.79E-21) *Coprinellus* (LFC=29.84, *P*=9.45E-17), and *Conocybe* (LFC=29.83, *P*=9.64E-17) had significantly higher relative abundance in plots within the canola field vs. the soybean field. We also found that *Dioszegia* (LFC=-14.91, *P*=6.10E-05) and *Exobasidium* (LFC=-17.40, *P*=2.28E-06) were the two pathogenic fungal ASVs within the phylum Basidiomycota that had significantly lower relative abundance in plots within the canola field vs. the soybean field in 2021.

In addition, differential abundance analysis clarified that the relative abundance of the only ASV affiliated with Chytridiomycota, *Rhizophlyctis* (LFC=4.43, *P*=2.47E-05), was significantly higher in plots from the canola field vs. the soybean field. However, the only ASV affiliated with Mucoromycota, *Mucor* (LFC= -9.34, *P*=0.0005), exhibited a significant lower abundance within the plots from the canola field vs. the soybean field in 2021.

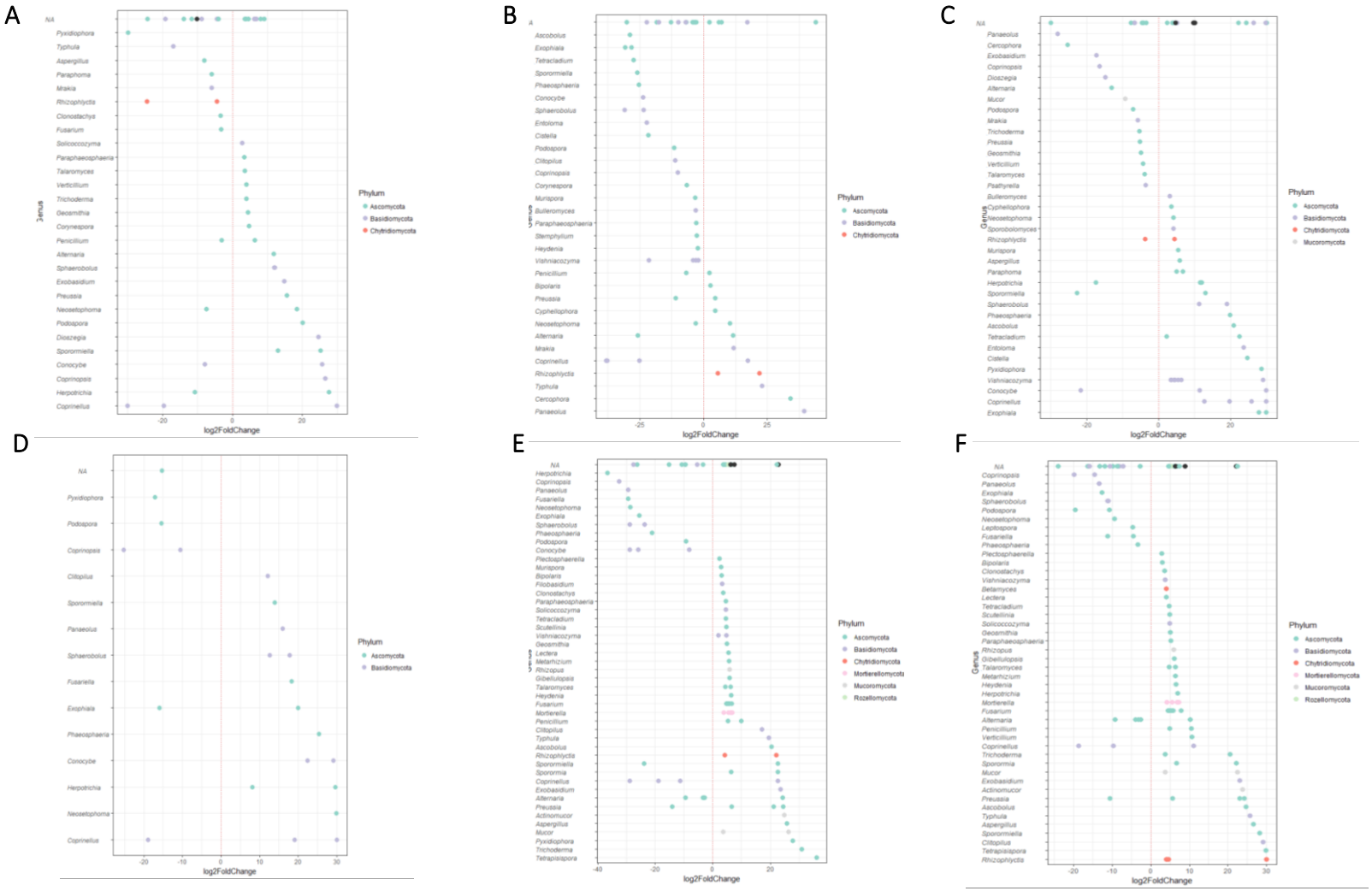

Figure 4. 16. Log_2 fold changes in the relative abundances of amplicon sequence variants (ASVs), in pairwise contrasts of the influence of crop in rotation and soil fractions in the first year of soil sampling. ASVs are colour coded by phylum. ASVs that were significantly differentially abundant between A) plots in the corn field compared to the canola field, B) plots in the soybean field compared to the corn field, C) plots in the canola field compared to the soybean field, D) large soil aggregates compared to small soil aggregates, E) small soil aggregates compared to particulate organic matter, and F) large soil aggregates compared to particulate organic matter. Adjusted P for significance < 0.05 and threshold for the log2FC was set at 1.0.

4.4.3 Differential Abundance among Soil Fractions; 2021

Differential abundance analysis clarified the responsiveness of individual ASVs to soil fractions in 2021. The abundances of 116 fungal ASVs spanning six phyla were significantly impacted by soil fraction. The majority of affected ASVs by soil fraction belonged to the phyla Ascomycota, Basidiomycota, Chytridiomycota, Mortierellomycota, Mucoromycota, and Rozellomycota. Table 4.8 shows the result of differential abundance of the five top-most strongly affected taxa in 2021 among soil fractions.

Table 4. 8. Results of differential abundance of the five top-most strongly affected taxa in 2021 among the soil fractions (based on the Adjusted *P*). Statistical significance was established at a threshold of p<0.05, following adjustments for comparisons utilizing the Benjamini-Hochberg false discovery rate (BH-FDR). The threshold for log2 fold change (log2FC) was defined as 1.0, with lfcSE representing the standard error of log2FC.

Soil Fraction Comparison; LSA vs SSA						
Base Mean	Log2FC	lfcSE	Adjusted *P*	Level 1; Phylum	Level 2; Family	Level 3; Genus
1.03	30	3.55	1.08E-13	Basidiomycota	Psathyrellaceae	*Coprinellus*
0.45	29.89	3.55	1.08E-13	Ascomycota	Phaeosphaeriaceae	*Neosetophoma*
0.70	29.59	3.55	1.44E-13	Ascomycota	Melanommataceae	*Herpotrichia*
4.22	29.09	3.55	3.30E-13	Basidiomycota	Bolbitiaceae	*Conocybe*
0.11	25.40	3.55	6.54E-10	Ascomycota	Phaeosphaeriaceae	*Phaeosphaeria*
Soil Fraction Comparison; SSA vs POM						
4.90	24.89	0.87	3.47E-163	Mucoromycota	Mucoraceae	*Actinomucor*
243.65	9.87	0.35	3.18E-133	Ascomycota	Aspergillaceae	*Penicillium*
28.01	6.69	0.28	1.74E-88	Mortierellomycota	Mortierellaceae	*Mortierella*
701.30	5.78	0.28	3.69E-63	Ascomycota	Nectriaceae	*Fusarium*
18.22	6.39	0.32	9.44E-59	Ascomycota	Sporormiaceae	*Sporormia*
Soil Fraction Comparison; LSA vs POM						
0.350	-24.03	3.57	1.56E-09	Ascomycota	NA	NA
29.03	-9.28	1.42	8.04E-08	Ascomycota	Pleosporaceae	*Alternaria*
133.3	-3.90	0.51	1.51E-07	Ascomycota	Pleosporaceae	*Alternaria*
52.08	-11.89	2.00	5.88E-07	Ascomycota	NA	NA
38.67	-9.88	1.64	7.13E-07	Ascomycota	NA	NA

4.4.3.1 Differential Abundance among Soil Fractions; LSA vs. SSA in 2021

Comparing LSA vs. SSA revealed that 22 fungal ASVs differed significanly in 2021, including 11 ASVs affiliated with the phylum Ascomycota, and 11 ASVs with the Basidiomycota phylum. Details of the top five fungi whose relative abundance differed most strongly in LSA vs. SSA. in 2021 are presented in Table 4.8.

Within the group of Ascomycotan fungal ASVs, *Neosetophoma* (LFC=29.89, *P*=4.45E-16), *Herpotrichia* (LFC=29.59, *P*=8.89E-16), and *Phaeosphaeria* (LFC=25.40, *P*=6.71E-12) exhibited a significant higher relative abundance in LSA vs. SSA, whereas the relative abundances of *Exophiala* (LFC=-15.90, *P*=2.78E-05), and *Pyxidiophora* (LFC=-17.06, *P*=6.26E-06) were significantly lower in LSA vs. SSA.

In addition, within the group of Basidiomycotan ASVs, *Coprinellus* (LFC=30.00, *P*=3.50E-16) and *Sphaerobolus* (LFC=17.71, *P-value*=2.62E-06) had significantly higher relative abundances in soils from LSA vs. SSA in 2021, while the relative abundance of *Coprinopsis* (LFC=-25.23, *P*=9.39E-12) was significantly lower in LSA vs. SSA. More details of significantly affected ASVs in the LSA vs SSA in 2021 are presented in Figure 4.16D.

4.4.3.2 Differential Abundance among Soil Fractions; SSA vs. POM in 2021

The analysis of differential abundance of fungal ASVs in SSA vs POM in 2021 showed that 95 ASVs were significantly affected. Among these fungal ASVs, 59 ASVs were identified in phylum Ascomycota, 21 ASVs in phylum Basidiomycota, 5 ASVs in phylum Mortierellomycota, 4 ASVs in phylum Mucoromycota, 2 ASVs in phylum Chytridiomycota, and 1 ASV in phylum Rozellomycota. Interestingly, the most significant fungal ASVs were identified in phylum Mucoromycota (*Actinomucor*). Details of the top five fungi whose relative abundance differed most strongly between SSA vs. POM in 2021 are presented in Table 4.8.

Within the Ascomycotan fungal ASVs, the relative abundance of several plant pathogens was significantly lower in SSA vs. POM. These plant pathogens included *Alternaria* (LFC=-2.86, *P*=0.0002), *Preussia* (LFC=-14.01, *P*=1.85E-10), *Sporormiella* (LFC=-23.85, *P*=7.81E-11), *Herpotrichia* (LFC=-36.54, *P*=5.54E-24), *Neosetophoma* (LFC=-28.65, *P*=3.52E-15), *Phaeosphaeria* (LFC=-21.14, *P*=9.98E-09), and *Exophiala* (LFC=-25.48, *P*=3.25E-12). However, importantly, the relative abundance of six plant pathogenic fungal ASVs were significantly higher within SSA vs.

POM: *Fusarium* (LFC=5.78, *P*=2.44E-65), *Gibellulopsis* (LFC=5.81, *P*=2.28E-56), *Penicillium* (LFC=9.87 , *P*=1.05E-135), *Plectosphaerella* (LFC=2.39 , *P*=6.93E-06), *Aspergillus* (LFC=25.77, *P*=2.71E-60), and *Bipolaris* (LFC=3.09, *P*=0.0001).

Moreover, in the Basidiomycotan group, the relative abundances of *Vishniacozyma* (LFC=1.99, *P*=0.006), *Coprinellus* (LFC=22.72, *P*=6.31E-10), *Clitopilus* (LFC=17.01, *P*=5.21E-06), and *Exobasidium* (LFC=23.48, *P*=1.58E-10) were significantly higher in soils from SSA vs. POM in 2021. We also found that *Sphaerobolus*, another plant pathogenic ASV from Basidiomycota, had significantly lower relative abundance in this contrast (LFC=-23.74, *P*=9.62E-11).

It is worth noting that fungal ASVs with biocontrol potential, such as *Talaromyces* (LFC=6.13, *P*=2.90E-25), *Metarhizium,* an endophytic plant pathogenic antagonism (Sasan and Bidochka 2013) (LFC=5.70, *P*=1.51E-16), *Pyxidiophora* (LFC=27.80, *P-value*=2.40E-14), and *Trichoderma* (LFC=30.98, *P-value*=1.33E-17) had significantly higher relative abundance in SSA vs. POM in the first year of soil sampling. We also found that *Mortierella,* a plant growth-promoting fungus (Ozimek and Hanaka 2020), was the only ASV identified in phylum Mortierellomycota, and its relative abundance was significantly higher in SSA vs. POM in 2021 (LFC=6.70, *P*=1.53E-45). Furthermore, *Rhizopus* (LFC=5.77, *P*=8.94E-22), *Actinomucor*, a fast-growing and enzyme-producing fungus (Kazerooni et al., 2022) (LFC=24.89, *P*=5.74E-166), and *Mucor,* a potential biocontrol agent (Nartey et al., 2022) (LFC=3.59, *P*=0.003) all had significantly higher relative abundance in SSA vs. POM in 2021. More details of significantly affected ASVs in the SSA vs POM in 2021 are presented in Figure 4.16E.

4.4.3.3 Differential Abundance among Soil Fractions; LSA vs. POM in 2021

The differential abundance comparison of fungal ASVs between the LSA vs. POM in 2021 showed that the relative abundances of 100 ASVs were significantly affected. Among these fungal ASVs, 65 ASVs were identified in phylum Ascomycota, 17 ASVs in phylum Basidiomycota, 5 ASVs in phylum Mortierellomycota, 5 ASVs in phylum Chytridiomycota, 4 ASVs in phylum Mucoromycota, and 1 ASV in phylum Rozellomycota.

Among the Ascomycotan fungal ASVs, the relative abundance of several plant pathogens was significantly higher in LSA vs. POM. These ASVs included *Penicillium* (LFC=10.47, *P*=4.11E-148),

Fusarium (LFC=5.79, *P*=2.56E-63), *Aspergillus* (LFC=26.63, *P*=2.09E-62), *Gibellulopsis* (LFC=6.11, *P*=3.28E-61), *Geosmithia* (LFC=5.01, *P*=7.18E-18), *Sporormiella* (LFC=28.24, *P*=2.52E-14), *Plectosphaerella* (LFC=2.81, *P*=7.89E-09), *Herpotrichia* (LFC=6.97, *P*=0.0005), *Bipolaris* (LFC=3.05, *P*=0.0002), and *Verticillium* (LFC=10.59, *P*=0.0007). Additionally, it is worth noting that within the Ascomycotan group, the relative abundance of several plant pathogenic fungal ASVs also were significantly lower in LSA vs. POM, including *Alternaria* (LFC=-2.66, *P*=6.80E-08), *Preussia* (LFC=-10.57, *P*=4.09E-06), *Neosetophoma* (LFC=-9.41, *P*=9.85E-05), *Phaeosphaeria* (LFC=-3.41, *P*=0.005), and *Exophiala* (LFC=-12.66, *P*=0.001).

Among the fungal ASVs belonging to the phylum Basidiomycota, *Exobasidium* was the only well-known plant pathogen that had significantly higher relative abundance in LSA vs. POM (LFC=23.08, *P*=6.67E-10). Also, *Vishniacozyma* was another fungal ASV within the Basidiomycotan group that had a significant higher relative abundance in soils from LSA vs. POM in 2021 (LFC=3.74, *P*<0.001). Conversely, *Coprinellus* (LFC=-9.74, *P*=0.008) and *Coprinopsis* (LFC=-14.57, *P*<0.0001) were two fungal ASVs in phylum Basidiomycota that demonstrated a significant lower relative abundance in LSA vs. POM in the first year of soil sampling.

Importantly, fungal ASVs with biocontrol potential had a significantly higher relative abundance in LSA vs. POM. These fungal ASVs included *Trichoderma* (LFC=3.79, *P*=0.0002), *Paraphaeosphaeria* (LFC=5.17, *P*<0.0001), *Talaromyces* (LFC=6.35, *P*<0.0001), and *Metarhizium* (LFC=6.35, *P*<0.0001). In addition, *Mortierella*, the only identified genus within the phylum Mortierellomycota, had significantly higher relative abundance in LSA vs. POM (LFC=7.24, *P*=8.29E-53). Furthermore, the relative abundance of *Rhizopus* (LFC=5.89, *P*=3.27E-22), *Actinomucor* (LFC=23.85, *P*=6.45E-146), and *Mucor* (LFC=22.53, *P*=6.47E-18) all were significantly higher in soils from LSA vs. POM. More details of significantly affected ASVs by LSA vs POM in 2021 are presented in Figure 4.16F.

4.4.4 Differential Abundance among Tillage Practices; 2022

In the second year of soil sampling, the relative abundances of 374 fungal ASVs spanning nine phyla were significantly impacted by tillage systems. The majority of affected ASVs by tillage systems in the second year belonged to the phylum Ascomycota and Basidiomycota. A smaller number of fungal ASVs within other phyla, such as Chytridiomycota, Mortierellomycota, Mucoromycota, Glomeromycota, Rozellomycota, Olpidiomycota, and Basidiobolomycota, were also affected by the tillage systems in 2022. The top five fungal ASVs whose relative abundance differed more significantly are presented in Table 4.9.

Table 4. 9. Differential abundance of the five top-most strongly affected taxa in 2022 among the tillage treatments (based on the Adjusted *P*). Statistical significance was established at a threshold of p<0.05, following adjustments for comparisons utilizing the Benjamini-Hochberg false discovery rate (BH-FDR). The threshold for log2 fold change (log2FC) was defined as 1.0, with lfcSE representing the standard error of log2FC.

Second Year of Soil Sampling						
Tillage Comparison; DT vs CT						
Base Mean	Log2FC	lfcSE	Adjusted *P*	Level 1; Phylum	Level 2; Family	Level 3; Genus
25.23	-29.99	2.11	1.06E-39	Ascomycota	Herpotrichiellaceae	*Exophiala*
3.38	-26.72	3.38	1.61E-11	Ascomycota	Pyronemataceae	*Heydenia*
4.27	-30	3.88	2.94E-11	Glomeromycota	Glomeraceae	NA
86.02	-30	4.10	1.15E-10	Ascomycota	NA	NA
17.26	-30	4.10	1.15E-10	Basidiomycota	Typhulaceae	*Typhula*
Tillage Comparison; VT vs CT						
1.89	-23.82	3.02	2.14E-11	NA	NA	NA
1.09	-29.60	3.77	2.14E-11	NA	NA	NA
17.26	-30	4.10	1.19E-10	Basidiomycota	Typhulaceae	*Typhula*
28.42	-30	4.10	1.19E-10	Basidiomycota	Exidiaceae	*Exidia*
7.33	-30	4.10	1.19E-10	Basidiomycota	NA	NA
Tillage Comparison; RB vs CT						
3.76	-24.17	2.70	1.27E-14	Basidiomycota	NA	NA
1.83	-28.48	3.68	5.00E-11	NA	NA	NA
17.26	-30	4.10	1.00E-10	Basidiomycota	Typhulaceae	*Typhula*
28.42	-30	4.10	1.00E-10	Basidiomycota	Exidiaceae	*Exidia*
18.39	-30	4.10	1.00E-10	Basidiomycota	Bolbitiaceae	*Conocybe*

Table 4.9 continued. Results of differential abundance of the five top-most strongly affected taxa in 2022 among the tillage treatments (based on the Adjusted P). Statistical significance was established at a threshold of p<0.05, following adjustments for comparisons utilizing the Benjamini-Hochberg false discovery rate (BH-FDR). The threshold for log2 fold change (log2FC) was defined as 1.0, with lfcSE representing the standard error of log2FC.

Tillage Comparison; VT vs DT						
Base Mean	Log2FC	lfcSE	Adjusted *P*	Level 1; Phylum	Level 2; Family	Level 3; Genus
25.23	32.22	2.11	2.23E-46	Ascomycota	Herpotrichiellaceae	*Exophiala*
4.95	-49.96	4.10	4.33E-30	Mortierellomycota	NA	NA
4.00	-45.58	4.10	5.54E-25	Glomeromycota	Glomeraceae	*Kamienskia*
0.67	-44.38	4.10	1.00E-23	NA	NA	NA
1.54	40.29	4.10	2.28E-19	Chytridiomycota	Alphamycetaceae	*Betamyces*
Tillage Comparison; RB vs DT						
1.54	46.98	4.10	3.90E-26	Chytridiomycota	Alphamycetaceae	*Betamyces*
1.47	44.93	4.10	4.61E-24	NA	NA	NA
4.68	-43.39	4.10	1.76E-22	Ascomycota	NA	NA
25.23	22.33	2.12	2.48E-21	Ascomycota	Herpotrichiellaceae	*Exophiala*
7.67	-39.86	4.09	4.90E-19	Basidiomycota	Ceratobasidiaceae	*Thanatephorus*
Tillage Comparison; RB vs VT						
470.43	-3.89	0.84	0.003317	Ascomycota	Microdochiaceae	*Microdochium*
582.82	31.17	4.10	4.93E-12	Ascomycota	Melanommataceae	*Herpotrichia*
4.67	-40.23	4.10	2.00E-19	Basidiomycota	Trichosporonaceae	*Cutaneotrichosporon*
212.92	-31.06	3.34	2.83E-17	Ascomycota	Cyphellophoraceae	NA
154.07	-14.35	2.59	2.32E-06	Ascomycota	Microascaceae	NA

4.4.4.1 Differential Abundance by Tillage; DT vs. CT in 2022

In 2022, the relative abundances of 187 ASVs were significantly impacted by the DT practice compared to the CT practice, including ASVs belonging to phylum Ascomycota (59 ASVs), Basidiomycota (38 ASVs), Glomeromycota (20 ASVs), Mortierellomycota (12 ASVs), Chytridiomycota (11 ASVs), Mucoromycota (4 ASVs), Olpidiomycota (1 ASV), Rozellomycota (1 ASV), and Basidiobolomycota (1 ASV).

There were several plant pathogenic ASVs within the phylum Ascomycota that had significantly higher relative abundances under DT than under CT in 2022. These ASVs included *Alternaria* (LFC=20.41, *P*=2.19E-06), *Diaporthe*, causing soybean stem canker (Mena et al., 2023) (LFC=17.73, *P*=4.50E-5), *Stemphylium* (LFC=26.09, *P*=1.28E-12), *Paraphoma* (LFC=12.18, *P*=0.0009), *Fusarium* (LFC=23.00, *P*=7.98E-08), and *Aspergillus* (LFC=12.51, *P*=0.005). Conversely, we also found that relative abundances of several plant pathogenic ASVs within the phylum Ascomycota were significantly lower under DT than under CT in 2022; including *Exophiala* (LFC=-89.99, *P*=1.01E-42), *Preussia* (LFC=-30.00, *P*=1.57E-12), *Plectosphaerella* (LFC=-24.73, *P*=7.48E09), *Gibellulopsis* (LFC=-23.80, *P*=2.81E-08), *Gaeumannomyces*, soil-borne pathogen of wheat (Zhao et al., 2023) (LFC=-17.73, *P*=1.21E-08), *Parastagonospora*, a necrotrophic pathogen of wheat (Kariyawasam et al., 2023) (LFC=-19.39, *P*=7.50E-06), *Geosmithia* (LFC=-5.06, *P*=0.002), and *Penicillium* (LFC=-10.23, *P*=0.002).

Interestingly, the relative abundances of some putatively beneficial fungal ASVs within the phylum Ascomycota were higher under DT than under CT in 2022, including *Phialocephala*, a dark septate endophyte (Grunig et al., 2008) (LFC=15.66, *P*=0.0003) and *Arthrobotrys* (LFC=13.33, *P*=0.002). However, the relative abundance of *Trichoderma* (LFC=-21.69, *P*=4.62E-07), and *Acremonium,* a biological agent against *Meloidogyne incognita* (Yao et al., 2023) (LFC=-29.83, *P*=2.15E-12) were significantly lower in soil samples under the DT vs. the CT system in 2022.

Among the Basidiomycotan ASVs that were responsive to this contrast, the relative abundances of some important plant pathogenic ASVs were higher under DT vs. CT; *Urocystis*, causing flag smut (Lal Kashyap et al., 2020) (LFC=17.96, *P*=3.55E-05) and *Thanatephorus*, a soybean blight pathogen (Kaur et al., 2022) (LFC=18.68, *P-value*=1.56E-05). However, the relative abundances of *Coprinopsis* (LFC=-26.70, *P*=3.56E-10) was significantly lower under the DT system vs. the CT system in 2022.

In addition, the relative abundance of fungal ASVs with biocontrol potential such as *Waitea*, a potential biocontrol mycorrhizal fungus (Carvalho et al., 2015) (LFC=17.80, *P*=4.22E-05) was higher. However, the relative abundance of *Vishniacozyma* (LFC=-28.45, *P*=2.25E-11) was significantly lower in soils under the DT vs. the CT system (Figure 4.17A).

We also identified several responsive fungal ASVs that were associated with the phylum Glomeromycota, which primarily consists of AMF. *Kamienskia*, known as AMF (Zhou, et al., 2023), was the only Glomeromycotan ASV with significantly higher relative abundance under the DT vs. the CT system (LFC=19.83, *P*=1.53E-12). However, other well-known Glomeromycotan fungal ASVs including *Rhizophagus*, known as AMF (Deja-Sikora et al., 2023) (LFC=-21.79, *P*=4.14E-07), *Dominikia*, known as AMF (Kaumbu et al., 2023), (LFC=-21.57, *P*=5.42E-07), and *Septoglomus*, known as AMF (Du et al., 2023) (LFC=-20.21, *P*=2.96E-06), had significantly lower relative abundances under the DT vs. the CT system. Details of relative abundances of individual microbial taxa that responded to DT vs. CT systems in the second year of soil sampling are presented in Figure 4.17A.

The relative abundance of *Olpidium* (LFC=21.99, *P*=3.09E-07), was higher in soils under DT vs. CT. In addition, the only ASV within the phylum Rozellomycota (an unclassified taxon) had lower relative abundance in DT vs. CT. The relative abundance of *Mortierella* was lower in soils under DT vs. CT in 2022 (LFC=-22.54, *P*=1.56E-07). Two ASVs belonging to Mucoromycota also were significantly impacted, with *Absidia* (LFC=-25.29, *P*=3.21E-09) showing a lower relative abundance in DT vs. CT while *Mucor* (LFC=18.49, *P*=2.04E-05) had higher relative abundance in soils under the DT vs. CT in 2022.

In general, the relative abundances of important plant pathogenic ASVs were greater in soil samples from DT vs. CT in 2022 (Table 4.4 and 4.5). It is important to highlight that some plant pathogens, like *Fusarium* and *Aspergillus*, which showed higher relative abundance in DT vs. CT in 2022, might actually be non-pathogenic fungi and have biocontrol potential against the plant pathogens. For example, various *Fusarium* species are known to effectively colonize plant roots without causing diseases (Benhamou and Grand 2001; Joshi et al. 2013; Reid et al. 2002; Silva and Wagner 2005). Non-pathogenic *Fusarium* species primarily colonize the root surface or outer root

tissues without inducing cell damage (Sajeena et al., 2020). Indeed, non-pathogenic and antagonistic *Fusarium* spp. serve as excellent examples of biocontrol agents inhibiting plant pathogens (Sajeena et al., 2020). Notably, our analysis also revealed a higher relative abundance of beneficial fungal ASVs within the Ascomycota in soils under DT compared to CT. Our findings from FUNGuild further supported an increased number of symbiotrophic ASVs in DT as opposed to CT (Table 4.4 and 4.5).

4.4.4.2 Differential Abundance by Tillage; RB vs CT in 2022

The differential abundance comparison of fungal ASVs between RB and CT practices showed that 99 fungal ASVs were significantly differentially abundant between these tillage treatments, including members of Ascomycota (62 ASVs) and Basidiomycota (37 ASVs).

Within the Ascomycotan fungal ASVs, the relative abundance of important plant pathogenic ASVs was significantly higher in soil samples under the RB vs. CT practices in 2022. These ASVs included *Herpotrichia* (LFC=17.86, *P*=3.99E-05), *Alternaria* (LFC=18.55, *P*=1.87E-05), *Diaporthe* (LFC=10.68, *P*=0.001), *Stemphylium* (LFC=24.22, *P*=5.30E-11), *Paraphoma* (LFC=19.31, *P*=8.08E-06), *Penicillium* (LFC=12.33, *P*=0.005), and *Fusarium* (LFC=19.46, *P*=6.78E-06). At the same time, the relative abundance of some other plant pathogens was significantly lower in RB vs. CT; *Preussia* (LFC=-25.37, *P-value*=2.91E-09), *Gaeumannomyces* (LFC=-22.45, *P*=1.74E-07), and *Gibellulopsis* (LFC=-23.34, *P*=5.28E-08).

Another important finding when we compared soils under the RB vs. CT practice was that the relative abundances of several fungal ASVs with biocontrol potential were significantly lower in RB; ASVs such as *Trichoderma* (LFC=-30.00, *P*=1.55E-12), *Metarhizium* (LFC=-24.20, *P*=1.59E-08), *Arthrobotrys* (LFC=-18.98, *P*=1.18E-05) are renowned for their exceptional biocontrol activity against soil-borne plant pathogens.

In addition, within the phylum Basidiomycota, the relative abundance of *Ustilago*, a plant pathogenic fungus causing corn smut (Yu et la., 2023), was significantly higher (LFC=29.61, *P-value*=3.12E-12) while the relative abundance of *Thanatephorus* (LFC=-30.00, *P*=1.56E-12) was significantly lower under the RB vs. CT practice in 2022. Furthermore, the relative abundances of *Dominikia* (LFC=-29.48, *P*=3.90E-12), *Rhizophagus* (LFC=-23.54, *P*=3.85E-08), and *Glomus,* an AMF

(Afrangan et al., 2023) (LFC=-24.24, *P*=1.48E-08) were significantly lower under the RB vs. CT practice. Details of abundances of individual microbial taxa respond to RB compared to the CT systems in the second year of soil sampling are presented in Figure 4.17B.

The relative abundance of *Olpidium* (LFC=-26.89, *P*=2.61E-10), was lower in soils under the RB vs. CT. In addition, the only ASV within the phylum Rozellomycota (an unclassified taxon) had lower relative abundance in this contrast (LFC=-24.38, *P*=1.20E-08). The relative abundance of *Mortierella* was lower in soils under the RB vs. CT in 2022 (LFC=-30.00, *P*=1.56E-12). The most significant impacted fungal ASV in phylum Mucoromycota was identified as *Mucor* and had significantly lower relative abundance in RB vs. CT (LFC=-29.98, *P*=1.61E-12).

In general, the soil samples from the RB treatment exhibited a higher relative abundance of crucial plant pathogenic ASVs compared to the CT in both 2021 and 2022. The analysis of functional trophic groups in FUNGuild for both years also indicated a greater number of pathotrophic ASVs in the soils under the RB (Table 4.4 and 4.5). Interestingly, in soils under the RB vs. CT practice, the relative abundance of beneficial fungi with biocontrol activity was significantly lower. This trend was particularly pronounced in 2022, where the relative abundance of AMF was significantly lower in soils under the RB vs. CT practice. Our analysis of functional trophic groups in FUNGuild during the second year further supported these findings, revealing a higher number of symbiotrophic ASVs associated with the soils under the CT compared to the RB system.

4.4.4.3 Differential Abundance by Tillage; VT vs. CT in 2022

Comparing soils under the VT vs. CT practices showed the relative abundance of 82 ASVs were significantly affected by this contrast in tillage practice, including members of Ascomycota (52 fungal ASVs) and Basidiomycota (30 fungal ASVs).

Within the Ascomycotan group, the relative abundance of six plant pathogenic fungi was higher in VT compared to the CT system. These ASVs included *Preussia* (LFC=5.88, *P*=0.004), *Alternaria* (LFC=15.29, *P*=0.0004), *Diaporthe* (LFC=18.82, *P*=5.97E-09), *Stemphylium* (LFC=22.32, *P*=1.72E-09), *Paraphoma* (LFC=15.51, *P*=0.0004), and *Fusarium* (LFC=24.36, *P*=1.18E-08). However, the relative abundances of *Corynespora* (LFC=-24.54, *P*=3.99E-12), *Penicillium* (LFC=-10.50, *P*=0.001), *Gibellulopsis* (LFC=-28.40, *P*=2.40E-11), *Verticillium* (LFC=-21.83, *P*=3.86E-07), *Bipolaris* (LFC=-

30.00, *P*=1.56E-12), and *Gaeumannomyces* (LFC=-21.63, *P*=4.72E-07) were significantly lower in VT vs. CT (Figure 4.17C). Interestingly, we found the relative abundance of *Chaetomium*, a versatile biological control agent for various plant pathogens (Ashwini 2019) was significantly lower in the soils under the VT vs. CT practice in 2022 (LFC=-26.82, *P*=2.96E-10).

Furthermore, among the Basidiomycotan fungal ASVs, the relative abundances of *Ustilago* (LFC=-21.73, *P-value*=4.46E-07), *Thanatephorus* (LFC=-21.87, *P*=1.98E-06), and *Urocystis* (LFC=-15.18, *P*=0.0005) were significantly lower in soils under the VT vs. the CT system in 2022. Also, the relative abundance of *Minimedusa*, a biological control agent of soilborne plant pathogens (Spinelli et al., 2022; Beale and Pitt 1992) (LFC=-25.84, *P*=1.45E-09) was lower in soils under the VT vs. CT systems.

Among fungal ASVs that were associated with the phylum Glomeromycota, *Dominikia* (LFC=-30.00, *P*=1.59E-12), *Microdominikia* (LFC=-29.96, *P*=1.69E-12), and *Glomus* (LFC=-24.21, *P*=1.53E-08) had lower relative abundances in VT vs. CT in 2022. However, the relative abundances of *Kamienskia* (LFC=22.91, *P*=9.28E-08) and *Rhizophagus* (LFC=20.29, *P*=2.56E-06) were significantly higher in VT vs. CT.

The relative abundances of *Olpidium* (LFC=-26.75, *P*=3.26E-10) and of *Mortierella* were lower in soils under the VT vs. CT in 2022 (LFC=-24.53, *P*=9.62E-09). Two significant ASVs belonging to Mucoromycota were both identified as *Mucor,* and both had higher relative abundance in soils under the VT vs. CT (LFC=21.99, *P*=3.11E-07 and LFC=20.65, *P*=1.66E-06). Details of how abundances of individual ASVs responded to VT compared to the CT system in the second year of soil sampling are presented in Figure 4.17C.

In general, the soil samples from the VT treatment exhibited a higher relative abundance of crucial plant pathogenic ASVs compared to the CT in both 2021 and 2022. The analysis of functional trophic groups in FUNGuild also indicated a greater number of pathotrophic ASVs in the soils under the VT in 2022 (Table 4.4 and 4.5). Interestingly, in soils under the VT vs. CT practice, the relative abundances of beneficial fungi with biocontrol activity such as *Chaetomium* and *Glomus,* were significantly lower. This trend was particularly pronounced in 2022, where the relative abundances of AMF were significantly lower in soils under the VT vs. CT practice. Our analysis of functional trophic groups in FUNGuild during the second year further supported these findings, revealing a

higher number of symbiotrophic ASVs corresponding to the soils under the CT compared to the VT system.

4.4.4.4 Differential Abundance by Tillage; VT vs. DT in 2022

Differential abundance analysis also clarified that 116 fungal ASVs were significantly affected by the contrast of VT vs. DT, including members of Ascomycota (77 fungal ASVs) and Basidiomycota (39 fungal ASVs).

Within the Ascomycotan group, the relative abundances of many plant pathogenic fungi were significantly lower in the soils under the VT vs. DT practice. These ASVs included *Alternaria* (LFC=-21.51, *P-value*=5.74E-07), *Corynespora* (LFC=-28.33, *P*=2.77E-11), *Paraphoma* (LFC=-9.95, *P*=0.007), *Gibellulopsis* (LFC=-20.39, *P*=2.32E-06), *Penicillium* (LFC=-22.11, *P*=2.74E-07), *Verticillium* (LFC=-19.47, *P*=6.81E-06), *Bipolaris* (LFC=-19.83, *P*=4.47E-06), *Gaeumannomyces* (LFC=-20.34, *P*=2.48E-06), and *Sporormiella* (LFC=-18.99, *P*=1.17E-05). The relative abundances of two plant pathogenic fungi, *Preussia* (LFC=20.26, *P*=2.70E-06) and *Neosetophoma* (LFC=12.79, *P*=0.004), were higher in soils under the VT vs. DT practice.

Among putatively beneficial taxa, the relative abundances of *Trichoderma* (LFC=18.42, *P*=2.2E-05) and *Arthrobotrys* (LFC=22.57, *P*=1.47E-07) were significantly higher in soils under VT vs. DT in 2022. However, the abundances of *Chaetomium* (LFC=-30.14, *P*=1.11E-12) and *Metarhizium* (LFC=-10.98, *P*=0.007) were significantly lower under VT vs. DT.

Within the Basidiomycotan group, the relative abundances of *Thanatephorus* (LFC=-32.15, *P*=2.78E-14), *Ustilago* (LFC=-28.63, *P*=1.66E-11), *Urocystis* (LFC=-16.51, *P*=0.0001) were significantly lower in the soil samples under the VT practice compared to the DT practice. In addition, *Vishniacozyma* (LFC=27.03, *P*=2.26E-10) had higher relative abundance in VT vs. DT in 2022.

Notably, soils subjected to the VT practice supported higher relative abundances of several AMF compared to the DT practice. This includes *Septoglomus* (LFC=30.74, P=1.21E-13), an unclassified taxon in the family Glomeraceae (LFC=28.59, P=1.23E-12), *Dominikia* (LFC=26.62, P=4.22E-10), *Rhizoglomus* (LFC=19.22, P=9.16E-06), and *Microdominikia* (LFC=18.04, P=3.30E-05).

The relative abundance of *Olpidium* (LFC=-30.58, *P*=5.51E-13) was significantly lower, while the relative abundance of *Mortierella* (LFC=29.35, *P*=4.83E-12) was higher in soils under the VT vs. DT in 2022. In 2022, there was only one ASV belonging to Rozellomycota, which was uncategorized at lower ranks (LFC=17.58, *P*=5.38E-05); its relative abundance was significantly higher in soils under the VT compared to the DT. Details of abundances of individual ASVs that responded to VT compared to the DT systems in the second year of soil sampling are presented in Figure 4.17D.

In both years of our study, soil samples from the VT demonstrated a lower relative abundance of critical plant pathogenic ASVs compared to the DT. Notably, the soils under the VT exhibited lower relative abundance of specific plant pathogens, such as *Alternaria*, *Olpidium*, *Phaeosphaeria*, *Bipolaris*, *Gaeumannomyces*, *Urocystis,* and *Ustilago* in comparison to the DT practice. Importantly, the relative abundance of beneficial fungi, such as members of AMF, *Trichoderma*, and other beneficial saprotrophs like *Mortierella* and *Vishniacozyma*, was significantly higher in soils under the VT compared to DT practices. The analysis of functional trophic groups in FUNGuild during the first year further confirmed the greater number of symbiotroph-species ASVs in VT compared to DT.

4.4.4.5 Differential Impact by Tillage; RB vs. VT in 2022

The analysis of differential ASV abundances between the RB and VT systems showed 113 responsive fungal ASVs, including members of Ascomycota (73 ASVs) and Basidiomycota (40 ASVs).

Within the Ascomycotan fungal ASVs, the relative abundance of several plant pathogens was significantly higher in RB compared to VT in 2022. These included *Herpotrichia* (LFC=31.17, *P*=1.96E-13), *Corynespora* (LFC=25.56, *P*=4.53E-13) *Penicillium* (LFC=9.16, *P*=0.006), *Gibellulopsis* (LFC=32.32, *P*=2.16E-14), *Verticillium* (LFC=31.92, *P*=4.65E-14), *Bipolaris* (LFC=19.77, *P*=4.79E-06), *Sporormiella* (LFC=27.22, *P*=1.67E-10), another *Sporormiella* ASV (LFC=27.22, *P*=1.05E10), and *Aspergillus* (LFC=22.41, *P*=1.82E-07). However, the relative abundances of *Microdochium*, a plant pathogenic fungus associated with cereal grains (Gagkaeva et al., 2020; Wen et al., 2014) (LFC=-3.89, *P-value*=0.006), *Alternaria* (LFC=-24.37, *P*=1.26E-08), *Gaeumannomyces* (LFC=-33.28, *P-value*=3.56E-15), and *Preussia* (LFC=-30.50, *P*=6.40E-13) were significantly lower in RB vs. VT

Furthermore, *Trichoderma* (LFC=-31.46, *P*=1.11E-13), *Arthrobotrys* (LFC=-41.00, *P*=1.76E-22), and *Metarhizium* (LFC=-24.92, *P*=1.40E-08) were the most significantly affected putative biocontrol agent fungal ASVs, all of which had lower relative abundance in the RB system compared to the VT system. The only putative biocontrol agent fungal ASV that exhibited a significantly higher relative abundance in RB vs. VT in 2022 was *Chaetomium* (LFC=23.53, *P*=3.93E-08).

Additionally, among the Basidiomycotan ASVs in soils under the RB vs. VT, there was a higher relative abundance of *Ustilago* (LFC=24.16, *P*=1.70E-08), while *Thanatephorus* (LFC=-21.95, *P*=3.35E-07) exhibited a lower relative abundance in this comparison. The abundance of *Minimedusa* also was higher significantly in RB vs. VT in 2022 (LFC=23.88, *P*=2.25E-08).

It is noteworthy that among the AMF ASVs within the Glomeromycotan group, the relative abundances of *Kamienskia* (LFC=-41.91, *P*=2.10E-23), *Rhizophagus* (LFC=-36.86, *P*=2.28E-18), and *Dominikia* (LFC=-25.74, P=1.63E-09) were lower in soils of the RB treatment when compared to the VT treatment in 2022. However, *Microdominikia* (LFC=35.06, *P*=1.01E-16), another AMF, had higher relative abundance in soils under the RB vs. VT in 2022. Details of abundances of individual microbial taxa respond to RB compared to the VT systems in the second year of soil sampling are presented in Figure 4.17E.

The relative abundances of *Olpidium* (LFC=27.57, *P*=9.41E-11) and of *Mortierella* (LFC=27.52, *P*=1.05E-10) were higher in soils under the RB vs. VT in 2022. In 2022, there was only one ASV belonging to Rozellomycota, which was not categorized at narrower ranks (LFC=-17.79, *P*=4.32E-05); its relative abundance was significantly lower in soils under the RB vs. VT. Five significantly responsive ASVs belonging to Mucoromycota were recognized as *Mucor* and *Actinomucor*. The relative abundance of *Mucor* was significantly lower (LFC=-35.60, *P*=3.43E-17), whereas the relative abundance of *Actinomucor* was significantly higher (LFC=22.37, *P*=1.92E-07) in RB vs. VT.

Overall, across both years of our study, soil samples from the RB practice displayed lower relative abundances of *Alternaria, Olpidium* and *Preussia*, indicating the detrimental effect of this tillage practice on these plant pathogens. However, soils subjected to RB systems exhibited higher relative abundances of several other plant pathogens compared to those under the VT practice. In both years, the relative abundances of beneficial fungi, including those playing a role in the biocontrol

of plant pathogens and those involved in decomposition, were significantly lower in soils under the RB practice.

4.4.4.6 Differential Abundance by Tillage; RB vs. DT in 2022

Differential abundance analysis also clarified that 105 fungal ASVs were significantly affected by the contrast of RB vs. DT in 2022, including members of Ascomycota (61 ASVs) and Basidiomycota (44 ASVs).

Among the Ascomycotan fungal ASVs, the relative abundances of *Herpotrichia* (LFC=30.87, *P*=3.35E-13), *Preussia* (LFC=17.30, *P*=7.19E-05), *Penicillium* (LFC=8.89, *P*=0.008), *Gibellulopsis* (LFC=11.92, *P*=0.007), and *Verticillium* (LFC=12.44, *P*=0.005) were significantly higher in soils under the RB vs. DT practice. However, the relative abundances of *Alternaria* (LFC=-37.32, *P-value*=8.89E-19), *Gaeumannomyces* (LFC=-28.33, *P*=2.57E-11), *Paraphoma* (LFC=-10.29, *P*=0.005), and *Sporormiella* (LFC=-14.74, *P*=0.0008) were significantly lower in RB vs. DT in 2022.

In addition, *Arthrobotrys* (LFC=-30.92, *P*=3.15E-13) and *Metarhizium* (LFC=-12.38, *P*=0.002) were the most significantly affected biocontrol agent fungal ASVs, both of which had lower relative abundance in the RB system compared to the DT system. However, other biocontrol agent fungal ASVs exhibited significantly higher relative abundances in RB vs. DT, including *Trichoderma* (LFC=30.28, *P*=9.21E-13) and *Pyxidiophora* (LFC=9.47, *P*<0.001).

Furthermore, among the Basidiomycotan ASVs, *Ustilago* (LFC=37.06, *P*=1.54E-18) had higher relative abundance, while *Thanatephorus* exhibited a significantly lower relative abundance (LFC=-39.86, *P*=2.31E-21) in the soils under the RB vs. DT practice. In addition, the relative abundances of *Minimedusa* and *Waitea* were significantly lower (LFC=-15.17, *P*=0.0005 and LFC=-26.58, *P*= 4.56E-10) in the RB system when compared to the DT system. However, *Vishniacozyma* exhibited significantly higher relative abundance in RB vs. DT in 2022 (LFC=28.99, *P*=8.96E-12).

It is worth highlighting that within the AMF ASVs of the Glomeromycotan group, the relative abundances *Kamienskia* (LFC=-38.83, *P*=3.12E-20), *Rhizophagus*, an AMF (Fresno, et al., 2023), (LFC=-37.89, *P*=2.32E-19), and *Glomus* (LFC=-20.61, *P*=1.78E-06) significantly decreased in soils under the RB system when compared to the DT system in 2022. However, the relative abundances

of *Dominikia* (LFC=22.89, *P*=9.61E-08), *Septoglomus* (LFC=21.35, *P*=7.20E-07) and *Microdominikia* (LFC=18.93, *P*=1.24E-05) were significantly higher in RB vs. DT.

The relative abundances of *Olpidium* (LFC=-17.58, *P*=4.06E-05) and of *Mortierella* (LFC=-35.78, *P*=2.28E-17) were lower in soils under the RB vs DT in 2022. Six significantly responsive ASVs belonging to Mucoromycota were recognized as *Mucor, Absidia* and *Rhizopus*; the relative abundance of *Mucor* was significantly lower (LFC=-32.09, *P*=3.66E-14), whereas the relative abundances of *Absidia* (LFC=25.08, *P*=4.43E-09) and *Rhizopus* (LFC=24.26, *P*=1.35E-08) were significantly higher in RB vs. DT in 2022. Details of abundances of individual microbial taxa respond to RB compared to the DT systems in the second year of soil sampling are presented in Figure 4.17F.

In summary, our two-year study revealed that soils under the RB practice, when compared to the DT practice, exhibited a lower relative abundance of plant pathogens. Specifically, certain plant pathogens such as *Alternaria, Olpidium, Gaeumannomyces, Paraphoma, Neosetophoma*, and *Fusariella* had lower relative abundance under the RB vs. DT. The analysis of functional trophic groups in FUNGuild further confirmed a higher number of pathotrophic ASVs (95 ASVs) in soils under the DT practice compared to the RB (79 ASVs). Additionally, soils subjected to RB systems showed a lower relative abundance of AMF. Our analysis of functional trophic groups in FUNGuild, especially in 2022, also revealed a higher number of symbiotrophic ASVs in soils under the DT practice. It is noteworthy that the FUNGuild results in 2021 for DT showed a slightly lower number of symbiotrophic ASVs compared to RB.

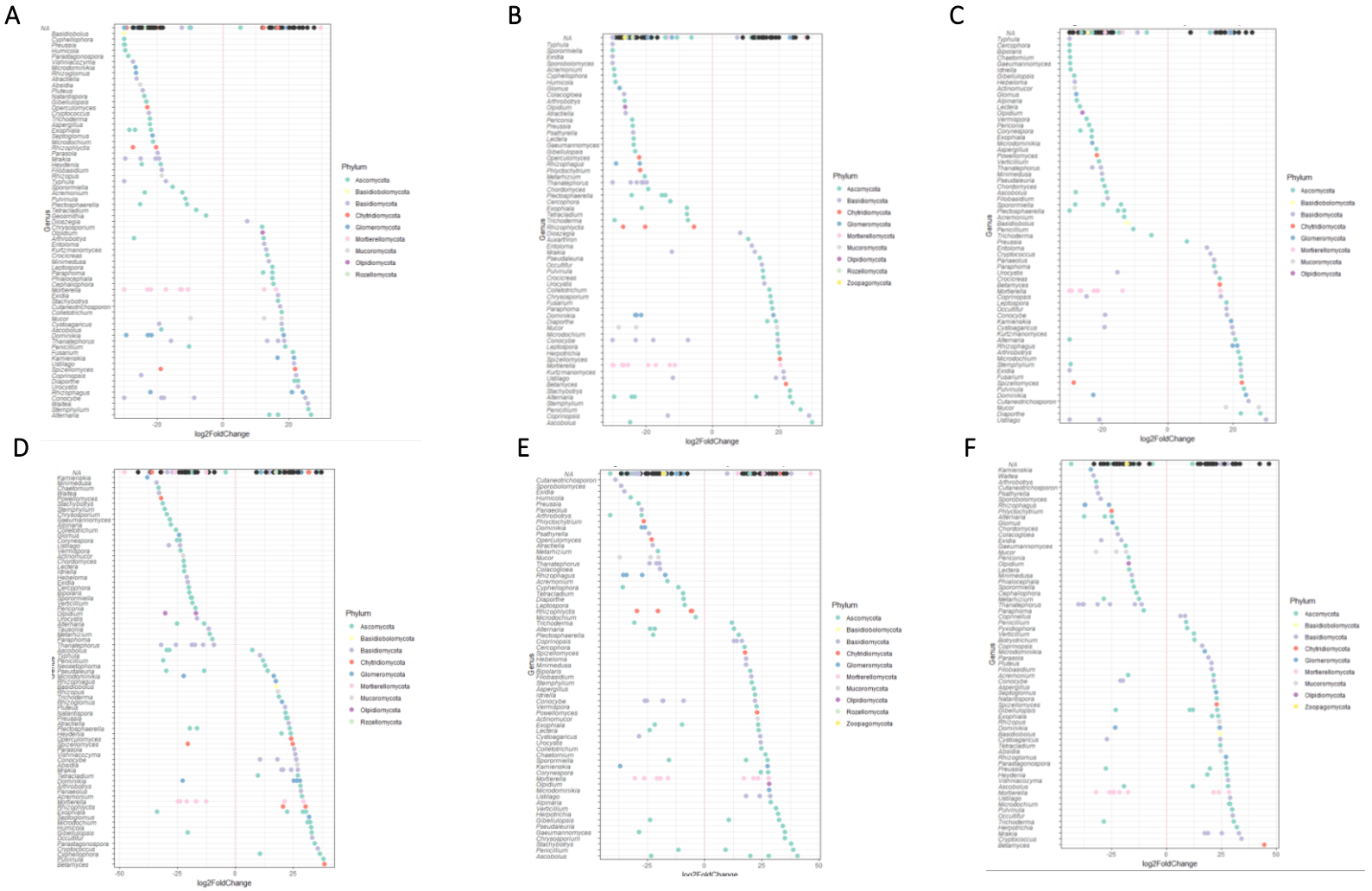

Figure 4. 17. Log_2 fold changes in the relative abundances of amplicon sequence variants (ASVs), in response to contrasting tillage regimes in the second year of soil sampling. ASVs are colour coded by phylum. ASVs were differentially abundant among contrasts between A) deep tillage compared to conventional tillage, B) raised-bed compared to conventional tillage, C) vertical tillage compared to conventional tillage D) vertical tillage compared to deep tillage, E) raised-bed compared to vertical tillage and F) raised-bed compared to deep tillage. Adjusted P for significance < 0.05 and threshold for the log2FC was set at 1.0.

4.4.5 Differential Abundance among Crop Species in Different Fields; 2022

In the second year of soil sampling, the relative abundances of 400 fungal ASVs spanning ten phyla were significantly impacted by crop rotation, with most belonging to phyla Ascomycota and Basidiomycota. A smaller number of fungal ASVs within other phyla, such as Chytridiomycota, Mortierellomycota, Mucoromycota, Glomeromycota, Rozellomycota, Kickxellomycota, Zoopagomycota, and Basidiobolomycota, were also affected by the crop rotation in 2022. The top five fungal ASVs whose relative abundance differed more significantly are presented in Table 4.10.

Table 4. 10. Differential abundance of the five top-most strongly affected ASVs in 2022 among plots with different crop species (based on the Adjusted *P*), which were also located in the different fields. Statistical significance was established at a threshold of $p<0.05$, following adjustments for comparisons utilizing the Benjamini-Hochberg false discovery rate (BH-FDR). The threshold for log2 fold change (log2FC) was defined as 1.0, with lfcSE representing the standard error of log2FC.

Field Comparison; Corn vs. Canola						
Base Mean	Log2FC	lfcSE	Adjusted *P*	Level 1; Phylum	Level 2; Family	Level 3; Genus
37.50	25.09	1.37	1.72E-65	Ascomycota	Aspergillaceae	*Penicillium*
21.29	-25.23	1.52	3.19E-54	Ascomycota	NA	NA
59.69	29.63	2.00	6.00E-44	Basidiomycota	Bolbitiaceae	*Conocybe*
606.01	14.03	1.00	2.14E-36	Ascomycota	NA	NA
19.92	-19.29	1.60	6.24E-28	Ascomycota	Hyaloscyphaceae	*Cistella*
Field Comparison; Canola vs. Soybean						
37.50	-24.70	1.37	2.28E-63	Ascomycota	Aspergillaceae	*Penicillium*
606.01	14.87	1.04	1.10E-37	Ascomycota	NA	NA
61.00	-27.66	2.10	2.51E-34	Basidiomycota	Cystobasidiaceae	*Occultifur*
5.70	26.54	2.11	3.57E-31	Basidiomycota	NA	NA
23.73	20.73	1.83	1.62E-24	Ascomycota	NA	NA
Field Comparison; Soybean vs. Corn						
606.0178	-30	1.00	1.61E-179	Ascomycota	NA	NA
19.92	27.43	1.57	2.83E-60	Ascomycota	Hyaloscyphaceae	*Cistella*
23.73	-28.5	1.82	9.55E-49	Ascomycota	NA	NA
21.29	23.20	1.52	1.35E-45	Ascomycota	NA	NA
25.23	25.53	1.82	9.06E-39	Ascomycota	Herpotrichiellaceae	*Exophiala*

4.4.5.1 Differential Abundance among Crop Species; Corn vs. Canola in 2022

The comparison of fungal ASVs in plots within the corn field vs. the canola field in 2022 revealed that the relative abundance of 152 fungal ASVs were strongly impacted, primarily among Ascomycota (82 ASVs), Basidiomycota (45 ASVs) and Glomeromycota (25 ASVs) (Figure 4.18A).

The analysis of differential abundance clarified that within the Ascomycotan group, the relative abundance of only two plant pathogenic ASVs significantly decreased among plots in the field planted to corn vs. plots in the field planted to canola in 2022: *Diaporthe* (LFC=-10.62, *P*=0.0002) and *Stemphylium* (LFC=-11.25, *P*=0.003). However, interestingly the relative abundances of many other plant pathogenic fungal ASVs were significantly higher in plots from the corn field vs. plots from the canola field. These ASVs included *Herpotrichia* (LFC=11.98, *P*=0.001), *Fusarium* (LFC=4.96, *P*=0.0002), *Penicillium* (LFC=25.09, *P*=1.62E-68), *Paraphoma* (LFC=6.19, *P*=0.001), *Alternaria* (LFC=24.62, *P*=3.05E-11), *Corynespora* (LFC=20, *P*=1.02E-10), *Preussia* (LFC=30, *P*=3.36E-16), *Gaeumannomyces* (LFC=30.00, *P*=3.36E-16), *Neosetophoma* (LFC=30.00, *P*=3.02E-16), *Gibellulopsis* (LFC=29.88, *P*=4.42E-16), *Bipolaris* (LFC=29.07, *P*=2.75.E-15), and *Aspergillus* (LFC=22.31, *P*=2.05E-09).

In addition, the relative abundances of *Paraphaeosphaeria* (LFC=-6.48, *P*=5.51E-09), *Trichoderma* (LFC=-10.37, *P*=0.008), and *Metarhizium* (LFC=-18.03, *P*=1.67E-06), were significantly lower while *Arthrobotrys* (LFC=27.33, *P*=1.30E-13) was the only ASVs with potential biocontrol activity that was present at significantly higher relative abundances among plots in the field planted to corn than among plots in the field planted to canola in 2022.

Moreover, among the Basidiomycotan ASVs, the relative abundances of *Ustilago* (LFC=29.84, *P*=4.90E-16), *Dioszegia* (LFC=8.22, *P*=0.004), *Vishniacozyma* (LFC=24.07, *P*=2.96E-22), *Thanatephorus* (LFC=29.54, *P*=9.86E-16), *Coprinopsis* (LFC=2.57, *P*=1.14E-11), and *Coprinellus* (LFC=8.54, *P*=0.0006) were significantly higher in abundances among plots in the field planted to corn than among plots in the field planted to canola in 2022. However, the relative abundances of *Urocystis* (LFC=-27.33, *P*=1.98E-07) and *Minimedusa* (LFC=-27.63, *P*=6.55E-14) were significantly lower among plots in the field planted to corn than among plots in the field planted to canola in 2022.

It is worth highlighting that within the AMF ASVs of the Glomeromycotan group, the most significant ASV, *Dominikia*, showed a significantly higher in relative abundance (LFC=30.00, *P*=5.24E-30) among plots in the field planted to corn than among plots in the field planted to canola in 2022. In addition, the relative abundances of well-known AMF ASVs such as *Glomus* (LFC=29.98, *P*=3.24E-16), *Rhizophagus* (LFC=26.38, *P*=8.64E-13), and *Rhizoglomus* (LFC=28.57, *P*=3.42E-19) were significantly higher among plots in this contrast in 2022. Details of relative abundances of individual microbial taxa respond among plots in the field planted to corn than among plots in the field planted to canola in 2022 are presented in Figure 4.18A.

Moreover, among plots in the field planted to corn compared to among plots in the field planted to canola in 2022, the relative abundances of *Olpidium* (LFC=22.60, *P*=1.22E-09) and *Mortierella* (LFC=30.00, *P*=3.07E-16) were significantly higher among plots in the field planted to corn than among plots in the field planted to canola in 2022. *Mucor* had significantly higher relative abundance of (LFC=23.68, *P*=1.80E-10) while *Absidia* (LFC=-26.01, *P*=1.86E-12) had significantly lower relative abundance among plots in the field planted to corn than among plots in the field planted to canola in 2022. Details of abundances of individual microbial taxa in this contrast in 2022 are presented in Figure 4.18A.

In summary, our study indicated a significantly higher relative abundance of plant pathogens and of saprotrophic fungi in plots within corn fields compared to those in canola fields in 2022. Furthermore, plots in the fields planted with corn exhibited a higher relative abundance of AMF compared to canola fields, with these differences being more pronounced in 2022. The analysis of functional trophic groups in FUNGuild in 2022 also confirmed a higher number of symbiotrophic ASVs (including mycorrhizal fungi) in plots within corn fields compared to canola fields.

4.4.5.2 Differential Abundance among Crop Species; Soybean vs. Corn in 2022

The comparison of fungal ASVs among plots in the soybean field vs. plots in the corn field in the 2022 revealed 167 ASVs that were significantly differentially abundant, including members of Ascomycota (92 ASVs), Basidiomycota (61 ASVs) and Glomeromycota (14 ASVs) (Figure 4.18B).

Important plant pathogenic ASVs were significantly impacted by soybean vs. corn field. Within the Ascomycotan group, the relative abundances of *Corynespora* (LFC=4.84, *P*=8.56E-08),

Stemphylium (LFC=2.73, *P*=0.006), *Alternaria* (LFC=6.31, *P*=0.0001), *Plectosphaerella* (LFC=17.60, *P*=2.81E-06), *Diaporthe* (LFC=18.88, *P*=1.53E-11), *Penicillium* (LFC=9.35, *P*=0.001), and *Paraphoma* (LFC=10.39, *P*=0.001) were significantly higher in the soybean field than in the corn field. However, the relative abundances of several other plant pathogenic ASVs displayed the opposite pattern, including *Herpotrichia* (LFC=-29.70, *P*=2.81E-16), *Verticillium* (LFC=-4.12,*P*=1.50E-07), *Geosmithia* (LFC=-7.64, *P*=9.97E-09), *Neosetophoma* (LFC=-12.69, *P*=0.0009), *Gaeumannomyces* (LFC=-27.69, *P*=5.76E-14), *Bipolaris* (LFC=-9.94, *P*=0.011), and *Preussia* (LFC=-18.77, *P*=5.81E-07). In addition, although the relative abundance of *Trichoderma* (LFC=-4.02, *P*=0.004) was significantly lower, the relative abundances of *Pyxidiophora* (LFC=6.78, *P*=0.008), *Arthrobotrys* (LFC=6.17, *P*=0.004), and *Metarhizium* (LFC=28.14, *P*=2.14E-14) were significantly higher among plots in the field planted to soybean vs. plots in the field planted to corn.

Moreover, among the Basidiomycotan ASVs, the relative abundance of *Ustilago* (LFC=-13.89, *P*=0.0002) was significantly lower, while relative abundance of *Urocystis* was significantly higher (LFC=23.19, *P*=4.36E-10) among plots in the field planted to soybean vs. corn. Additionally, the relative abundance of *Coprinopsis* (LFC=-8.70, *P*=0.0001) was lower while *Vishniacozyma* (LFC=20.38, *P*=4.99E-08) and *Minimedusa* (LFC=16.82, *P*=8.65E-06) showed significantly higher relative abundances in this contrast in 2022.

Among AMF ASVs impacted among plots in the field planted to soybean vs. plots in the field planted to corn in 2022, a non-assigned taxon exhibited a significantly higher relative abundance (LFC=7.84, *P*<0.001), while *Dominikia* (LFC=-10.05, *P*=0.0003), *Rhizophagus* (LFC=-17.74, *P*=1.63E-12), Kamienski (LFC=-10.49, *P*=0.007), and *Microdominikia* (LFC=-22.12, *P*=2.81E-09) exhibited significantly lower relative abundances in this contrast in 2022.

Moreover, the relative abundance of *Mortierella* was significantly lower (LFC=-8.84, *P*=0.0009) among plots in the field planted to soybean than among plots in the field planted to corn in 2022. Among the ASVs identified under the phylum Mucoromycota, *Mucor* and *Absidia* had significantly higher relative abundances (LFC=20.64, *P*=3.22E-08 and LFC=17.55, *P*=3.25E-06) among plots in the field planted to soybean than among plots in the field planted to corn in 2022. However, the relative abundance of *Actinomucor* (LFC=-3.71, *P*=0.0003) was lower in this contrast. Importantly,

Olpidium was not among those ASVs whose relative abundance was strongly affected in this contrast. Details of abundances of individual microbial taxa among plots in the field planted to soybean vs plots in the field planted to corn in 2022 are presented in Figure 4.18B.

In summary, our two-year study indicated that although the relative abundance of some plant pathogens was significantly higher in plots within fields planted with soybeans compared to those planted with corn in 2022, the majority of plant pathogens showed a significantly lower relative abundance in soybean plots. Surprisingly, *Olpidium* was not among the ASVs whose relative abundance was affected in this contrast (while it was appeared in other contrasts). Our analysis of functional trophic groups in FUNGuild in 2022 also showed a higher number of pathotrophic ASVs in plots within corn fields compared to soybean fields. Additionally, the analysis of differential abundance demonstrated that the relative abundance of AMF was significantly lower in soybean plots compared to corn plots. Furthermore, the analysis of functional trophic groups in FUNGuild for both 2021 and 2022 indicated a higher number of symbiotrophic ASVs associated with plots in corn fields compared to soybean fields.

4.4.5.3 Differential Abundance among Crop Species; Canola vs. Soybean in 2022

The analysis of differential abundance indicated that a total of 200 ASVs were significantly affected among plots in the field planted to canola compared to soybean in 2022, including Ascomycota (94 ASVs), Basidiomycota (58 ASVs), Glomeromycota (27 ASVs), and Chytridiomycota (21 ASVs).

The relative abundances of three plant pathogenic ASVs within the phylum Ascomycota were significantly higher among plots in the field planted to canola vs. soybean. These ASVs included *Herpotrichia* (LFC=18.59, *P*=5.60E-07), *Verticillium* (LFC=5.20, *P*=1.52E-12), and *Geosmithia* (LFC=9.67, *P*=1.02E-13). However, the abundances of several other plant pathogenic ASVs within Ascomycota were oppositely impacted, including *Corynespora* (LFC=-28.08, *P*=2.97E-20), *Fusarium* (LFC=-4.61, *P*=0.009), *Alternaria* (LFC=-17.82, *P*=2.20E-06), *Penicillium* (LFC=-24.70, *P*=2.15E-66), *Plectosphaerella* (LFC=-24.28, *P*=5.70E-11), *Diaporthe* (LFC=-8.81, *P*=0.003), *Exophiala* (LFC=-24.29, *P*=5.56-E11), *Preussia* (LFC=-15.21, *P*=6.36E-05), *Paraphoma* (LFC=-18.12, *P*=1.40E-06), *Stemphylium* (LFC=-11.11, *P*=0.004), *Gibellulopsis* (LFC=-21.08, *P*=1.64E-08), and *Bipolaris* (LFC=-19.99, *P*=9.29E-08).

In addition, although the relative abundances of *Trichoderma* (LFC=4.36, *P*=0.001) and *Talaromyces* (LFC=6.91, *P*=3.59E-11) were significantly higher, the relative abundances of *Pyxidiophora* (LFC=-7.51, *P*=0.002), *Arthrobotrys* (LFC=-5.74, *P*=0.009), and *Metarhizium* (LFC=-10.10, *P*=0.01) were significantly lower among plots in the field planted to canola vs. soybean in 2022.

Within the phylum Basidiomycota, ASVs with significantly higher relative abundance in the field planted to canola vs. soybean were *Ustilago* (LFC=26.79, *P*=4.00E-13), *Urocystis* (LFC=25.64, *P*=4.30E-12), *Coprinopsis* (LFC=30.00, *P*=3.38E-16), and *Coprinellus* (LFC=-15.85, *P*=2.21E-11). In contrast, *Thanatephorus* (LFC=-30.00, *P*=3.38E-16) and *Vishniacozyma* (LFC=-17.03, *P*=1.58E-11) showed significantly lower relative abundances in the field planted to canola vs. soybean.

Additionally, a non-assigned taxon (LFC=-24.83, *P*=8.76E-17), *Dominikia* (LFC=-19.43, *P*=6.06E-13), *Rhizoglomus* (LFC=-25.51, *P*=1.79E-15), *Kamienskia* (LFC=-21.00, *P*=1.81E-08) and *Glomus* (LFC=-29.71, *P*=6.11E-16) were among the AMF within the phylum Glomeromycota whose relative abundances were significantly lower among plots in the field planted to canola vs. soybean in 2022. However, the relative abundance of *Microdominikia* (LFC=28.40, *P*=1.20E-14) was significantly higher in this contrast.

Moreover, the relative abundances of *Olpidium* (LFC=12.39, *P*=0.001) and of *Mortierella* (LFC=30.00, *P*=3.38E-16) were significantly higher in this contrast in 2022. The relative abundances of *Mucor* (LFC=23.66, *P*=1.85E-10) and *Actinomucor* (LFC=18.23, *P*=1.28E-06), the ASVS within the phylum Mucoromycota, were significantly higher in this contrast. The only ASV within the phylum Rozellomycota was a non-assigned taxon (LFC=29.97, *P*=2.14E-12) and its relative abundance was significantly higher in this contrast in 2022. Details of abundances of individual microbial taxa in this contrast in 2022 are presented in Figure 4.18C.

In summary, our two-year study indicated that the relative abundance of plant pathogens was significantly lower in plots within fields planted with soybeans compared to those planted with canola in 2022. The relative abundance of beneficial fugal ASVs such as *Trichoderma* was higher in this contrast. The relative abundance of *Mortierella* was higher in plots within fields planted with soybeans vs. canola. Additionally, the analysis of differential abundance demonstrated that the relative abundance of AMF was significantly lower in soybean plots compared to canola plots. Our

analysis of functional trophic groups in FUNGuild in 2021 also confirmed a higher number of symbiotrophic ASVs in canola vs soybean.

4.4.6 Differential Abundance among Soil Fractions; 2022

In the second year of soil sampling, the relative abundances of 305 fungal ASVs spanning ten phyla were significantly differentially abundant among soil fractions. The majority of affected ASVs by soil aggregates in the second year belonged to the phylum Ascomycota and Basidiomycota. A smaller number of fungal ASVs within other phyla, such as Chytridiomycota, Mortierellomycota, Mucoromycota, Glomeromycota, Kickxellomycota, Olpidiomycota, Zoopagomycota, and Basidiobolomycota, were also affected by the crop rotation in 2022. The most significant ASVs (top five) were grouped on three levels including phylum, family, and genus. We also calculated the $\log_2$ LFC as presented in Table 4.11.

Table 4. 11. Differential abundance of the five top-most strongly affected taxa in 2022 among the soil fractions (based on the Adjusted *P*). Statistical significance was established at a threshold of p<0.05, following adjustments for comparisons utilizing the Benjamini-Hochberg false discovery rate (BH-FDR). The threshold for log2 fold change (log2FC) was defined as 1.0, with lfcSE representing the standard error of log2FC.

Soil Aggregates Comparison; LSA vs. SSA						
Base Mean	Log2FC	lfcSE	Adjusted *P*	Level 1; Phylum	Level 2; Family	Level 3; Genus
34.31	-29.91	2.52	2.84E-27	Basidiomycota	Ceratobasidiaceae	NA
11.31	-24.32	2.37	5.54E-20	Basidiomycota	Bulleribasidiaceae	*Vishniacozyma*
0.98	-29.98	3.47	2.49E-14	Basidiomycota	NA	NA
112.51	-30	3.54	2.94E-14	NA	NA	NA
24.51	30	3.55	2.94E-14	Ascomycota	Pleosporaceae	*Alternaria*
Soil Aggregates Comparison; SSA vs. POM						
37.50	27.07	1.37	6.43E-77	Ascomycota	Aspergillaceae	*Penicillium*
2.65	34.24	2.33	2.37E-43	Kickxellomycota	NA	NA
1.16	-41.28	3.55	3.24E-27	Basidiomycota	Mrakiaceae	*Mrakia*
212.92	-33.18	2.89	2.88E-26	Ascomycota	Cyphellophoraceae	NA
27.82	34.56	3.16	6.83E-24	Basidiomycota	Entolomataceae	*Entoloma*
Soil Aggregates Comparison; LSA vs. POM						
37.50	27.47	1.37	2.56E-79	Ascomycota	Aspergillaceae	*Penicillium*
2.65	29.77	2.33	4.37E-32	Kickxellomycota	NA	NA
9.13	-29.76	2.58	3.50E-26	Ascomycota	Cyphellophoraceae	*Cyphellophora*
10.38	30	2.61	3.61E-26	Ascomycota	Aspergillaceae	*Penicillium*
4.49	28.55	2.63	2.48E-23	NA	NA	NA

4.4.6.1 Differential Abundance among Soil Fractions; LSA vs. SSA in 2022

Comparing LSA vs. SSA revealed that the relative abundance of 128 fungal ASVs in 2022 were significantly different between these aggregate size classes. The majority of fungal ASVs whose relative abundance were significantly differed in this contrast were affiliated with the Ascomycota (59 ASVs) and Basidiomycota (42 ASVs). In addition, other impacted ASVs in this contrast were affiliated with the Mortierellomycota (10 ASVs), Chytridiomycota (9 ASVs), Glomeromycota (6 ASVs), Mucoromycota (1 ASV), and Olpidiomycota (1 ASV) phyla.

Within the Ascomycotan group, the relative abundances of *Herpotrichia* (LFC=12.69, *P*=0.0008), *Alternaria* (LFC=30.00, *P*=3.43E-16), *Corynespora* (LFC=24.74, *P*=6.65E-16), *Verticillium* (LFC=12.10, *P*=0.001), *Gibellulopsis* (LFC=29.26, *P*=1.81E-15), *Sporormiella* (LFC=28.48, *P*=1.03E-14), and *Microdochium* (LFC=26.71, *P*=4.701E-13) were significantly higher in LSA vs. SSA in 2022. Additionally, the relative abundances of *Preussia* (LFC=-24.72, *P*=2.56E-11), *Gaeumannomyces* (LFC=-26.63, *P*=5.63E-13), *Penicillium* (LFC=-12.39, *P*=0.001), and *Aspergillus* (LFC=-22.21, *P*=2.39E-09) were significantly lower in this contrast. The relative abundance of *Trichoderma* (LFC=-12.73, *P*=0.0009) also was significantly lower.

Among the Basidiomycotan ASVs, the relative abundances of *Ustilago* (LFC=17.32, *P*=4.42E-06) and *Urocystis* (LFC=17.44, *P*=3.76E-06) were significantly higher in LSA compared to SSA. However, the relative abundances of *Thanatephorus* (LFC=-30.00, *P*=3.76E-06), *Vishniacozyma* (LFC=-24.32, *P*=1.05E-22), *Conocybe* (LFC=-22.05, *P*=3.21E-09), *Coprinopsis* (LFC=-19.38, *P*=2.36E-07), and *Dioszegia* (LFC=-9.68, *P*=4.03E-05) were significantly lower in LSA vs. SSA in 2022. The relative abundance of all AMF ASVs within the Glomeromycota phylum showed significantly higher in LSA compared to the SSA. Among these ASVs, the relative abundances of *Microdominikia* (LFC=27.60, *P*=6.63E-14), *Rhizophagus* (LFC=21.00, *P*=1.87E-08) and *Glomus* (LFC=17.80, *P*=2.29E-06) were significantly higher in LSA compared to SSA in 2022.

Moreover, the relative abundance of *Olpidium* (LFC=-23.87, *P*=1.23E-10) was significantly lower, while the relative abundance of *Mortierella* was significantly higher (LFC=30.00, *P*=3.37E-16) in LSA vs. SSA in 2022. The relative abundance of *Mucor* (LFC=-18.60, *P*=7.43E-07) was significantly lower in this contrast. Among the ASVs belonged to Chytridiomycota, the relative abundances of *Betamyces* (LFC=-30.00, *P*=3.38E-16), *Spizellomyces* (LFC=-28.11, *P*=2.42E-14), and *Rhizophlyctis*

(LFC=-20.46, *P*=4.40E-08) were significantly lower in this contrast. Details of relative abundances of individual microbial taxa in this contrast in 2022 are presented in Figure 4.18D.

In summary, our two-year study revealed higher relative abundances of filamentous fungi in LSA compared to SSA. Among the strongly affected ASVs in LSA vs. SSA, there was a notably higher relative abundance of Ascomycota. As another example for filamentous fungi, *Mortierella* exhibited significantly higher relative abundance in LSA compared to SSA. Additionally, the relative abundance of AMF was higher in LSA than SSA. The analysis of functional trophic groups in FUNGuild also consistently confirmed a higher number of symbiotrophic ASVs in both 2021 and 2022 in LSA.

4.4.6.2 Differential Abundance among Soil Fractions; SSA vs. POM in 2022

The analysis of differential abundance in soils from SSA vs. POM in 2022 showed that 193 ASVs were significantly affected. Among these fungal ASVs, 68 ASVs were identified in phylum Ascomycota, 38 ASVs in phylum Basidiomycota, and 15 ASVs in phylum Glomeromycota. In addition, other impacted ASVs in this contrast were affiliated with the Chytridiomycota (11 ASVs), Mortierellomycota (10 ASVs), Kickxellomycota (1 ASV), Mucoromycota (4 ASVs), Zoopagomycota (1 ASV), Basidiobolomycota (1 ASV) and Olpidiomycota (2 ASVs).

Within the Ascomycotan group, the relative abundances of *Penicillium* (LFC=27.07, *P*=6.08E-80), *Bipolaris* (LFC=28.53, *P*=9.44E-15), *Aspergillus* (LFC=32.68, *P*=4.92E-19), and *Preussia* (LFC=31.33, *P*=1.40E-17) were significantly higher in SSA vs. POM. In addition, the relative abundances of ASVs with putative biocontrol activity such as *Pyxidiophora* (LFC=7.47, *P*=0.003), *Trichoderma* (LFC=30.41, *P*=1.20E-16), *Metarhizium* (LFC=27.69, *P*=5.95E-14), and *Arthrobotrys* (LFC=26.67, *P*=9.11E-17) were significantly higher in SSA vs. POM in 2022.

Within the Basidiomycota group, the relative abundances of *Urocystis* (LFC=24.86, *P*=1.93E-11), *Conocybe* (LFC=22.68, *P*=1.05E-09), *Dioszegia* (LFC=9.69, *P*=4.03E-05), and *Coprinellus* (LFC=7.58, *P*=0.002) were significantly higher in SSA vs. POM in 2022. It is noteworthy that among the AMF ASVs whose relative abundances were significantly impacted when comparing SSA to POM, *Dominikia* (LFC=31.28, *P*=1.62E-17), *Septoglomus* (LFC=24.52, *P*=3.76E-11), *Kamienskia* (LFC=23.40, *P*=3.03E-10) had higher relative abundances in SSA compared to POM in 2022.

Moreover, the relative abundance of *Olpidium* (LFC=3.05, *P*=0.0008) was significantly higher in SSA vs POM in 2022. In addition, the relative abundance of *Mortierella* was significantly lower in this contrast in 2022 (LFC=-34.49, *P*=4.62E-21). The relative abundance of *Mucor* (LFC=30.22, *P*=2.00E-16) and *Absidia* (LFC=10.81, *P*=0.005), both ASVs in phylum Mucoromycota were significantly higher in this contrast. Among the ASVs belonging to Chytridiomycota, the relative abundances of *Betamyces* (LFC=4.42, *P*=0.0005), *Spizellomyces* (LFC=23.20, *P*=4.31E-10), and *Rhizophlyctis* (LFC=30.93, *P*=3.84E-17) were significantly higher in this contrast. Details of relative abundances of individual microbial taxa in this contrast in 2022 are presented in Figure 4.18E.

In summary, our two-year study uncovered a higher relative abundance of symbiotrophic ASVs in SSA compared to POM. Additionally, in both years of the study, there was a higher relative abundance of plant pathogens in SSA vs. POM. The analysis of functional trophic groups in FUNGuild for both 2021 and 2022 further confirmed a higher number of pathotrophic and symbiotrophic ASVs in SSA compared to POM. The analysis of differential abundance in soils from SSA versus POM revealed a higher relative abundance of saprotrophic fungal ASVs in SSA compared to POM. Members of the Chytridiomycota and Basidiomycota phyla, well-known saprotrophic fungi, were identified. The analysis of functional trophic groups in FUNGuild in both years also showed a higher number of saprotrophic ASVs in SSA compared to POM.

4.4.6.3 Differential Abundance among Soil Fractions; LSA vs. POM in 2022

The analysis of differential abundance in soils from LSA vs. POM in 2022 showed that 165 ASVs were significantly affected. Among these fungal ASVs, 65 ASVs were identified in phylum Ascomycota, 52 ASVs in phylum Basidiomycota, and 18 ASVs in phylum Glomeromycota. In addition, other impacted ASVs in this contrast were affiliated with the Chytridiomycota (15 ASVs), Mortierellomycota (7 ASVs), Kickxellomycota (1 ASV), Mucoromycota (5 ASVs), Zoopagomycota (1 ASV), and Olpidiomycota (1 ASVs).

Within the Ascomycotan group, the relative abundances of *Penicillium* (LFC=27.40, *P*=2.42E-82), *Plectosphaerella* (LFC=25.60, *P*=4.59E-12), *Verticillium* (LFC=12.83, *P*=0.0008), *Bipolaris* (LFC=30,.00 *P*=3.73E-16), *Gibellulopsis* (LFC=22.86, *P*=7.71E-10), *Chrysosporium* (LFC=28.27, *P*=1.72E-14), *Preussia* (LFC=20.04, *P*=8.68E-08), *Alternaria* (LFC=13.41, *P*=0.0004), and *Sporormiella* (LFC=10.22, *P*=0.009) were significantly higher in LSA vs. POM in 2022. Additionally,

putative beneficial taxa *Trichoderma* (LFC=21.95, *P*=3.80E-09), *Metarhizium* (LFC=28.70, *P*=6.51E-15), and *Arthrobotrys* (LFC=25.80, *P*=9.94E-16) had significantly higher relative abundance in LSA vs. POM.

Among the Basidiomycotan ASVs, the relative abundances of *Ustilago* (LFC=30.00, *P*=3.39E-16), *Urocystis* (LFC=20.59, *P*=3.60E-08), and *Waitea* (LFC=24.68, *P*=2.68E-11) were significantly higher in LSA compared to the POM in 2022. However, *Thanatephorus* (LFC=-22.56, *P*=1.32E-09), *Vishniacozyma* (LFC=-26.55, *P*=9.37E-15), and *Coprinopsis* (LFC=-30.00, *P*=3.14E-16) had significantly lower relative abundances in LSA vs. POM. The relative abundance of AMF ASVs within the Glomeromycota phylum showed significantly higher in LSA compared to the POM: *Kamienskia* (LFC=29.81, *P*=5.24E-16), *Septoglomus* (LFC=29.75, *P*=6.06E-16), *Dominikia* (LFC=29.38, *P*=1.38E-15), *Rhizoglomus* (LFC=27.73, *P*=5.43E-14), and *Microdominikia* (LFC=10.50, *P*=0.007).

Moreover, the relative abundance of *Olpidium* (LFC=-11.36, *P*=0.003), was significantly lower in SSA vs. POM in 2022. In addition, the relative abundance of *Mortierella* was significantly higher in this contrast in 2022 (LFC=30.00, *P*=3.12E-16). The relative abundances of *Mucor* (LFC=29.89, *P*=4.36E-16) and *Absidia* (LFC=11.07, *P*=0.004), both ASVs in phylum Mucoromycota, were significantly higher in LSA vs. POM. Among the ASVs belonged to Chytridiomycota, the relative abundances of *Operculomyces* (LFC=29.96, *P*=3.71E-16), *Spizellomyces* (LFC=29.81, *P*=4.31E-10), and *Powellomyces* (LFC=29.76, *P*=5.86E-16) were significantly higher in LSA vs. POM. Details of abundances of individual microbial taxa that responded to LSA compared to the POM in the second year of soil sampling are presented in Figure 4.18F.

In summary, our two-year study revealed a higher relative abundance of plant pathogens in LSA compared to POM. Additionally, there was an elevated relative abundance of filamentous fungi, including saprotrophic ASVs, in LSA compared to POM. Notably, filamentous fungi such as *Penicillium*, *Verticillium*, and *Mortierella* exhibited higher relative abundance in LSA compared to POM. In both years, the relative abundance of beneficial fungi, including AMF and those with biocontrol activity, was higher in LSA compared to POM.

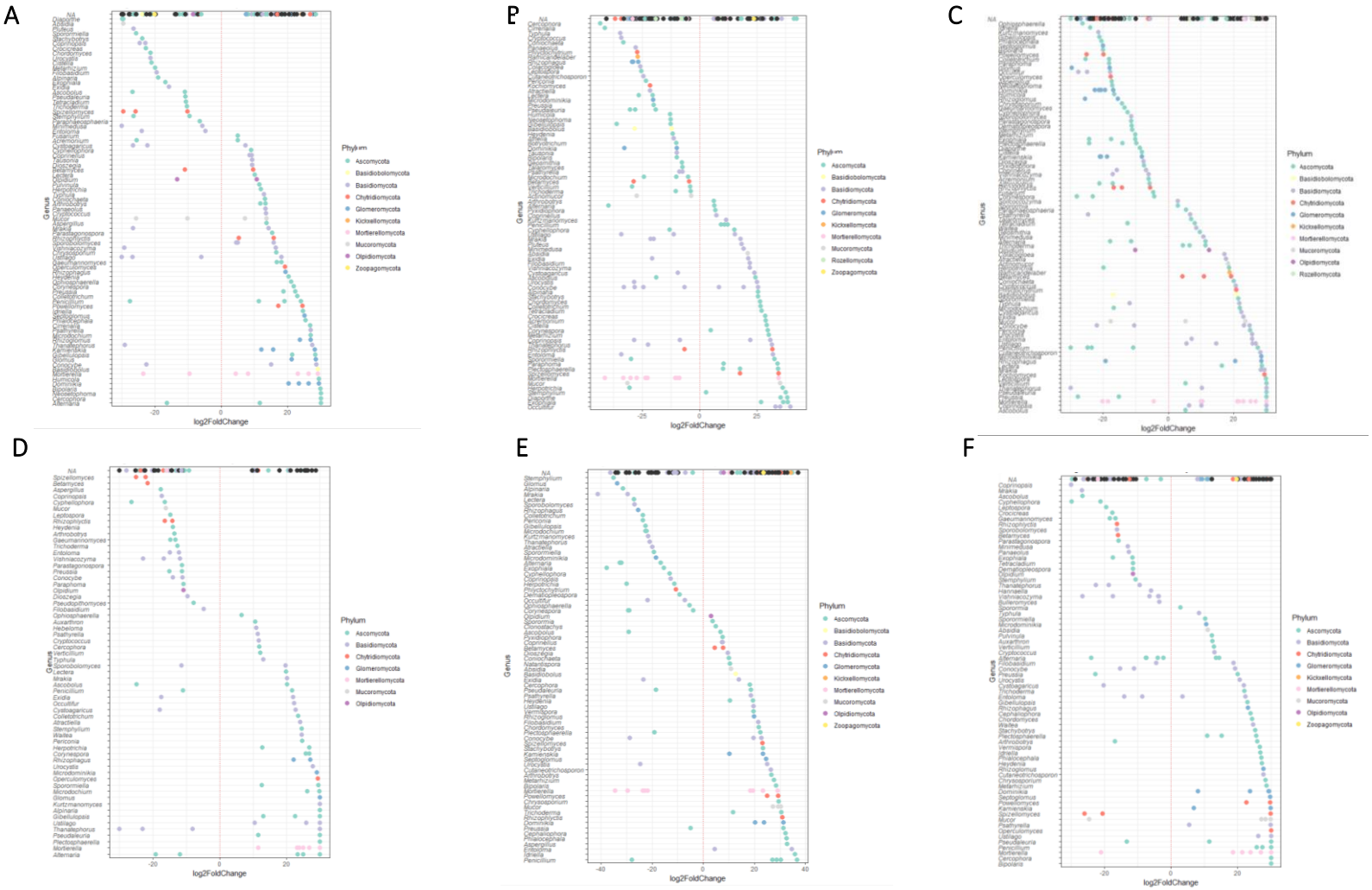

Figure 4. 18. Log2 fold changes of amplicon sequence variants (ASVs) in pairwise contrasts among crop species in rotation and soil fractions in the second year of soil sampling. ASVs are coloured by phylum. ASVs were differentially abundant among contrasts between A) corn field compared to canola field, B) soybean field compared to corn field, C) canola field compared to soybean field, D) large soil aggregates compared to small soil aggregates, E) small soil aggregates compared to particulate organic matter, and F) large soil aggregates particulate organic matter. Adjusted P for significance < .05 and threshold for the log2FC was set at 1.0.

5 Discussion

This study describes the effects of the rotation of three crops (corn, canola, and soybean) and four tillage regimes [conventional tillage (CT), deep tillage (DT), raised bed (RB), and vertical tillage (VT)] on the soil fungal community diversity and compositional structure in two consecutive years. In addition, the fungal community structure was evaluated within three distinct soil fractions (LSA, SSA, and POM) under these effects. In 2021, soil samples were collected during the spring, while in 2022, samples were collected in the fall. This allowed us to assess shifts in soil fungal communities across two distinct years in this study.

In both years of soil sampling, the analyses conducted to assess both Shannon and Simpson alpha diversity indices across various tillage treatments did not reveal statistically significant differences. However, as shown in Figure 4.7 and 4.8, the biodiversity tended to be higher in fields under VT and CT practice in 2021 and CT practice in 2022. In addition, both the Shannon and Simpson alpha diversity indices indicated reduced biodiversity in the RB system in both years of soil sampling. We also found a significantly higher alpha diversity in small and large soil aggregates compared to the particulate organic matters in both years of soil sampling. Moreover, in 2021, greater alpha diversity was observed in plots within the soybean field, whereas in 2022, plots within the corn field exhibited higher alpha diversity. In addition, Ascomycota and Basidiomycota were the dominant fungal phyla emerged as prominent players across all these treatments.

Our results showed that alpha diversity indices were higher in 2022 across all treatments. We also observed a higher relative abundance of plant pathogens and AMF within the treatments in 2022 compared to 2021. Previous studies indicate that microbial community composition may be significantly influenced by environmental factors such as temperature, water availability, and substrate quality (Voriskova et al., 2013). Seasonal processes govern the seasonality of soil carbon allocation and its accessibility to the soil biota. The belowground allocation of carbon through plant roots shows significant seasonal variations with a peak occurring during the late vegetative season. Shifts in the abundance of key fungal functional groups are expected to occur significantly when soil carbon is involved.

The drought experienced in 2021 (prior of our soil sampling in May), accompanied by high temperatures, could have adversely affected the growth and activity of fungal community particularly AMF. This condition might have created an inhospitable environment for AMF, leading to a decline in their abundance and diversity in 2021 in our soil samples. Furthermore, the presence of corn in the field at the time of sampling in 2022 could have contributed to the observed differences. The absence of harvest-related soil disturbance in the corn-covered plots might have provided a more stable and favorable environment for soil microbial communities. It is plausible that the altered environmental conditions and the presence of corn residues created a microenvironment favoring the proliferation of certain microbial groups.

5.1 Soil Disturbance by Tillage Impacts Nutrient Availability for Fungal Communities

In our system, practicing CT involved minimal soil disturbance (5 cm) using a highspeed disc, whereas other tillage systems caused more substantial soil disturbance. In soils under the VT practice, we had a moderate disturbance (15 cm) via rotary disc while soils in RB (raised bed maker) and DT (deep tiller) practices were highly disturbed (20 and 40 cm depth of tilling, respectively).

High-speed disc tillage at a 5 cm depth can effectively cut and incorporate crop residues into the soil. The residues are mixed with the topsoil, leading to their decomposition and gradual breakdown. This can improve soil organic matter content and may increase biodiversity. As the discs work close to the soil surface, they tend to bury or break down most of the crop residue. Indeed, incorporation of crop residues into the soil can serve as a nutrient-rich substrate for a diverse range of microorganisms. The availability of organic matter in the soil encourages a broader spectrum of microbial species to thrive.

In addition, several studies have demonstrated that tillage with higher level of disturbance can potentially disturb fungal mycelial networks and disrupt opportunistic decomposers as a result of soil disturbance (Mozafar et al 2000; Drijber et al 2000; Wortmann et al 2008). Kraut-Cohen et al. (2020) reported that in soils under no-tillage treatments, high levels of soil microbial activity are maintained. The absence of soil disturbance and high raw carbon sources availability could have favored the presence of saprotrophic fungi in topsoil. Our findings also indicate that biodiversity tended to be higher in soils subjected to low disturbance, specifically under the CT practice using a high-speed disc with a 5 cm disturbance. Several experimental studies have demonstrated that

soil fungal community diversity is greater in reduced or no-tillage systems when compared to systems involving intensive soil disturbance through tillage (Doran et al., 1998; Kandeler et al., 1999; Souza et al., 2018).

We found that soils under the VT and DT systems exhibited higher Shannon and Simpson alpha diversity indices in comparison to the RB systems. Many research studies have consistently shown that the utilization of deep tillers and rotary discs in deep tillage practices typically led to a reduction in fungal richness, primarily attributed to the disturbance of fungal mycelial networks (Lienhard et al., 2013; Essel et al., 2019; Wang et al., 2020; Orru et al., 2021). However, it is important to note that fungi also produce asexual or sexual spores in addition to hyphae. These spores provide them with increased resilience to tillage disturbance compared to hyphae alone (Guan et al., 2022). Furthermore, it is worth noting that tillage practices can lead to an increase in soil aeration (Khan 1996). This increased aeration can raise the oxygen content in the soil, thereby enhancing soil respiration (Bridgham and Richardson, 1992; Schlesinger and Andrews, 2000; Stępniewski and Stępniewska, 2009). Since the majority of soil-dwelling fungi are aerobic (Gruninger et al., 2014), the enhanced soil aeration in soils subjected to deep tillage practices can be advantageous for fungal growth (Guan et al., 2022*)*.

5.2 Soil Disturbance; Populations of AMF vs. Plant Pathogens

The relative abundance of AMF in phylum Glomeromycota in our study exhibited a higher relative abundance in CT (soil disturbance to 5 cm depth) compared to VT, RB and DT (soil disturbance to 15-40 cm depth). In our study, *Rhizoglomus* and *Glomus* had significantly lower relative abundance across all DT, RB and VT soils compared to the CT soils. These results are in line with previous research findings. For example, Brito et al (2012) reported that non-disturbed soils exhibited higher species richness of AMF. They speculated that in no-tillage or reduced tillage systems, the absence of disturbance might enable the development of a hyphal network. Therefore, the diversity of AMF in such systems could be influenced by the presence of intact mycelia.

Furthermore, studies have demonstrated that tillage affects all types of AMF propagules to varying degrees through multiple mechanisms: (i) disruption of the hyphal network; (ii) dilution of the propagule-rich topsoil; and (iii) accelerated root decomposition (Schalamuk and Cabello, 2010). Indeed, soil disturbance is a crucial factor that disrupts extraradical mycelium (Brito et al., 2012)

and incorporates surface residues into the soil profile (Kabir et al., 2002). This disturbance selectively interferes with different AMF. It can either promote or impair specific groups of AMF (Brito et al., 2012).

Moreover, tillage may consequently diminish soil mycorrhizal infectivity, leading to a reduction in AMF root colonization during the early stages of crop growth. The primary impact of soil disturbance on the decrease in AMF propagule density occurs when soil tillage disrupts the AMF hyphal network. In reduced tillage, an intact hyphal network may persist, while soil disturbance with deep tiller or rotary discs could result in damaged hyphae that are noninfective (Schalamuk and Cabello, 2010).

In our study, the implementation of CT with minimal soil disturbance revealed a notable reduction in the relative abundance of potentially pathogenic ASVs associated with plants. Various tillage practices have a significant impact on soil environments and influence the habitats of soil microorganisms (Zuber and Villamil, 2016). Non-disturbance of soil in no-till or reduced till practices likely promotes fungal hyphal growth, which is commonly observed. On the other hand, tillage practices with more soil disturbance, especially when crop residues are buried deeper into the soil, could negatively impact fungal communities. Several studies indicate that, in comparison to deep tillage, reduced tillage enhances microbial diversity (Chen et al., 2020; Schmidt et al., 2019). Furthermore, as rhizosphere microorganisms coevolve with plant root growth, they exert significant influences on plant nutrient uptake, soil carbon sequestration, pathogen suppression, and abiotic stress tolerance (Philippot et al., 2013; Wei et al., 2015). Reduced tillage, typically associated with retaining a minimum of 30% crop residues on the soil surface after planting (Conservation Tillage Information Center CTIC, 2004), may increase the biodiversity of microbial communities in the soil and soil organic carbon. In addition, as we discussed earlier, a higher abundance of AMF due to an intact hyphal network as well as available carbon source may persist in systems with reduced disturbance. Consequently, higher AMF populations are expected to reduce the success of plant pathogens. These may explain the higher abundance of plant pathogens, including *Fusarium*, *Alternaria*, *Pythium*, and *Rhizoctonia* in the soil with higher degree of disturbance.

In addition, our results indicated a notable increase of AMF ASVs in 2022 compared to 2021, with a significant impact on their relative abundance. One plausible explanation for this is the timing of soil sampling. In 2022, samples were collected immediately after a period of dense crop growth, potentially influencing the prevalence of AMF. In contrast, the 2021 samples were taken following a prolonged winter when no living host plants were present, suggesting a different ecological context that likely affected the AMF populations.

5.3 AMF; Soil Temperature

There is clear evidence indicating that the community structure and diversity of AMF, a group within the Glomeromycota phylum, plays a crucial role in enhancing plant growth performance (Hart and Forsythe 2012). It has been proposed that a high richness of AMF in agricultural ecosystems can contribute to improved crop performance (Verbruggen et al., 2013). A previous investigation has revealed that soil temperature exerts an influence on both the structure and distribution of the AMF hyphal network. This effect remains consistent across varying soil temperature conditions, including cooled soils (14°C), ambient soils (20°C), and warmed soils (26°C) (Hawkes et al., 2008, Higo et al., 2020). These could be possible reasons of higher relative abundance of Glomeromycota phylum in the CT soils in the second year of soil sampling. Tillage methods can impact soil temperature by modifying the surface conditions. In no-tillage and reduced-tillage systems, the presence of crop residues on the soil surface can mitigate the pace of soil temperature changes. This is due to the fact that these surface residues more efficiently reflect incoming solar radiation compared to bare soil, resulting in a more stable temperature environment (Li et al., 2013). The relative abundance of the Glomeromycota phylum in CT soils was significantly higher in the second year of soil sampling when compared to the other tillage systems. It also showed at very low abundance (rare phylum) in the first year of soil sampling (Table 4.1).

5.4 AMF; Plant Preference

Our findings revealed a lower relative abundance of the Glomeromycota phylum in plots within the canola field in the second year of soil sampling. This observation aligns with prior research that quantified the impact of canola cultivation intensity on the diversity of AMF in the soil (Floc'h et al., 2022; Town et al., 2023). Canola is among the minority of plants that do not form associations with AMF (Mocali et al., 2015).

Recent findings from amplicon sequencing have indicated that Glomeromycota members, particularly within the Glomeraceae family, are typically fungal constituents in various agricultural field settings (Higo et al., 2010; Vasar et al., 2017; Xiao et al., 2019; Higo et al., 2020). This predominance can be attributed to their proficiency in sporulation, which enables rapid recovery, and their adaptability to disrupted environmental conditions (Oehl et al., 2003). The adoption of crop rotation has a notable impact on the abundance of AMF in the soil. This influence becomes particularly pronounced after corn cultivation, leading to an augmentation in the total length of external mycelium and the number of spores (Moitinho et al., 2020). Additionally, there is an increase in the overall production of glomalin, a glycoprotein produced by fungi that plays a key role in soil aggregate formation, indicating the positive effects of crop rotation, especially after corn cultivation, on these important soil components (Kabir et al., 2002; Higo et al., 2017).

5.5 Plant Residue Degradation

According to some previous research studies, tillage regimes have a significant impact on soil fungi richness (Wang et al., 2010; Miura et al., 2013; Cohen et al., 2020). However, our findings suggest that there was only a slight difference in the composition of soil fungal communities among the various tillage treatments, which was consistent with other studies on fungal community composition and richness (Wang et al., 2021; Orru et al., 2021). In both years of our study, the analysis of differential abundance showed that most of the fungal ASVs whose relative abundances were significantly affected by various experimental contrasts were saprotrophic ASVs within the phylum Ascomycota and Basidiomycota. Additionally, results from taxonomical compositions of soil fungi at the phylum level further confirmed this fact (Table 4.1).

Ascomycota and Basidiomycota each harbor significant genera of saprotrophic fungi within their respective classifications. It is important to mention that their presence and behavior in the soil are notably affected by the plant species and the process of organic residue degradation (Boer et al., 2005; van Groenigen et al., 2010). VT (with a 15 cm deep rotary disc) and CT (with a 5 cm highspeed disc) systems created a favorable soil environment for saprotrophic fungi in the soil. This environment may facilitate the ability of saprotrophic fungi to exploit the readily decomposable portion of crop residues more effectively, thereby promoting their rapid growth (Maet al., 2013). This may explain a higher relative abundance of Ascomycota and Basidiomycota in soils under

these tillage systems. Higo et al (2020) also stated the rotary disc can effectively mix crop residues into the soil, promoting population growth of Ascomycota with a readily available source of organic matter for decomposition. Brennan et al. (2014) stated that in reduced tillage systems, the rates of crop residue decomposition and nutrient release into the soil tend to be slower when compared to tillage systems that cause high disturbance. This slower decomposition and nutrient release can have an impact on the structure of the soil fungal communities (Navarro-Noya et al., 2013).

Basidiomycota, the second dominant phylum in our study, includes many saprotrophic fungi. The members of Basidiomycota fungi are known for their ability to decompose complex organic matter in the environment, including wood, leaf litter, and other plant materials. Also, crop residues found in the soils under reduced tillage practice may contain a comparatively higher lignin content, potentially leading to an increased prevalence of the Basidiomycota phylum (Blackwood et al., 2007; Boer et al., 2005). Some Basidiomycota are also known for their ability to break down lignin and cellulose under anaerobic conditions. A possible reason for increasing the population of Basidiomycota in our study may be because the available substrates necessary to support fungal growth are primarily concentrated in the soil (Zhang et al., 2012). Additionally, reduced tillage practices (like CT in our experiment) may encourage the development of fungal hyphae by minimizing soil disturbance and enhancing specific soil physicochemical characteristics like moisture levels and aggregate size. These combined factors contribute to an increase in soil fungal populations in phylum Basidiomycota in soils under the CT as we observed in the second year of our soil sampling.

5.5.1 Microbiome Survival in Plant Residue

In 2022, Ascomycota exhibited a slightly higher relative abundance in the plots within the corn vs. canola fields. A higher relative abundance of Basidiomycota was also detected in plots within the soybean field vs. the canola and corn fields in 2022 (Table 4.1). In our study, crucial plant pathogenic ASVs were identified within the genera of the phylum Ascomycota. Notably, Basidiomycota exhibited a higher number of genera associated with saprotrophic functions.

The analysis of differential abundance also showed that plots within the soybean field compared to the plots within the corn and canola field had lower relative abundance of several plant pathogens (Figure 4.18B). Even the relative abundance of several plant pathogens in rare phyla

was lower in plots within the soybean field vs corn. For instance, plots planted to canola had higher relative abundance of *Olpidium* compared to plots planted to the soybean. We observed a greater relative abundance of *Mortierella* in plots within the soybean field, while plots within the corn field showed a significantly higher abundance of AMF. Despite limited AMF support in plots within the canola fields, the relative abundance of other beneficial fungi, including *Trichoderma* and *Talaromyces*, was significantly higher in canola plots. Thus, we found that different cropping systems can change the composition and structure of important fungal phyla such as Ascomycota, Basidiomycota, Mortierellomycota and Glomeromycota.

One possible explanation is associated with variations in the quantity and quality of crop residues within the cropping systems. In general, corn plant tends to leave a greater amount of plant residue in the field following harvest (Buyanovsky and Wagner 1986; Wang et al., 2021). The increased presence of crop residues in the corn field can result in a higher availability of carbon substrates. This increased availability is likely accountable for the rise in the population of fungi, such as Ascomycota which can more effectively exploit the readily decomposable fraction of crop residues compared to other fungi (Zhang et al., 2014). Furthermore, corn residues are characterized by high cellulose and hemicellulose contents, which may create a favorable environment for the growth of specific fungi with robust cellulase activity (Liang et al., 2017). Challacombe et al. (2019) demonstrated that members of the Ascomycota phylum possess multiple copies of carbohydrate-active enzymes (CAZymes) crucial for breaking down plant biomass. Their study revealed that all Ascomycota genomes in their study contain numerous genes with CAZyme domains, encompassing all the necessary enzymes for the decomposition of various types of hemicellulose, including xylan, xyloglucan, mannan, and galactomannan. This likely explains the higher relative abundance of Ascomycota in the corn field compared to the soybean field.

Furthermore, in our study, a plot combine was employed to harvest crops from all three fields. When harvesting soybeans with a plot combine, residual plant material remains in the field after the soybeans have been harvested. This residue encompasses stems, leaves, and any other parts of the soybean plants that were not gathered during the harvest. This also can potentially explain the greater relative abundance of the *Mortierella* phylum in the soybean field compared to canola and corn in the second year of soil sampling. *Mortierella* recognized as fast-growing fungi that

predominantly rely on easily decomposable carbon compounds (Espana et al., 2011). In addition, *Mortierella* species are recognized as significant contributors to the decomposition of organic residues and utilize readily decomposable carbon compounds in soybean treatments (Montgomery et al., 2000, Kjøller and Struwe, 2002). Moreover, the slightly higher relative abundance of Ascomycota in the corn field compared to the canola field is likely attributed to the presence of simpler compounds like starch in canola residue (Pascault et al., 2010). According to Kerdraon et al. (2019), the residues in canola contain simpler compounds, while corn residues consist of more complex compounds, including cellulose and lignin (Fathallah Eida et al., 2012). Therefore, these complex compounds in corn residues break down less quickly, resulting in a relatively higher prevalence of Ascomycota in corn fields compared to canola fields. Ascomycota are well-known cellulose decomposers and thrive when complex carbon compounds take longer to decompose. In addition, although members of the phylum Basidiomycota are also recognized for their cellulose-degrading capabilities, they are primarily known as lignocellulose decomposers. These fungi exhibit their ability to break down not only lignin but also carbohydrates in various types of litter. Soybean residues, with a higher proportion of readily available carbohydrates, tend to promote the proliferation of fungal decomposers that specialize in the utilization of easily decomposable carbon compounds (Roldan et al., 2007; Marschner et al., 2003). This also likely can explain the higher relative abundance of Basidiomycota in the soybean field compared to the corn field in both years of soil sampling. In addition, specialized plant compounds play a crucial role in helping crops resist diseases. For example, Benzoxazinoids are common and extensively researched chemical defenses found in grasses. Studies showed that Benzoxazinoids play a role in protecting against fungi, including northern corn leaf blight (Ding et al., 2020).

5.6 Microbiome; Plots in the Corn Field

The corn microbiome has evolved into an exceptionally diverse and environment-specific ecosystem (Singh and Goodwin 2022). The below-ground microbiome of corn primarily includes microorganisms at the plant-soil interface, encompassing the rhizoplane and rhizosphere, along with the surrounding soils and root endosphere. Several studies have demonstrated significant differences in the relative abundance of fungi within the orders Agaricales, Mortierellales, and Pleosporales, as well as unidentified species of Ascomycota in aerial roots and bulk soil

microbiomes within the fields planted to corn (Wang et al., 2016). In our study, the fungal ASVs whose relative abundance strongly affected in plots within the corn field belonged to the orders Agaricales, Mortierellales, and Pleosporales, along with unidentified species of Ascomycota, further confirming findings from previous investigations.

In addition, fungal communities within corn residues typically consist of saprotrophic, phytopathogenic, and mycotoxin-producing fungi, belonging to the classes Dothideomycetes and Sordariomycetes (Szilagyi-Zecchin et al., 2016). In our study, *Verticillium* and unclassified taxa within the Sordariomycetes class, along with *Herpotrichia* within the Dothideomycetes class, had higher relative abundance in plots within the corn field. Furthermore, studies consistently affirm that *Alternaria*, *Paraphaeosphaeria*, and *Fusarium* are frequently identified in corn residues (Singh and Goodwin, 2022). For instance, corn residue post-harvest represents micro-scale ecosystems that support associated fungal communities both externally and internally, showcasing mutualistic and pathogenic activities. Fungi belonging to the phylum Ascomycota dominate the corn residue microbiome. Xing et al., (2018) showed within this phylum, species including *Aspergillus niger*, *A. flavus, Bipolaris zeicola, Chaetomium murorum, Cladosporium sphaerospermum, Penicillium aurantiogriseum*, and *Trichoderma gamsii* were exclusively isolated from residue of corn. Moreover, species including *Alternaria alternata*, *Fusarium verticillioides, F. proliferatum, Penicillium oxalicum,* and *P. polonicum* were found in both external and internal corn residues (Xing et al., 2018).

5.7 Microbiome; Plots in the Soybean Field

Several studies have demonstrated that the continuous cultivation of soybeans leads to an increase in the populations of *Alternaria* and *Fusarium* (Liu et al., 2019). *Fusarium* is recognized as a pathogenic fungus that causes Fusarium root rot in soybeans (Chang et al., 2018). Liu et al. (2019) also demonstrated a positive correlation between *Alternaria* and the incidence of soybean root disease. In our study, *Alternaria* had a higher relative abundance in plots within the soybean fields compared to the plots in either the corn or the canola field. Additionally, there was a significantly lower relative abundance of plant pathogens such as *Ustilago* and *Bipolaris* in plots within the soybean field, aligning with findings from other studies. This phenomenon is likely attributed to the preference of *Ustilago* and *Bipolaris* for infecting gramineous crops. Consequently, this may

explain their lower relative abundance in the soybean field. Furthermore, we noted a higher relative abundance of *Penicillium* and *Metarhizium* in soybean fields. While certain *Penicillium* species can indeed cause crop diseases, many species within the *Penicillium* genus have been identified as beneficial fungi. These beneficial species have the capability to inhibit the growth of pathogenic organisms, contributing to the control of plant diseases. Liu et al. (2019) similarly noted a higher relative abundance of *Metarhizium* in long-term soybean cropping systems. Additionally, we observed that the relative abundance *Corynespora*, a genus predominantly comprising plant pathogens, was significantly higher in plots within the soybean field. Notably, a fungal pathogen belonging to the genus *Corynespora* is identified as the causal agent of soybean frogeye leaf spot disease (Dashiell and Akem 1991).

5.8 Microbiome; Plots in the Canola Field

In our study, plots in canola field did not support AMF populations very well. Our finding showed that the relative abundance of other beneficial fungi such as *Talaromyces*, *Trichoderma*, and *Exophiala* increased significantly in the canola field. Floc'h et al. (2020) reported that the core microbiome of canola comprised two potentially endophytic taxa: *Trichoderma koningii* and *Exophiala* sp. The genus *Trichoderma* is recognized for its antagonistic and hyperparasitic capabilities, making certain strains suitable for use as biocontrol agents. Notably, *Trichoderma* was reported to exhibit a suppressive effect on *Fusarium* spp., *Alternaria* spp., and *Sclerotinia* spp., pathogens known to affect canola (Hirpara et al., 2017). Fungi within the genus *Trichoderma* have been documented to enhance the growth and development of both monocotyledonous and dicotyledonous plants, leading to increased yields. This positive impact on plant growth is attributed to various mechanisms, including the production of phytohormones and siderophores, as well as the facilitation of the solubilization and mineralization of micro- and macro-elements (Contreras-Cornejo et al., 2011).

Exophiala belongs to the category of dark septate endophytes (DSE). Some DSE are recognized for establishing symbiotic relationships that enhance the overall fitness of their host plants. The genus *Exophiala* has been demonstrated to enhance drought resistance in sorghum (Zhang et al., 2017). In 2020, Floc'h et al. reported the presence of this DSE in the canola rhizosphere for the first time.

5.8.1 *Olpidium brassicae*; Canola Field

Previous studies have revealed that *Olpidium brassicae* is notably abundant in the rhizosphere of canola (Lay et al., 2018). Studies consistently identified *O. brassicae* as primarily a plant root pathogen of the Brassicaceae family (Hilton et al., 2013; Lay et al., 2018). Li et al. (2023) have identified the presence of *O. brassicae* in both the root and rhizosphere of *Brassica napus*. Notably, in monoculture systems, it is frequently the most dominant species in the roots of *B. napus* plants (Hilton et al., 2013). Our study also validated these observations, as we found that the relative abundance of *Olpidium* was significantly higher in plots within the canola field compared to the corn or soybean fields. *O. brassicae* is an obligate root-infecting pathogen, consistently present in the roots and rhizosphere of canola. *O. brassicae* can endure for extended periods in the soil because the causal agent is carried within the resting spores of *O. brassicae*. (Li et al., 2023).

5.9 Fusarium and *Alternaria*; Relative Abundance

At the genus level, *Fusarium* showed a higher relative abundance in plots within the canola compared to the plots in soybean field. We also found that plots within the corn had higher relative abundance of same ASVs (Fusarium, ASV 70) compared to the plots within canola. This observation aligns with findings from the literatures, which have identified the dominance of the genus *Fusarium* in the rhizosphere of canola plants (Gkarmiri et al., 2017; Lay et al., 2018). The colonization of the canola rhizosphere by *Fusarium* has the potential to result in yield losses, although this is not always the case (Fernandez 2007; Fernandez et al., 2008). *Fusarium* can also adapt to soils that are abundant in organic matter and function as a saprotroph without necessarily inducing plant diseases (Hamel et al., 2005; Yergeau et al., 2006; Floc'h et al., 2020). Residues from both corn and canola can contribute to an increase in soil organic matter (Abdullah 2014). In addition, several species of *Fusarium* have been associated with diseases of corn at all growth stages, causing seed, root, stalk, and ear rot (Dodd et al., 1999; Broders et al., 2007). Broders et al. (2007) reported a higher level of pathogenicity associated with *Fusarium* in the context of corn compared to soybean. In their experiment, they recovered 112 isolates of *Fusarium* from inoculated plants, and all of these isolates exhibited high pathogenicity on corn seeds and moderate pathogenicity on soybean seeds. These potential reasons could explain the higher relative abundance of *Fusarium* in the corn and canola fields compared to the soybean field.

In addition, research has demonstrated that short-term continuous soybean cropping results in an elevation in the relative abundance of *Alternaria* (Song et al., 2022). *Alternaria* is the predominant pathogenic fungi in soybean fields (Bai et al., 2015; Cheng et al., 2017). In our study, the analysis of differential abundance also showed that relative abundance of *Alternaria* was higher in plots within the soybean field compared to plots within canola field. *Alternaria* has the capacity to infect a range of crops, leading to associated diseases, such as soybean Alternaria leaf spot (Li and Yang, 2009; Logrieco et al., 2009).

5.10 Gibellulopsis; Relative Abundance

The genus *Gibellulopsis* comprises saprotrophic fungi and opportunistic plant pathogens, including *Gibellulopsis nigrescens* (formerly known as *Verticillium nigrescens* Pethybridge) (Zare et al., 2007). This species is responsible for causing vascular wilt diseases in a wide range of host plants and is capable of surviving in soil or on deceased plant material (Klosterman et al., 2011). In our years of study, the relative abundance of *Gibellulopsis* was higher in plots within the corn fields compared to the plots in canola field. *Gibellulopsis* species are consistently found at notably high levels in agricultural soils, as indicated by various microbiome studies (Idbella and Bonanomi 2023; Kawaradani et al., 2013). *Gibellulopsis* is known to cause seedling rot, and the symptoms observed, such as the appearance of light brown spots on the lower leaves of seedlings followed by leaf blight or rot, resemble those typically associated with root rot in plants (Tian et al.,2021). It is probable that these fungi are integral components of the typical microbial community in agricultural soils, especially in areas where corn, canola, and soybean rotation practices are in effect (Idbella and Bonanomi 2023).

5.11 Filamentous Fungi; Soil Aggregates

The analysis of differential abundance showed a higher relative abundance of *Aspergillus*, *Penicillium*, *Fusarium*, and *Verticillium* in LSA and SSA compared to the POM in both years. We also observed a higher relative abundance of Mortierellomycota in the SSA and LSA compared to the POM. Filamentous fungi are present in a wide array of terrestrial environments, existing as free-living microbes, symbionts, commensals, and pathogens. The documented contributions of filamentous fungi to soil aggregation highlight their significant role in this ecological process (Lehmann et al., 2020). Filamentous fungi exhibit a well-documented influence on soil structure,

particularly at the macroaggregate (>250 µm) scale. The capacity of filamentous fungi to aggregate soil is hypothesized to result from a variety of physical, chemical, and biotic traits (Six et al., 2004). As these fungi forage and grow through the soil, their filamentous growth form is believed to entangle and enmesh soil particles and aggregates (Lehmann et al., 2020). Filamentous fungi further release extracellular biopolymers, functioning as both cements and surface sealants for soil aggregates (Daynes et al., 2012). Additionally, they produce enzymes that degrade organic matter (Lehmann et al., 2020), potentially acting as agents to disintegrate aggregates. Among the molecules released are hydrophobins, known to alter the wettability of aggregates, likely playing a stabilizing role (Zheng et al., 2016). This may explain the significantly higher relative abundance of filamentous Ascomycota and Basidiomycota fungi in soil aggregates, particularly in LSA, compared to the POM.

In addition, we also found that alpha diversity was significantly higher in small and large soil aggregates compared to the POM in both years of study. Microbial access to soil organic matter represents a critical limitation that impacts the decomposition, as well as the cycling and retention of carbon (C) and nitrogen (N) in soil (Schimel and Schaeffer, 2012; Lehmann and Kleber, 2015). Organic matter has the potential to become embedded within soil aggregates, which can vary in size from larger than 2 mm to smaller than 0.25 mm (Tisdall and Oades, 1982). Therefore, the physical separation within aggregates can restrict microbial access to organic matter. Once microorganisms establish themselves within an aggregate, the composition and metabolic capabilities of the microbial community can significantly influence the processing and preservation of soil organic matter (Bach et al., 2018).

Soil aggregates, along with the pores within and around them, generate microhabitats that provide a conducive environment for diverse microbial communities (Ruamps et al., 2011; Rabbi et al., 2016). In these microhabitats, disparities in the abundance and chemical composition of organic substrates are highly probable factors contributing to variations in microbial community composition (Gupta and Germida, 1988; Hattori, 1988; Davinic et al., 2012; Lagomarsino et al., 2012; Smith et al., 2014). Moreover, variations in environmental conditions, including oxygen concentration, exist within and between aggregates, creating a spectrum of niches that accommodate distinct groups of microorganisms (Bach et al., 2018). Several studies also have

demonstrated that lower total levels of C and N, as well as a reduced carbon-to-nitrogen (C:N) ratio within microaggregates, are indicative of a higher degree of processing of organic matter by microorganisms (Six et al., 2000; Grandy and Neff, 2008; Hofmockel et al., 2011). The variations observed in microbial communities among different aggregate fractions are a reflection of the distinctions in C and N content, as well as the cycling processes within these specific aggregate fractions. According to Bach et al. (2018), it was found that there is greater extracellular enzyme activity related to cellulose degradation (such as β-glucosidase and cellohydrobiolase) within microaggregates, while higher enzyme activity associated with nitrogen cycling is observed in large macroaggregates.

6 Conclusions

In this study, we analyzed the impact of combined tillage and crop rotation regimes on soil fungal communities within the three soil fractions. Soils under tillage treatments and crop rotation showed dense populations of fungal decomposers and plant pathogens mostly represented by the phylum Ascomycota and Basidiomycota. Higher biodiversity of fungi characterized soils under lower disturbance. Among the soil fractions, we found that soil aggregates had significantly higher fungal diversity compared to the POM.

The analysis of differential abundance revealed that although there was a limited AMF support in canola fields, beneficial fungi including *Trichoderma* had significantly higher relative abundance in canola plots. We know that canola is among the minority of plants that do not form associations with AMF. Hence, we conclude that the cultivation of canola in the field may impact negatively on these essential symbiotic organisms, creating an opportunistic environment for other microorganisms to proliferate in the absence of AMF. We also found plots within the corn field had a great support of AMF while plots within the soybean field had significantly higher relative abundance of important saprotrophic fungi such as of *Mortierella* and *Vishniacozyma.*

The results in our study suggest that quantity and quality of crop residues after harvest within our fields impact the soil fungal communities. The presence of crop residues in the fields in our study can result in a higher availability of carbon substrates which may be responsible for the increase in soil fungal population.

In our study, rotary disc and raised bed maker soil management disturbed the soil profile up to 20-40 cm. This magnitude of disturbance could potentially create an inhospitable environment for numerous AMF and other fungal mycelial networks. Intensive soil disturbance can also impact AMF propagules by disrupting the hyphal network and causing a dilution of their propagules within the soil. In contrast, in soils subjected to CT and VT practices, the use of high-speed disc and rotary disc disturbed the soil profile much less, to depths of only 5-15 cm. This not only facilitates the efficient cutting and incorporation of crop residues into the soil but also ensures the maintenance of high level of soil microbial activity. Therefore, a higher soil fungal diversity was observed in these soils with low level of disturbance.

Our study revealed higher relative abundance of filamentous fungi in LSA and SSA compared to the POM in both years. Soil aggregates provide a more stable and favorable microenvironment for filamentous fungi. The structure of aggregates offers protection from external stresses, such as fluctuations in temperature and moisture, creating conditions conducive to fungal growth. The intricate network of hyphae can efficiently explore and exploit resources within the soil aggregates, allowing the fungi to access nutrients and organic matter. Additionally, filamentous fungi can engage in relationships with other microorganisms, further enhancing their ability to thrive within soil aggregates compared to the POM.

Future studies should focus on the differences in carbon resources in soils under these tillage practice combined with crop rotation to accurately evaluate the relationship between organic carbon source utilization and fungal communities. Moreover, more studies are necessary to uncover the roles of soil fungi in the overall health of the soil. Also, understanding of how deliberate interventions can optimize soil microbial communities and may hold the key to unlocking enhanced agricultural productivity.

7 Appendix

Appendix A: Average soil properties in different fields (corn, canola, soybean) for the Years 2021 and 2022.

Table A1. The table presents the average values of pH, nutrient concentrations (N, P, K, S), and soil texture (Sand, Silt, Clay percentages) for each field (corn, canola, and soybean) during the years 2021 and 2022.

Year	Field	pH	N (ppm)	P (ppm)	K (ppm)	S (ppm)	Sand (%)	Silt (%)	Clay (%)
2021	Corn	7.83	22.15	23	435.59	14.35	19	45.93	35.06
	Canola	7.79	17.37	21.56	447.12	40	27.15	40.71	32.12
	Soybean	7.63	66.40	35.15	585.5	34.38	25.18	44.43	30.37
2022	Corn	7.83	11.12	25.25	272.31	10.25	22.37	44.37	33.25
	Canola	7.85	9.96	13.5	373.25	14.5	20.75	46.75	32.5
	Soybean	7.93	10.40	7.56	272.31	31.76	25.87	42	32.12

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