EFFECTS OF INTENSIVE AGRICULTURAL MANAGEMENT PRACTICES ON SOIL MICROBIAL ASSEMBLY AND RECRUITMENT

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

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ABSTRACT

Soil microbial communities play an important role in ecosystems in various ways to promote healthy and fertile soil. However, intensive agricultural practices with excessive tillage and fertilizer applications can affect the abundance and community structure of microbial communities in soil as well as their assembly and recruitment by plant roots. Using amplicon sequencing and microscopy, we have examined bacterial and fungal communities under different tillage and fertilizer treatments in a 34-year-old field-trial at the Carrington Research Extension Center of NDSU. We observed that fertilizer application has a significantly stronger effect than tillage on soil properties, as well as the overall soil microbial abundance and community structure. Significantly higher mycorrhizal colonization was found under organic manure application. Overall, the results of this study can improve our understanding of the effects of fertilizer application on soil microbial communities and how management practices can be optimized to reduce the imprints of intensive agriculture.

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INTRODUCTION

Intensive Agriculture

Over the last century, intensive agricultural techniques spread fast all over the world with the induction of farm machines run by internal combustion engines and the availability of commercially produced fertilizers, pesticides, and herbicides (www2.palomar.edu). Agricultural intensification is a configuration of change in land utilization with a common aspect of improved use of the same resources for agrarian production (Meyer and Turner, 1992; Schneider et al., 2022; Spangler et al., 2020). This is typically a result of a shift from occasional to continuous farming of the same area of land. Some accompanying things are change in utilized crop or livestock, improved supervision, involvement and greater reliance on markets (Matson et al., 1997). In order to support the growing human population, total cultivated land area in the world has increased over 500% in last five decades. The usage of fertilizer has been increased 700% and pesticide usage has increased several folds than before (Aktar et al., 2009; Srivastava et al., 2021; gro-intelligence.com). In this way, intensive agriculture has fostered an extensive array of environmental distresses, for example- poor nutrient-use efficacy, greenhouse gas emissions, groundwater contamination and surface water eutrophication, degradation of soil quality, soil erosion etc., and has turned out to be one of the most persistent problems of twenty-first century (Matson et al., 1997).

Agricultural intensification started in the 1960s in the developing countries under an overall banner of “The Green Revolution”, with allocation and distribution of seeds with a high yield capacity (Cycon, 2013; Sebby, 2010; www.toppr.com). The Green Revolution is a notable achievement of science and technology based on agricultural intensification which was achieved through utilization of varieties of crops with high production rate, chemical fertilizers, pesticides,

irrigation, and automation (Matson et al., 1997). Agricultural intensification has many environmental consequences, such as- increased soil erosion, lowered soil fertility, reduced soil biodiversity, groundwater pollution, eutrophication of water bodies, and adverse impacts on climate (Tilman, 1999; www.fao.org). Whether a longstanding intensive agriculture can be maintained, is growing concern. For instance, the conservative rice-wheat cropping system in Punjab, India, have started to show signs of a serious deterioration with damaged soil quality and additional plant health issues (N. Kumar et al., 2021). One important attribute of intensive agriculture is monoculture which shapes the abundance and composition of soil microorganisms and soil invertebrates, and thus, affects plant and soil developments (Joos and De Tender, 2022; Tibbett et al., 2020). Because of intensive agriculture, richness in terrestrial species gets relegated and that leads to variation in community composition of crop-attacking microbes and herbivorous insects along with their natural predators and parasites. Monoculture leads to insect pest which is less diverse but profuse and results in more crop loss. However, the aptitude of crop diversity to oversee insect and microbial pests still need to be developed widely (Matson et al., 1997).

Over five million tons of pesticides are being spread over in agricultural fields globally for pest control, which is causing pesticide resistance (Matson et al., 1997; Sud, 2020). This has become an omnipresent issue; and as pesticide gets transported to water and air, nature and human health are under threat. This large quantity of pesticides is altering the natural composition, abundance, and function of soil microbial community (Muturi et al., 2017). Intensive farming is responsible for loss of soil organic matter and decreasing the amount of soil carbon linked with cultivation (Matson et al., 1997). Tons of chemical inorganic fertilizers are used in conventional agriculture. Nitrogen leaches from excess nitrate fertilizer application into water bodies from soil and increases nitrate concentration in surface water system as well as in drinking water (Maghanga

et al., 2013)- leading to methemoglobinemia which is a severe concern for human health. Because of eutrophication, oxygen level drops in stratified water, leading to algal bloom, loss of fish and shellfish, and rise of organism toxic to fish. Inorganic fertilizers increase emission of harmful gases which are responsible for air pollution (www.water-pollution.org.uk; www.epa.gov). For example, nitrogen oxides get released from the farmland soils and reacts with atmospheric ozone to create smog which is not only harmful for human health, but also for crop health and ecosystem (Chameides et al., 1994). Nitrogen oxides and ammonia emitted from farmlands get transferred and piled up in terrestrial and aquatic ecosystems and give rise to acidification, eutrophication, and change species diversity besides effecting the predator and parasite system (Galloway et al., 1995). At the end, agricultural lands where inorganic chemical fertilizers are used for intensive farming, work as an important source of greenhouse gases including carbon dioxide, methane, and nitrous oxide (Galloway et al., 1995).

Tillage is employed to make the soil ready for sowing and to control weeds and pests and has been a part of crop farming for a long time. Intensive tillage practice can increase the chances of soil erosion, runoff of nutrient in adjacent water bodies, and emission of greenhouse gases in atmosphere (Bhattacharyya et al., 2022). However, if the frequency and intensity of tillage is brought down, cropland soil can preserve more organic matter, becomes less prone to erosion, and helps in carbon storage. No-tillage is commonly the least rigorous form of tillage and conventional tillage is the most intensive form. Conservation tillage is less intensive than conventional tillage (www.ers.usda.gov). Even though the notion of tillage is to administer soil in a workable kind for farming, nonstop apply of conventional tillage has steered to some adverse effects such as loss of organic matter, degradation of soil mass, loss of carbon fixed in soil in humus form, drop in useful soil microbes and other macroorganisms etc. (Gupta and Seth, 2007). Moreover, conventional

tillage practice has amplified soil erosion, and liberation of greenhouse gas (Alam et al., 2014; Subbulakshmi et al., 2009). Conversely, reduced tillage was found to be the utmost appropriate tillage preference under a rice-based branched-out cropping system in India (Kar et al., 2021). Distress about supporting a fast-developing human population and easing hunger is mounting simultaneously as other environmental apprehensions are increasing. According to the calculations of demographers, population will rise between 8 billion to 10 billion in the 21st century (www.un.org). As a result, agricultural intensification continues to be a foremost focus of research (Matson et al., 1997).

Sustainable Agriculture

Soil is not just important for growing crops, rather it is complex and diverse, and demands to be cared for to make sure that production is long-lasting and steady. Importance of soil needs to be understood to get a solid perception about the justification of sustainable agriculture (Reganold et al., 1990). A sustainable agriculture aims to protect the nature, to multiply natural resource of earth, and to preserve and develop soil fertility (nifa.usda.gov). Sustainable agriculture pursues to increase production to meet food and fiber need of human population, develop, and support a healthy soil, a thoughtful water management, decreasing water and climate pollution, and uphold biodiversity (www.ucsusa.org; nifa.usda.gov). Sustainable agriculture involves a broad variety of production systems which are both conventional and organic. Examples of some key sustainable agricultural practices are- crop rotation, cultivating cover crops, reducing tillage, using an integrated pest management, taking up agroforestry systems, managing landscapes etc. (nifa.usda.gov). Crop rotation involves sowing a variety of crops, which might be beneficial for a healthy soil and pest control. Intercropping and a complex multi-year crop rotation are some decent examples of practicing crop diversity (www.nrcs.usda.gov; rodaleinstitute.org; Bybee-Finley and

Ryan, 2018). Cover crops such as clover, milk vetch, hairy vetch protect soil health and fertility by preventing erosion, and replenishing soil nutrients (www.almanac.com; extension.psu.edu; www.ufseeds.com). These plants can be raised in off-season, and they are able to keep off weed, thus, herbicidal usage can be lessened (www.farmers.gov; lgpress.clemson.edu; extension.psu.edu). Conventional tillage is responsible for a great soil loss. No tillage or reduced tillage aid to stop soil erosion and recover soil health (Zuazo et al., 2020; www.nrcs.usda.gov). By a sustainable farming practice, uncultivated or less intensively cultivated areas such as buffer and strip regions between two farmlands are treated vital to a farm (www.eos.com; www.fao.org). Those uncultivated lands are appreciated for their contribution in controlling soil erosion, reducing runoff of nutrients, and supporting pollinators and other biodiversity (nifa.usda.gov).

Agricultural Microbiomes and Ecological Intensification

By 2050, global population is going to increase by at least 2.3 billion. Due to the growing human population, the need for agricultural crops is increasing and it will keep going for decades (Tilman et al., 2011). To meet such a high demand, clearing lands and more intense usage of farmlands have been useful, however, how these alternative paths contribute to agricultural development is still under research. Some major impressions of agriculture on global environment include jeopardizing biodiversity by destruction of habitation and land clearing, and emission of greenhouse gas due to cultivation, land clearing and fertilization (Alinovi et al., 2008). That is why, it is important to realize the environmental impacts of a huge amount of crop production for upcoming population, how different crop farming practices can affect crop yield along with several ecological variables (Pretty and Bharucha, 2014). Awareness for agricultural sustainability started to emerge in the 1950s and 1960s (Pretty, 2008). Over the 20th century, as agricultural intensification rose significantly, use of synthetic pesticides and fertilizers also increased two-fold

(Pretty, 2005). The sheer need of more food production without damaging ecosystem demand a wide range of diverse types of agriculture which are more sustainable. The key features of this sustainable production scheme include using crop variabilities of high productivity rate, sidestepping excessive use of chemical fertilizers, pesticides, insecticides, fungicides, and herbicides, utilizing various ecological processes such as nutrient cycles, nitrogen fixation, predation, parasitism, reducing or minimizing practices or technologies which have unfavorable effects on nature and human health, implementing water, soil, and pest management to resolve system-wide issues, minimizing emission of greenhouse gas, maintaining biodiversity, unpolluted water, sequestering carbon, and disbanding pests, weeds, and pathogens (Pretty, 2008; Warwick, 2009). These distinct and harmonious approaches with superior aptitude and awareness display qualities that discriminate them from the procedures and consequences of a conventional farming system. Sustainable intensification is a process where production is increased without adverse environmental effects and without tilling extra lands (Pretty and Bharucha, 2014). The fundamental principles of sustainable intensification are- developing powerful systems for a strong and effective food system by increasing food production and reducing wastage of food, (Foley et al., 2011; Prosekov and Ivanova, 2018), saving biodiversity by reducing emission of greenhouse gas and reflecting other possible concerns (Ki-Moon, 2009; Stern, 2013).

SOIL MICROBIOME

Soil Microbiota

Soil is complex, dynamic, and contains an unprecedented number and diversity of microorganisms. These prokaryotic and eukaryotic soil microorganisms interact with each other as well as the other soil components (Islam and Wright, 2004). Being the most biologically diverse habitat on earth, there can be at least 10 billion of microorganisms in one gram of soil (Carey, 2016). Although soil contains a huge density and diversity of microbes, most of them still need to be explored (Torsvik and Øvreås, 2002). According to current literature, microbiomes now consist of bacteria, fungi, archaea, and protists (Berg et al., 2020). One of the most heterogenous and adapted groups in soil are bacteria. They are prokaryotic, unicellular, and of different shapes and size ranging from 0.1 to 5.0 micrometers (Fierer and Jackson, 2006). On the contrary, soil fungi are eukaryotic. Their filamentous branching makes them crucial for soil microbial community and various soil metabolic activities. Soil microbes are the cornerstone in soil and cover a large portion of the genetic diversity of earth. Their role is important in many critically important ecosystem processes such as nutrient acquisition, soil formation, regulation of soil fertility, plant health, and biogeochemical cycles like carbon and nitrogen cycle (Fierer et al., 2012; van der Heijden et al., 2008). Despite their crucial role on nature and ecosystem, how soil microbes influence crop yield and productivity remains underexplored. A single gram of soil can contain billions of bacteria, and up to two hundred meters of fungal hyphae (Van Der Heijden et al., 2008). Mycorrhiza are a mutualistic symbiosis between plant roots and soil fungi (Genre et al., 2020). Two main categories of mycorrhiza are: ectomycorrhiza and endomycorrhiza. Soil fungi receives carbohydrate from their host plants through ectomycorrhizal association and in return, improves mineral and water uptake to their host plants. Ectomycorrhizal fungi protects plant roots from pathogens and other

abiotic environmental stresses (Amaranthus, 1998). Endomycorrhizal fungi take part in nutrient exchange between soil and their hot plants. Endomycorrhiza is commonly known as Arbuscular Mycorrhizal Fungi or AMF. Positive effects of AM fungi on plant performance and soil strength makes them indispensable for a sustainable agricultural ecosystem (Gianinazzi et al., 2010a). Recent literature show that AM fungi can reduce the amount of plant nutrients leached from soil, and the amount of N2O released from soil during denitrification (Bender et al., 2016). AM fungi also influence on soil phosphorus availability indirectly by boosting the activity of phosphate-solubilizing rhizobacteria (Chen et al., 2022; Wahid et al., 2022).

Impacts of Soil Microbes on Plant Productivity

Soil microbes can directly affect plants by forming a mutualistic or pathogenic relationship with them via organisms associated with plant root system. Beside this, free-living soil microbes can indirectly affect plants by shifting nutrient supply rate and resource partitioning (Schimel and Bennett, 2004). Nitrogen-fixing symbiotic soil microbes provide limiting nutrients by forming a close symbiotic correlation with plants to promote plant productivity. Soil microbes produce exoenzymes and split complex insoluble proteins from dead organic matter into dissolved organic nitrogen. Plants gather up dissolved organic nitrogen in the form of amino acid straight from soil (Cleveland et al., 1999; Schimel and Bennett, 2004). Soil microbes can impact on plant productivity by solubilizing different forms of precipitated phosphorus, secreting organic acid to erode soil and protecting plants by subduing many plant diseases (Kucey, 1983; Renske Landeweert et al., 2013; Weller et al., 2002). Thus, prompting nutrient availability to plants, soil microbes influence nutrient storage in ecosystem and sequential partitioning of nutrients between plants and soil microbial pool. However, soil microbes can also reduce plant productivity by acting

as soil pathogens, rivalling with plants for soil nutrients, and by lowering nitrogen availability in ecosystem (Van Der Heijden et al., 2008).

The Role of Soil Microbiomes in Sustainable Agriculture

Generally, microorganisms that promote plant growth and attain nutrients, are used widely in agriculture to reduce the application of inorganic fertilizers. However, researchers have revealed that microbial groups with a discrete operative ecological niche play a critical role in holding and absorbing inorganic nutrients to plant surface and split organic matter to integrate them into soil (Finkel et al., 2017; Kumar and Dubey, 2020; Lakshmanan et al., 2014). Previous studies have shown that the aboveground plant diversity supports belowground microorganisms through root exudation and rhizodeposition (Bais et al., 2006; Eisenhauer et al., 2017; Morella et al., 2020). Microbial composition is more abundant and complex in the rhizosphere area. Applying an efficient and diverse soil microbiome backed by modern technologies can facilitate and promote sustainable agriculture and can effectively contribute to meet the economic, social, and environmental sustainability requirements (Ray et al., 2020).

The USDA has recommended using rhizobacteria for an improved nitrogen fertility in case of leguminous crops since the 1800s (Schneider, 1892). Currently, trials to fix nitrogen in non-leguminous plants are going on using artificial biological methods. It is being assumed that these approaches are going to have significant impacts on global food supplies (Rogers and Oldroyd, 2014; Ryu et al., 2020). Still, a lot remains to learn about the function of rhizobacteria and their host plants in global nitrogen cycle and the best way to exploit these microbes for a progress in plant productivity (Ray et al., 2020). Microbes also make plants resistant against different biotic and abiotic stresses (Oleńska et al., 2020; Singh et al., 2016). Recently, next-generation sequencing is being used to get a hold of the community composition and functions of microbes. This has been

helpful along with improved culture methods in the field of microbial employment in agriculture. Methods based on metagenomics have revealed a wide range of previously undiscovered microorganisms that could have new enhanced powers and might be potential to be used in agriculture, bioremediation, and human health (Panke-Buisse et al., 2015; Pelegrin et al., 2015; Schweitzer et al., 2008). Through a number of recent studies, it has been demonstrated how microorganisms control ecosystem and how excessive using of chemical fertilizers for a long time have suppressed their ability to improve plant and soil health (Ray et al., 2020; Kwak et al., 2018). The primary focus of a sustainable agriculture is to reduce plant reliance on chemical fertilizers and to improve plant growth. For this, plant growth promoting microbes have been emphasized in growth and development by enriching acquisition of nitrogen, iron, and phosphorus from nature, and by altering plant hormone intensity (Hayat et al., 2010). Microbes can alleviate the deleterious effects of plant pathogens by acting as biocontrol agents, and thus, lower yield loss from diseases (Glick, 2012).

Soil contains a variety of ecological niches which allow assorted microbial strains to live together and form complex microbial communities (Bulgarelli et al., 2013; Kemen, 2014). Next-generation sequencing techniques allow researchers to assess how microbial populations vary spatiotemporally, and to identify core microbiomes which are preserved among host genotypes (Sergaki et al., 2018). Soil microbiota plays a key role in soil organic matter decomposition. Several types of soil carbon change belowground ecosystem function by using selective soil microbial community to affect an overall plant growth and productivity (Orwin et al., 2006). Studies on how tillage affects soil fungal communities have shown mixed results. Recent studies showed that soil fungal communities are harmed by tillage (Yin et al., 2017). In addition to tillage, crop rotation also plays a crucial role in growing belowground microbial diversity. Previous

studies suggested that crop rotation helps in increasing soil quality, organic matter content, microbial biomass, and respiration (Campbell et al., 1991; St. Luce et al., 2013). Cover crops are basically non reaped crops planted between main crops that improve delivering carbon to soil system that can support many microbes in plant rhizosphere during the active growing season of the cover crop via unharvested residues and root exudates (Fernandez et al., 2016). Cover crops enrich soil carbon, Nitrogen, and microbial biomass to support soil quality and productivity. Arbuscular mycorrhizal (AM) fungi can be copiously found beneath oat and cereal rye (Secale cereale L.) cover crops, while non-AM fungi are affiliated with hairy vetch (Vicia villosa L.) (Finney et al., 2017). Clover as a cover crop is reputed to repress pathogenic fungi, affecting soil carbon positively, and increase the diversity of beneficial fungi (Ray et al., 2020). Soil-dwelling microorganisms are critical components of soil health, plant productivity, and stress tolerance. It is exceptionally smart to employ microbes to improve agriculture productivity. As soil microbes play a part in improving soil health and productivity, they are indispensable in sustainable agriculture (Ray et al., 2020).

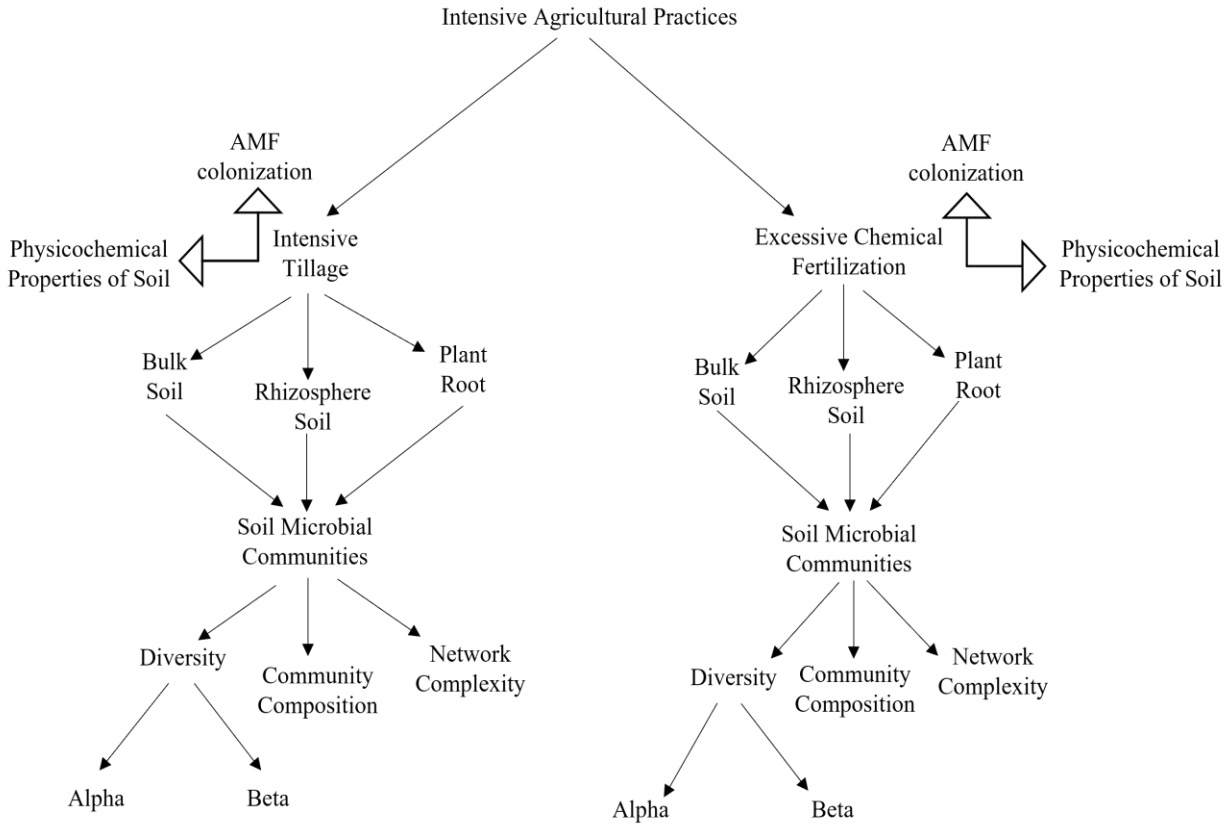

Figure 1: Overview of the research objectives

Overall Objectives and Assumptions

The overall objective of this project was to assess the impacts of intensive agricultural practices on soil biochemistry and AMF colonization in soil. The project also investigated the effects of intensive agricultural practices on the microbial diversity, community composition and network complexities of bulk soil, rhizosphere soil and plant roots. I hypothesized that intensive agricultural techniques would have adverse effects on soil health indicators and will decrease AMF colonization in root. I also hypothesized that microbial assembly in the bulk soil and recruitment in the rhizosphere and plant roots will differ owing to intensive agricultural practices.

EFFECTS OF INTENSIVE AGRICULTURAL TREATMENTS ON SOIL BIOCHEMICAL PROPERTIES AND AMF COLONIZATION

Introduction

Soil Biochemical Properties

To understand the basic soil properties, it is important to be familiar with the physical, chemical, and biological characteristics of this fundamental pool of global biodiversity and food production. There are solid inorganic segments as well as organic portions which aggregate and outline the texture of soil- making the soil structure more diverse. Important biochemical properties of soil are soil pH, nutrients, carbon, nitrogen, and cation exchange capacity. Generally, the inorganic or abiotic soil properties function as the base for the biotic components of soil (FAO, 2015). Soil quality changes with its physiochemical and biological conditions. Being a highly dynamic entity, alteration in soil environment is constant (Liu et al., 2018). Soil pH signifies whether the soil is acidic or alkaline by showing the extent of the hydrogen ion concentration in soil. The most favorable soil pH to obtain nutrients is 6.5. If the pH drops less than 5.5, the concentrations of hydrogen and aluminum in soil get toxic. A pH value above 7.2 might restrain the soil nutrients. pH value above 8.5 may disrupt the soil particles. Salts present in soil can form hard layers in soil and make it difficult for plant roots to grow deeper (FAO, 2015). Soil organic matter signals soil fertility and can develop soil construction and nutrient adjustment (M. Kumar et al., 2021). Plant litter, organic matter or humus, and soil creatures which decay organic matter comprise the soil organic matter together. Soil organic matter determines the physiochemical properties of soil, and nutrient circulation in it (Wojciech et al., 2019). A major part of soil organic matter is carbon- which works as a great source of nutrients and trace elements, and aids in plant growth (FAO, 2015). Soil carbon is a crucial soil factor (Wang et al., 2015). Total carbon in soil

can increase soil quality, soil fertility and water retention, and can enhance crop yield (Muñoz and Kravchenko, 2011). Soil carbon pool works as the largest carbon reservoir in extraterrestrial ecosystem, therefore, minute deviations in total soil carbon might stimulate an overall climatic change. Shortfall of total soil carbon due to farming might lead to impaired soil fertility, demote biomass productivity and negatively impact on water quality (Lal, 2004; McDowell et al., 2012). Soil organic carbon is comprehended in a wide variety of organic portions of soil such as microorganismal cells, plant and animal remainders at various stages of decaying, highly disintegrated forms such as humus, and abundantly carbonated compounds like charcoal, coal and graphite (Sparks et al., 1996). Water-extractable organic matter is the most labile and biodegradable form of soil organic matter and essential for agriculture because plant nutrient uptake and soil microbial activities are gained from such organic matter (M. Zhang et al., 2011). Water-extractable organic carbon and nitrogen are two important constituents of water-extractable organic matter (Chantigny, 2003). Nitrogen works as an essential mineral nutrient of soil and is a basic component of biogeochemical cycles. The primary forms of nitrogen found in nitrogen fertilizers are nitrate and ammonium. Plants rely on these available forms of nitrogen for growth (Dari et al., 2019). Nitrogen loss from croplands are critical environmental concerns. A previous study from (Angle et al., 1993) have shown that nitrogen fertilizer applied excessively can leach a significant portion of mineral nitrogen to contaminate groundwater. Phosphorus is a vital macronutrient needed by plants, and like nitrogen and potassium, can be taken via nutrition. Phosphorus is an important plant nutrient and works as the chief limiting factor for plants (Hinsinger, 2001). A previous study from (Ott and Rechberger, 2012) has proved the impacts of phosphorus in stimulating plant and root growth. Potassium is a vital nutrient and is involved in many significant biological developments in plants. Potassium supports plants to persist against

adverse environmental conditions (Marschner, 2011; Pettigrew, 2008). Adequate amount of potassium in soil ensures a high crop production (Dong et al., 2010; Zhang et al., 2011). Exchangeable bases in soil such as Calcium, Magnesium and Sodium are usually described as the alkaline earth metals and primarily stay attached to the clay and organic elements of soil (Black et al., 1996). Cation exchange capacity (CEC) of soil is crucial to determine how much supplemental potassium is needed, and how much herbicides will be appropriate to add in soil. CEC assessed by summation is a good estimation of the actual CEC in different types of soil (www.soilquality.org.au). Sulfur is one of the fundamental plant nutrients (Scherer, 2001), and takes place in both inorganic and organic forms in soil. In many areas around the world, sulfur has become a key limiting factor and insufficiency of sulfur might reduce crop yield (Scherer, 2009). Copper, Manganese and zinc are the essential plant micronutrients to support plant growth (Westerman et al., 1990). Total nitrogen in soil is a major factor in agricultural environment and denotes soil fertility. Soil total nitrogen is strongly associated with soil productivity (Wang et al., 2009). Decline in total nitrogen in soil might reduce nutrient retention, fertility, and productivity of soil (Huang et al., 2007). Soil physiochemical properties can be considered as important attributes to evaluate soil health as well (Liu et al., 2018).

Arbuscular Mycorrhizal Fungi

Effects of abiotic stresses intensify on ecosystem and crop yield from climatic difference and dereliction in agriculture. Arbuscular mycorrhizal fungi are an ecofriendly managing approach which can be harnessed for an enhanced crop productivity (van der Heijden et al., 1998; Smith and Read, 1984; van der Heijden et al., 2015). Arbuscular mycorrhizal fungi (AMF) are soil borne microorganisms and belong to the phylum Glomeromycota. The mutualistic association AMF have with the host plants is highly established as a classic example of a symbiotic relationship.

Arbuscular mycorrhizal fungi are obligate biotrophs, and thus, AMF need the photosynthetic byproducts and lipids from the host plants to complete their life cycle. In exchange, AMF significantly contribute in plant growth, nutrient uptake, gaining water from adjacent soil, and provide an enhanced resistance to the host plants from pathogenic fungi and various environmental abiotic stresses (Sun et al., 2018). AMF accelerate the growth of the host plants even under stressful conditions by boosting the rate of photosynthesis and gas exchange, creating a complex interchange between themselves and the host plants, and increasing the rate of water uptake. AMF make vesicles, arbuscules, and hyphae in plant root system (Begum et al., 2019). The hyphal network of AMF in plant root system improves plant growth by ascribing a large soil surface area to the roots, therefore, allowing the roots to access much more water and nutrients (Bowles et al., 2016; van der Heijden et al., 2015). Secreting phosphatase enzyme to hydrolyze phosphate from organic phosphorus compounds and accepting insoluble phosphate ions from soil, AMF aid to improve crop production under a phosphorus deficient condition. Hyphae of AMF are an expansion of the host plant's root system. Hyphae help in picking up inert soil nutrients, for instance, phosphorus from those area of soil which cannot be approached by host plant (Scheublin et al., 2010). The widespread extraradical hyphae of AMF increase the uptake of several other soil micronutrients and mineral ions (Rouphael et al., 2015), keep the total soil mass together, and improve the water holding capacity of soil and maintain soil health (Thirkell et al., 2017). Under adverse and stressful conditions, hyphae can make use of water and minerals from soils better than host plant roots and can effectively transfer to the plant macrosymbiont (Hildebrandt et al., 2002). Vesicles work as a storage organ for arbuscular mycorrhizal fungi. Vesicles have a thick wall and can store lipid within (Müller et al., 2017). Arbuscules are fungal formation created by arbuscular mycorrhizal fungi, and can contribute to trade inorganic minerals and carbon and phosphorus

compounds for the growth and development of host plant (Begum et al., 2019; Li et al., 2016). For the beneficial effects on soil health, plant productivity and ecosystem, Arbuscular mycorrhizal fungi are considered a key factor for a sustainable agriculture (Begum et al., 2019; Gianinazzi et al., 2010b).

Research Questions

The overall research questions for this chapter were:

1. How do intensive agricultural treatments such as tillage and inorganic fertilizer application influence soil biochemistry?
2. How do intensive agricultural treatments and organic fertilizer application influence the AMF colonization in soil?

Hypotheses

1. Intensive agricultural treatments have adverse effects while organic fertilizer application have beneficial effects on soil health and fertility.
2. Intensive agricultural treatments reduce AMF root colonization.

Materials and Methods

Soil and Plant Sampling

Soil and plant samples were collected from agricultural plots located at the Carrington Research Extension Center in Foster County, North Dakota. These plots are a part of a long-term field trial of 33 years. Samples were collected from a total of 45 plots in June- 2020. The field design consisted of three replicate plots (111, 205, 301). Plot 111 was about 73 meters apart from plot 205. Plot 205 was about 232 meters apart from plot 301. Distances among the targeted plots ensured their independence. Each replicate plot was being treated with three different tillage levels, and at the same time, were divided into three groups of 15 subplots based on fertilizer application.

The subplots were adjacent to each other. However, sampling was performed 2 meters away from the edges to avoid possible edge effects. Soil in each targeted plot was primarily loamy. Three different tillage treatment were defined by tillage intensity: no tillage, minimum tillage, and conventional tillage. No tillage was basically a farming practice where crop is produced without distressing the soil through tillage (https://regenerationinternational.org/). Minimum tillage could be defined as a farming system where soil tilling is kept at its minimum for crop production (https://soilcare-project.eu/). Conventional tillage was defined as a more old-fashioned farming method where soil is entirely overturned with agricultural machineries for crop production following an added tillage to make the soil surface even for cultivation (https://www.ctc-n.org/). For the targeted CREC plots, minimum tillage consisted of a disk in the fall (tillage depth was 10-15 centimeters based on the soil conditions at that time) and a field cultivator in the spring just prior to sowing (tillage depth was 8-13 centimeters based on the soil conditions at that time). Simultaneously, conventional tillage treatment on the targeted plots also consisted of a disk in the fall (tillage depth was 10- 15 centimeters based on the soil conditions at that time), a chisel plow in late fall (tillage depth was 15-20 centimeters based on the soil conditions at that time), and a field cultivator in the spring just prior to sowing (tillage depth was 8-13 centimeters based on the soil conditions at that time). Plots were also treated with inorganic and organic fertilizers. As for the inorganic fertilizer, a urea broadcast was applied each spring to all plots at 0 lbs, 50 lbs, 100 lbs, and 150 lbs of nitrogen per acre to 36 plots (Figure 2). The remaining nine plots were treated with organic fertilizer which was composted beef feedlot manure at 200 lbs of nitrogen at the beginning of the year. From each plot, 20 full-grown wheat plant samples were collected. Whole plants were sampled using a shovel. Simultaneously, soil cores were collected at the top 15 cm depth where plants were sampled.. Five soil cores were sampled around each plant. Each soil core

was sampled from the top 20 centimeters of topsoil. To represent the whole area of each plot, sampling locations were chosen randomly.

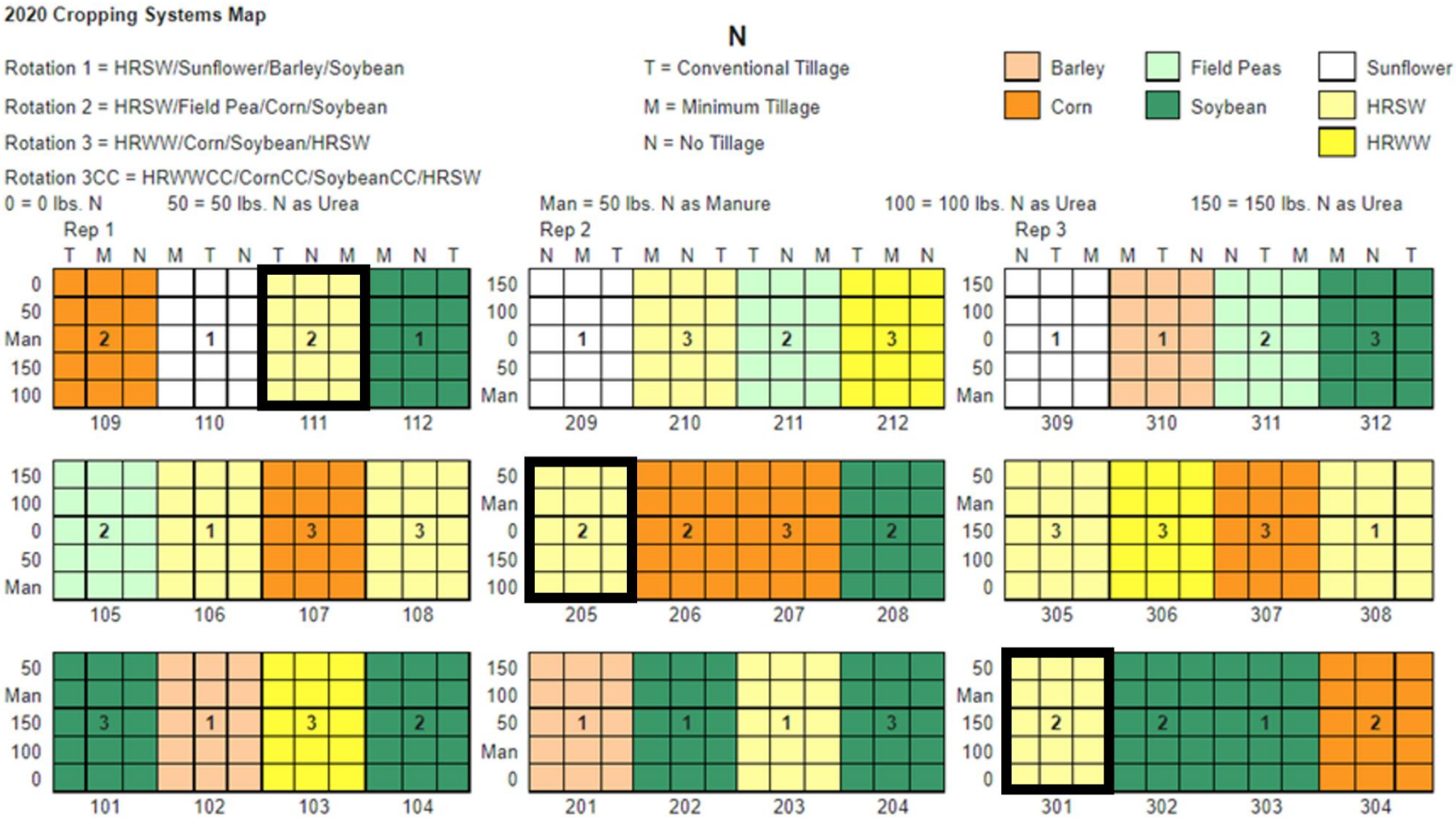

Figure 2: Field Plot Map from Carrington Research Educational Center, 2020

Collected soil cores were kept into Ziplock bags and put onto ice packs in coolers. The soil samples were transported back to the lab for processing. After transportation, the soil cores in each Ziplock bag were broken and sieved with 2 mm sieves to homogenize. Next, each homogenized soil sample was divided in one 15 ml and two 50 ml conical tubes. Ten grams of soil was measured immediately from each soil sample and dried in a VWR forced air oven at 120°C for 24 hours for a dry moisture analysis. Finally, the Ziplock bags and conical tubes were stored in a freezer at -20°C for molecular analysis. Collected wheat plants were immediately kept into brown paper bags and put onto ice packs in coolers. The plant samples were transported back to the lab for processing. Initially, the crown roots were washed with tap water to remove the soil particles. Next, the roots were dipped into a 10% bleach solution for approximately 20 seconds to disinfect. Finally, the roots are rinsed with distilled water. The processed roots were cut from just below the crown of the plants using a scissor. Excess water was blotted from the roots using paper towels. Washed and cleaned roots were cut into pieces of approximately 1.5 cm length using scalpels. Approximately 30% of the cut roots from each plot were kept in one, 15 ml conical tube. These conical tubes were stored in a freezer at -20°C for performing molecular analysis. Comparatively thinner roots were chosen for mycorrhizal slide preparation. These cut root pieces were kept in 15 ml conical tubes, and 50% EtOH was added to it for longer storage timing.

Assessment of Soil Properties

Soil samples were sent to a soil analyzing facility "AgVise' at Grand Forks, North Dakota, to analyze different soil physical and chemical properties including: pH, inorganic material content for nitrate, ammonium, phosphorus, potassium, calcium, magnesium, sodium, sulfur, zinc, manganese, copper, total inorganic nitrogen content, cation exchange capacity, sand, silt, clay, total carbon content, total organic carbon content, amount of water extractable organic carbon and

water extractable total nitrogen, and ratio of carbon and nitrogen. Soil pH was measured using the 1:1 soil: water method. To assess the soil pH, 7.65 g of soil sample was measured (Peters et al., 2015). Soil organic matter was estimated following the loss of weight on ignition (LOI) method. Five g of dried and ground soil sample was taken to measure soil organic matter (Combs et al., 2015). The amount of inorganic nitrogen present in soil was determined by the two M KCl extraction method (Black et al., 1996; Dorich and Nelson, 1984). An amount of 7.65 g of air-dried, ground, and sieved soil sample was used for measurement of nitrate present in soil using the cadmium reduction method (Gelderman, et al., 2015). Two g of soil was measured to determine the ammonium present in soil (Sparks et al., 1996; Mulvaney, R.L. 1996). Soil phosphorus was determined by the sodium bicarbonate method of the Olsen Phosphorus Test (Olsen et al., 1954). One g of soil was measured and air dried for the extraction of soil phosphorus (Frank et al., 2015). Quantities of potassium, and other basic cations in soil, e.g., calcium, magnesium, and sodium were measured with the neutral ammonium acetate extraction method. One g of soil sample was measured for each cation extraction test (Warnacke et al., 2015). Cation exchange capacity (CEC) of soil was determined using the summation method (Warnacke et al., 2015). Sulfur in soil was determined by the turbidimetric procedure. Seven g and 650 mg of soil was measured, air-dried, crushed, and sieved to determine sulfur concentration (Franzen, 2015). Amounts of micronutrients such as zinc, manganese, and copper in the test soil sample was verified by the widely accepted DTPA (diethylenetriaminepentaacetic acid) test (Lindsay and Norvell, 1978). Ten g of soil sample was measured, air-dried, crushed, and sieved for each micronutrient (Whitney, 2015). Total nitrogen amount in soil was determined by the classical Dumas method. One g of test soil sample was heated to assess the amount (Sparks et al., 1996; Bremner, J.R. 1996). Soil particle size was analyzed with the help of a hydrometer. Fifty g of air-dried soil was measured for the assessment

(Campbell et al., 2002; Gee et al., 2002). A dry combustion method was used to determine the amount of total carbon in soil. One g soil was measured and ground to assess the amount (Sparks et al., 1996; Nelson et al., 1996). Amount of organic carbon in soil was determined by subtracting the volume of inorganic carbon from the amount of total carbon in soil. Organic C = Total C - Inorganic C (Sparks et al., 1996; Nelson et al., 1996). Water extractable organic carbon was determined using a dry combustion method. Two g of soil was measured for the assessment (Haney et al., 2012). Water extractable total nitrogen was determined using a dry combustion method. Two g of soil was measured for the assessment (Haney et al., 2012). Ten g of the sieved soil samples were weighed and dried in a VWR forced air oven at 120°C for 24 hours. Then the dried soil samples were taken out of the oven and dry soil moisture was calculated according to the formula: Soil dry moisture = (Soil weight - Dry soil weight)/ (Dry soil weight) * 100%.

Assessment of Mycorrhizal Colonization

Processed roots were further washed using one ml of a one M solution of (NaPO3)6, to remove any excess soil that remained. Approximately 15 root pieces were removed from each 15 ml conical tubes and kept into a 1.5 ml microcentrifuge tube. Labeling was done accordingly. (NaPO3)6 was added to each 1.5 ml microcentrifuge tubes, and the closed tubes were shaken for two hours. After two hours, the roots were removed and washed in deionized water. A 40% w/v KOH solution was added to the roots, and the mixture were incubated at 80°C for 30-40 minutes. After that, the roots were again rinsed with deionized water. Then, a 5% ink-vinegar solution was added to the roots and kept for 30-40 minutes at 80°C for staining. For the ink-vinegar stock solution, 50 ml ink (black from Parker Quink) was mixed with 950 ml of household vinegar (5% acetic acid). After the staining, the roots were rinsed with tap water and a drop of vinegar (Vierheilig et al., 1998).

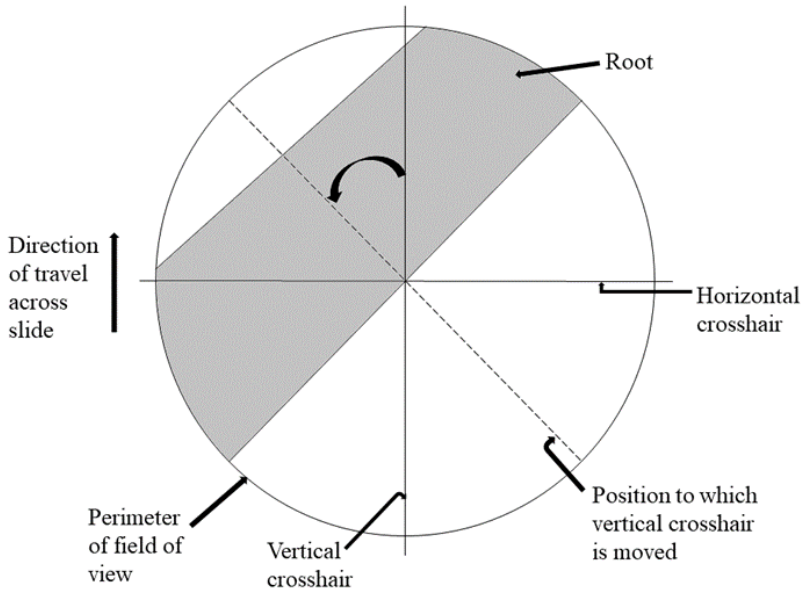

Figure 3: Diagram of a magnified intersection (Adopted from McGonigle et al., (1990))

The stained roots were aligned parallel to the long axis of a microscopy slide. On each slide, there were five rows of roots- each having two to three pieces. Roots were fixed into place using 50% glycerol and a cover slip. The edges of the cover slips were securely sealed with coats of clear nail polish. The arbuscular mycorrhizal colonization was observed under a compound microscope at a magnification of 40X. The spot on the root surface where the center of the horizontal and vertical crosshairs entered through the side of the root was taken as the point of intersection. All intersections between the roots and the vertical crosshair were taken into consideration. Several categories were made to count the intersections: ‘arbuscules’, ‘vesicles’, ‘arbuscules and vesicles’, ‘only hyphae’, and ‘none’ i.e., there is no fungal material present in the root. To assess each intersection, the plane of focus was moved completely through the root. If the vertical crosshair cut one or more arbuscules or vesicles or hyphae, the appropriate category was incremented by one. For the arbuscular and vesicular colonization, the sum of the corresponding category was divided by the total number of intersections studied. The hyphal colonization was

estimated as the ratio of non-negative intersections. One hundred intersections were calculated per sample to get the representative numbers (McGonigle et al., 1990b).

Statistical Analyses

All statistical analyses were performed in R (R version 4.0.2). The R packages used were: dplyr (Wickham et al., 2022), ggplot2 (Wickham H (2016). ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York. ISBN 978-3-319-24277-4), tidyverse (Hadley Wickham et al., 2019), readxl (Wickham et al., 2019), phyloseq (McMurdie and Holmes, 2013), vegan (Oksanen et al., 2020), Desctools (Signorell, 2018), PMCMR (Pohlert, 2016), and ggpubr (Kassambara, 2020). Data used for the statistical analysis was the soil biochemical properties from 45 bulk soil samples and the mycorrhizal colonization data, corresponding to the three wheat farming plots (111, 205, 301). The target variables were the soil biochemical properties and the mycorrhizal colonization abundance data. For non-normally distributed data, the non-parametric analysis Kruskal-Wallis test, Dunn post-hoc test using the Benjamini-Hochberg correction method, Scheirer-Ray-Hare test (factor= tillage and fertilizer regime, n= 45, df= 44), the Spearman rank correlation (n= 45), and the regression analysis (n= 45) were performed (Benjamini and Hochberg, 1995; Best and Roberts, 1975; Jean Dunn, 1964; Yutao Wang et al., 2010). Boxplots were created for each examined soil biochemical properties against three different tillage treatments and five different fertilizer regimes respectively. An illustrating software Inkscape (Inkscape version 1.1), was used to arrange the boxplots together to compare.

Result

Soil Biochemical Properties

Effect of Tillage Regime on Soil Properties

Intensive agricultural management techniques are having a multifaceted effect on mean values of different soil properties. Levels of pH, calcium, clay, carbon-nitrogen ratio, cation exchange capacity, dry soil weight and the base saturation of calcium in soil rose higher from no tillage to conventional tillage. Levels of nitrate, potassium, magnesium, sulfur, sand, total carbon, and water extractable total nitrogen in soil got lower from no tillage to minimum tillage and rose again for conventional tillage. Levels of ammonium, phosphorus, sodium, silt, and base saturation of sodium in soil got higher from no tillage to minimum tillage and dropped in conventional tillage. Levels of zinc, manganese, copper, base saturation of magnesium, total organic carbon, and water extractable organic carbon in soil dropped down from no tillage to conventional tillage. Level of total inorganic nitrogen in soil decreased from no tillage to minimum tillage and stayed same for conventional tillage. Level of base saturation of potassium in soil stayed same at both no tillage and minimum tillage and dropped for conventional tillage. Level of dry soil weight increased from no-tillage to minimum-tillage and stayed same at both minimum-tillage and conventional-tillage. No significant difference was found in the levels of soil properties for three different tillage treatment (Table 1, Figure 4). However, in general, several key soil properties were observed to have different amounts detected under three different tillage treatments. Soil pH was the lowest under no tillage (mean= 6.00) and the highest under conventional tillage (mean= 6.30). Ammonium levels were the lowest under conventional tillage (mean= 5.70) and the highest under minimum tillage (mean= 9.70). Nitrate levels were the lowest under minimum tillage (mean= 31.80), and the highest under no tillage (mean= 38.30). Phosphorus levels were the lowest under

conventional tillage (mean= 23.10) and the highest under minimum tillage (mean= 26.00). Potassium levels were the lowest under minimum tillage (mean= 222.70), and the highest under no tillage (mean= 227.30). Range of total carbon was the lowest under minimum tillage (mean= 1.78), and the highest under no tillage (mean= 1.94). Range of water extractible organic carbon was the lowest under conventional tillage (mean= 166.79), and the highest under no tillage (mean= 176.58). Range of total inorganic nitrogen level was the highest under no tillage (mean= 0.19) and was at equal under minimum and conventional tillage (mean= 0.17). The range of the ratio between carbon and nitrogen was the lowest under no tillage (mean= 10.24), and the highest under conventional tillage (mean= 10.49). The results showed that the comprehensive range of overall organic and inorganic nitrogen and carbon was at the maximum level in plots with no tillage, however, there was a tendency of decrease as the soil was conventionally tilled.

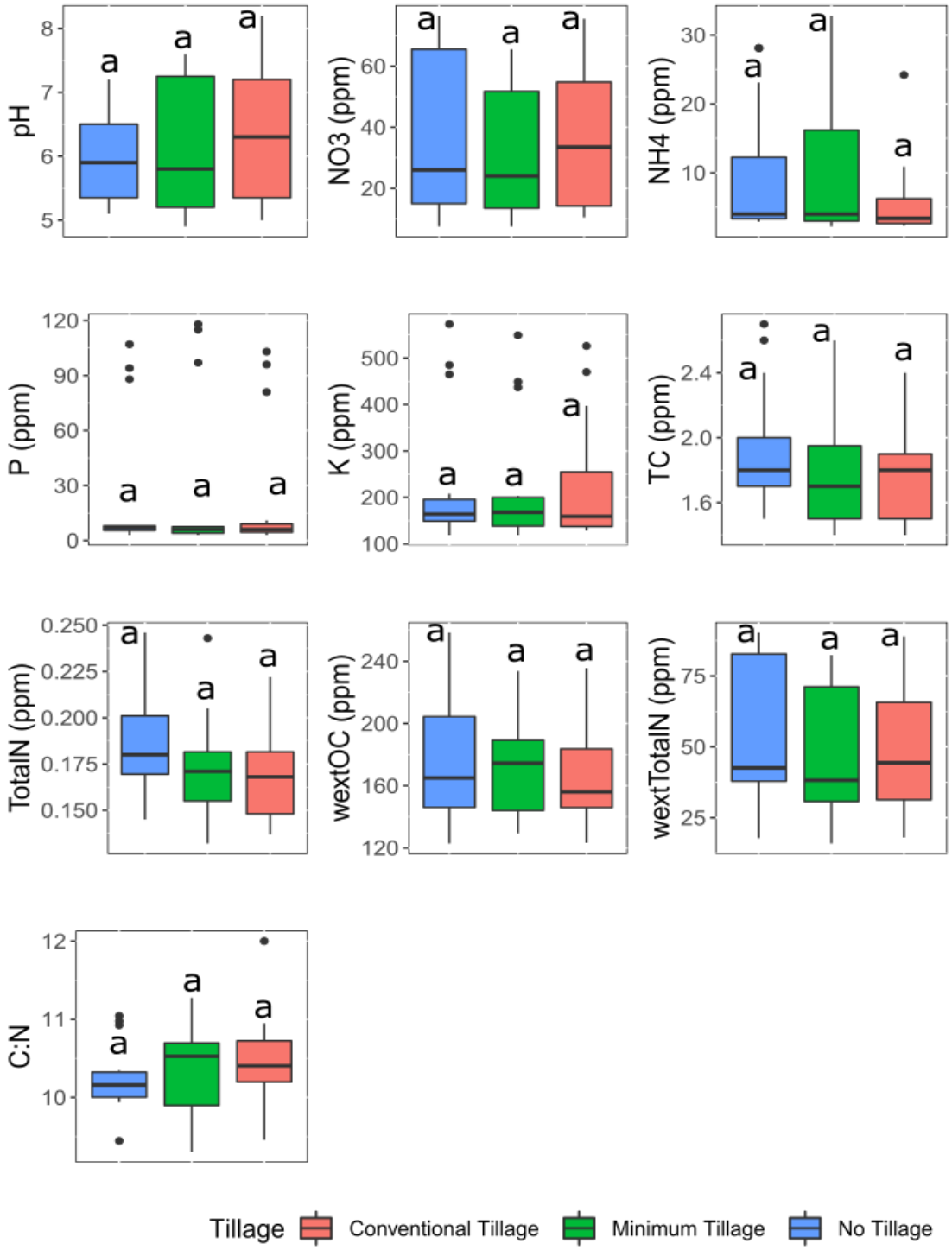

Figure 4: Change in several soil biochemical properties with respect to three types of tillage management. No tillage ( ), Minimum tillage ( ), and Conventional tillage ( ). NO3: nitrate, NH4: ammonium, P: phosphorus, K: potassium, TC: total carbon, TotalN: total inorganic nitrogen, wextOC: water extractible organic carbon, wextTotalN: water extractible total nitrogen, C: N: carbon-nitrogen ratio. Statistical significance is denoted here with the letter "a" at ($p < 0.05$) level.

Effect of Fertilizer Application on Soil Properties

Levels of pH, calcium, zinc, and cation exchange capacity in soil rose from 0 (control) to 50 lbs of inorganic fertilizer, lowered from 50 lbs to 150 lbs, and again rose for organic fertilizer application. Levels of nitrate, ammonium, manganese, and water extractable total nitrogen in soil rose from 0 (control) to 150 lbs of inorganic fertilizer and lowered at organic fertilizer application. Levels of phosphorus, potassium, copper, base saturation of potassium, and total organic carbon in soil got higher from 0 (control) to 100 lbs of inorganic fertilizer, lowered at 150 lbs and again rose for organic fertilizer. Levels of magnesium and carbon-nitrogen ratio in soil dropped from 0 (control) to 150 lbs of inorganic fertilizer and rose for organic fertilizer. Level of sodium in soil dropped from 0 (control) to 50 lbs of inorganic fertilizer and rose from 50 lbs to organic fertilizer. Levels of sulfur and sand in soil rose from 0 (control) to 50 lbs of inorganic fertilizer, dropped from 50 lbs to 100 lbs and rose from 100 lbs to organic fertilizer. Level of total inorganic nitrogen in soil stayed same at 0 (control) and 50 lbs of inorganic fertilizer, rose from 50 lbs to 100 lbs, dropped from 100 lbs to 150 lbs and again rose from 150 lbs to organic fertilizer. Level of soil silt lowered from 0 (control) to 50 lbs of inorganic fertilizer, rose from 50 lbs to 100 lbs, and again dropped from 100 lbs to organic fertilizer. Level of clay in soil rose from 0 (control) to 100 lbs of inorganic fertilizer and dropped from 100 lbs to organic fertilizer. Levels of base saturation of calcium in soil rose from 0 (control) to 50 lbs of inorganic fertilizer, lowered from 50 lbs to 100 lbs, rose from 100 lbs to 150 lbs and again dropped from 150 lbs to organic fertilizer. Levels of base saturation of magnesium and total carbon in soil lowered from 0 (control) to 50 lbs of inorganic fertilizer, rose from 50 lbs to 100 lbs, lowered from 100 lbs to 150 lbs and again rose from 150 lbs to organic fertilizer. Level of base saturation of sodium in soil lowered from 0 (control) to 50 lbs of inorganic fertilizer, rose from 50 lbs to 150 lbs and again dropped from 150

lbs to organic fertilizer. Levels of water extractable organic carbon in soil increased from 0 (control) to organic fertilizer application. Level of dry soil weight decreased from 0 (control) to 50 lbs of inorganic fertilizer, stayed same for 50 lbs to 150 lbs, and increased from 150 lbs to organic fertilizer application. A statistically significant difference was found in most cases where organic fertilizer was applied (Table 1, Figure 5). The p-values derived from the post hoc test for both tillage and fertilizer application managements showed that fertilizer treatment was statistically significant (p-value < 0.05, 0.01, 0.001) in case of all tested soil properties, except silt, base saturations of calcium and magnesium, and dry soil weight. Fertilizer application was not statistically significant for silt, base saturations of calcium and magnesium, and dry soil weight. However, in general, several key soil properties were observed to have different amounts detected under five different fertilizer treatments. Soil pH was significantly higher under organic fertilizer (mean= 7.4). Ammonium level was significantly higher under 150 lbs of inorganic fertilizer (mean= 19.0). Nitrate level was significantly higher under 150 lbs of inorganic fertilizer (mean= 63.3). Phosphorus level was significantly higher under organic fertilizer (mean= 99.9). Potassium level was significantly higher under organic fertilizer (mean= 483.4). Range of total carbon significantly higher under organic fertilizer (mean= 2.43). Range of water extractible total nitrogen was significantly higher under 150 lbs of inorganic fertilizer (mean= 78.39). Range of water extractible organic carbon was significantly higher under organic fertilizer (mean= 217.48). Range of total inorganic nitrogen level was significantly higher under organic fertilizer (mean= 0.223). The range of the ratio between carbon and nitrogen was significantly higher under organic fertilizer (mean= 0.91). The results showed that the comprehensive range of overall organic carbon and nitrogen was at the maximum level in plots with organic fertilization and inorganic nitrogen was at the maximum level in plots where 150 lbs of inorganic fertilizer was applied.

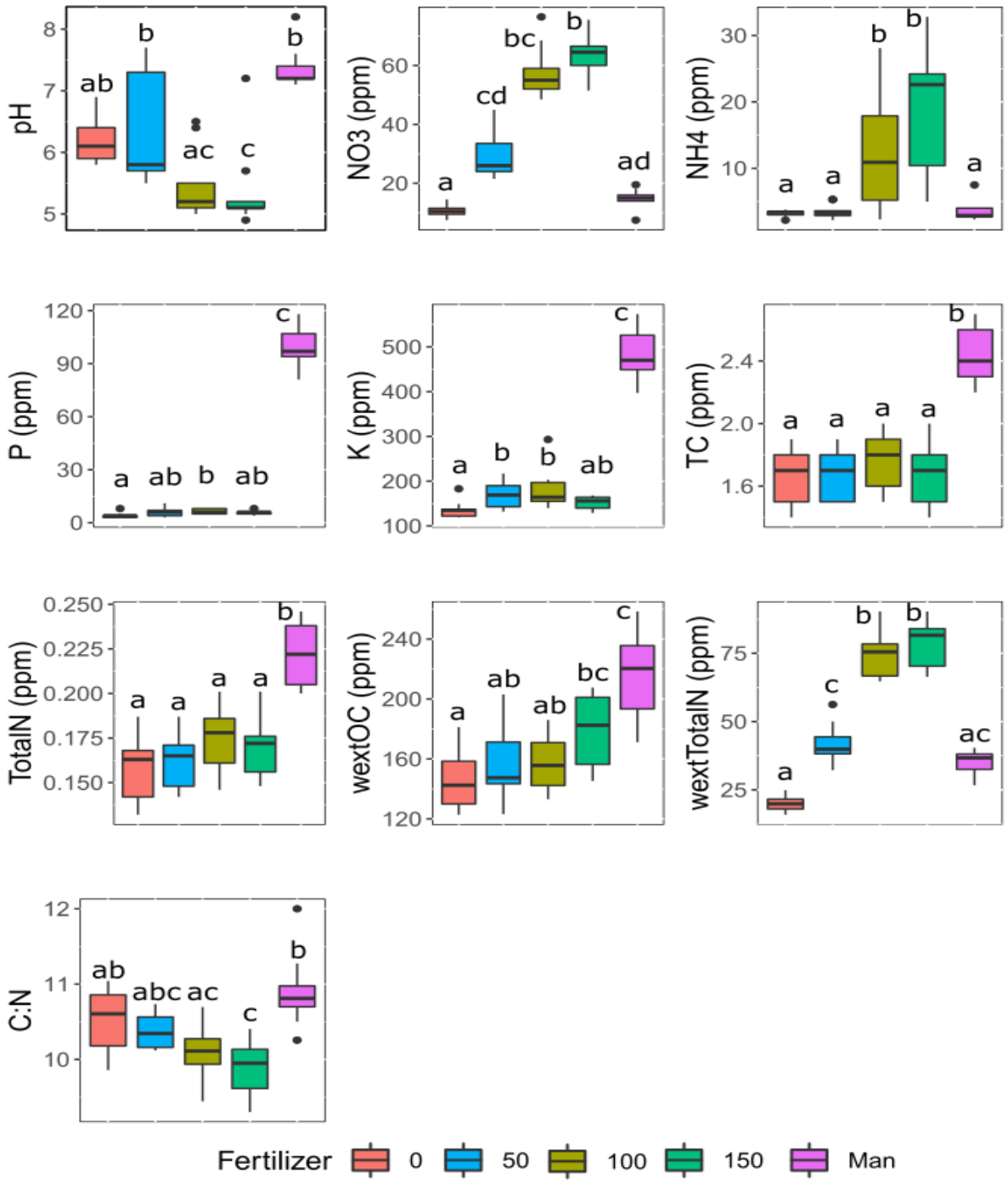

Figure 5: Change in several soil biochemical properties with respect to five types of fertilizer application. 0 ( ), 50 ( ), 100 ( ), 150 ( ), Manure ( ). NO3: nitrate, NH4: ammonium, P: phosphorus, K: potassium, TC: total carbon, TotalN: total inorganic nitrogen, wextOC: water extractible organic carbon, wextTotalN: water extractible nitrogen, C: N: carbon-nitrogen ratio. Statistical significance is represented here with two different letters "a" and "b" at ($p < 0.05, 0.01, 0.001$) level.

The table below (Table 1) contains the mean values of each soil property for three different tillage and five different fertilizer practices. Statistical significance is represented with different letters “a”, “b”, “c” and “d” at ($p < 0.05, 0.01, 0.001$) level.

Table 1: Mean values of soil properties for three tillage types and five fertilizer regimes. Letters “a” , “b”, “c”, and “d” indicate statistical significance at ($p < 0.05, 0.01, 0.001$) across columns for different tillage and fertilizer treatments.

Soil Properties	NT	MT	CT	0	50	100	150	Manure
pH	6.00^{a}	6.20^{a}	6.30^{a}	6.20^{ab}	6.40^{b}	5.50^{ac}	5.40^{c}	7.40^{b}
NO_3	38.30^{a}	31.80^{a}	35.60^{a}	10.60^{a}	29.40^{cd}	58.10^{bc}	63.30^{b}	14.80^{ad}
NH_4	9.40^{a}	9.70^{a}	5.70^{a}	3.20^{a}	3.50^{a}	12.00^{b}	19.00^{b}	3.60^{a}
P	24.20^{a}	26.00^{a}	23.10^{a}	4.10^{a}	6.00^{ab}	6.40^{b}	5.80^{ab}	99.90^{c}
K	227.30^{a}	222.70^{a}	224.40^{a}	137.00^{a}	170.60^{b}	181.30^{b}	151.60^{ab}	483.40^{c}
Ca	1777.90^{a}	1844.20^{a}	2125.20^{a}	1785.30^{ab}	2113.70^{ab}	1644.30^{a}	1636.10^{a}	2399.40^{b}
Mg	422.10^{a}	399.90^{a}	425.50^{a}	418.60^{a}	409.60^{a}	362.60^{ab}	348.90^{b}	539.70^{c}
Na	18.10^{a}	19.70^{a}	17.70^{a}	17.90^{ab}	16.20^{a}	18.80^{ab}	19.20^{ab}	20.30^{b}
S	7.30^{a}	5.00^{a}	5.10^{a}	3.60^{a}	4.20^{a}	3.30^{a}	4.60^{a}	13.40^{b}
Zn	1.30^{a}	1.00^{a}	0.90^{a}	0.50^{a}	0.60^{a}	0.59^{a}	0.54^{a}	3.20^{b}
Mn	15.80^{a}	14.80^{a}	11.20^{a}	6.58^{a}	7.74^{a}	22.61^{b}	29.88^{b}	2.76^{a}
Cu	0.62^{a}	0.60^{a}	0.58^{a}	0.51^{a}	0.55^{ab}	0.65^{b}	0.62^{b}	0.67^{b}
TotalN	0.19^{a}	0.17^{a}	0.17^{a}	0.16^{a}	0.16^{a}	0.17^{a}	0.16^{a}	0.22^{b}
CEC	13.06^{a}	13.20^{a}	14.82^{a}	12.83^{ab}	14.47^{bc}	11.77^{ab}	11.55^{a}	17.83^{c}
Sand	47.13^{a}	43.66^{a}	43.73^{a}	41.33^{a}	44.11^{ab}	42.66^{ab}	44.11^{ab}	52.00^{b}
Silt	33.13^{a}	35.40^{a}	34.73^{a}	37.11^{a}	34.22^{a}	35.55^{a}	34.66^{a}	30.55^{a}
Clay	19.73^{a}	20.93^{a}	21.53^{a}	21.55^{ab}	21.66^{a}	21.77^{ab}	21.22^{ab}	17.44^{b}
bsK	4.22^{a}	4.22^{a}	3.87^{a}	2.74^{a}	3.20^{ab}	4.00^{b}	3.40^{ab}	7.20^{c}
bsCa	68.36^{a}	69.64^{a}	70.68^{a}	69.60^{a}	71.90^{a}	69.60^{a}	70.30^{a}	66.40^{a}
bsMg	26.82^{a}	25.54^{a}	24.91^{a}	27.20^{a}	24.50^{a}	25.80^{a}	25.50^{a}	25.90^{a}
bsNa	0.62^{a}	0.66^{a}	0.54^{a}	0.60^{ab}	0.50^{b}	0.70^{a}	0.80^{a}	0.50^{b}
TC	1.94^{a}	1.78^{a}	1.79^{a}	1.70^{a}	1.69^{a}	1.76^{a}	1.68^{a}	2.43^{b}
TOrC	1.93^{a}	1.74^{a}	1.72^{a}	1.62^{a}	1.66^{a}	1.73^{a}	1.67^{a}	2.32^{b}
wextOC	176.58^{a}	170.42^{a}	166.79^{a}	146.96^{a}	156.34^{ab}	158.28^{ab}	177.28^{bc}	217.48^{c}
wextTotalN	55.11^{a}	47.27^{a}	48.06^{a}	19.99^{a}	42.07^{c}	75.13^{b}	78.39^{b}	35.18^{ac}
C: N	10.24^{a}	10.33^{a}	10.49^{a}	10.52^{ab}	10.39^{abc}	10.07^{ac}	9.90^{c}	10.91^{b}
DSW	9.00^{a}	9.10^{a}	9.10^{a}	9.10^{a}	9.00^{a}	9.00^{a}	9.00^{a}	9.10^{a}

Note: NT = no tillage, MT= minimum tillage, CT= conventional tillage. NO_3: nitrate, NH_4: ammonium, P: phosphorus, K: potassium, Ca: calcium, Mg: magnesium, Na: Sodium, S: sulfur, Zn: zinc, Mn: manganese, Cu: copper, TotalN: total inorganic nitrogen, CEC: cation exchange capacity, bsK: base saturation of potassium, bsCa: base saturation of calcium, bsMg: base saturation of magnesium, bsNa: base saturation of sodium, TC: total carbon, TOrC: total organic carbon, wextOC: water extractible organic carbon, wextTotalN: water extractible total nitrogen, C: N: carbon-nitrogen ratio, DSW: dry soil weight

The table below (Table 2) contains the p-values derived after post hoc test of each soil property for three different tillage and five different fertilizer practices to show statistical significance for each treatment.

Table 2: Soil properties for three tillage types and five fertilizer regimes with respective p-values. *, **, and *** indicate statistical significance at ($p < 0.05$, 0.01 and 0.001) correspondingly. NS: non-significant.

Soil Properties	Tillage p-value	Fertilizer p-value
pH	0.77629 (NS)	0.00002 (***)
NO3	0.69244 (NS)	0.00000 (***)
NH4	0.33904 (NS)	0.00007 (***)
P	0.54543 (NS)	0.00001 (***)
K	0.95623 (NS)	0.00001 (***)
Ca	0.47982 (NS)	0.00044 (***)
Mg	0.60661 (NS)	0.00000 (***)
Na	0.45785 (NS)	0.02004 (*)
S	0.57053 (NS)	0.00002 (***)
Zn	0.64085 (NS)	0.00016 (***)
Mn	0.5864 (NS)	0.00000 (***)
Cu	0.73133 (NS)	0.00413 (**)
TotalN	0.11288 (NS)	0.00008 (***)
CEC	0.47709 (NS)	0.00003 (***)
Sand	0.93475 (NS)	0.01971 (*)
Silt	0.89696 (NS)	0.14281 (NS)
Clay	0.48722 (NS)	0.04676 (*)
bsK	0.73363 (NS)	0.00005 (***)
bsCa	0.69672 (NS)	0.07618 (NS)
bsMg	0.5793 (NS)	0.71433 (NS)
bsNa	0.18248 (NS)	0.00053 (***)
TC	0.25337 (NS)	0.00017 (***)
TOrC	0.14171 (NS)	0.0003 (***)
wextOC	0.88811 (NS)	0.00027 (***)
wextTotalN	0.46936 (NS)	0.00000 (***)
C: N	0.36924 (NS)	0.00018 (***)
DSW	0.46247 (NS)	0.41549 (NS)

Note: NT = no tillage, MT= minimum tillage, CT= conventional tillage. NO3: nitrate, NH4: ammonium, P: phosphorus, K: potassium, Ca: calcium, Mg: magnesium, Na: Sodium, S: sulfur, Zn: zinc, Mn: manganese, Cu: copper, TotalN: total inorganic nitrogen, CEC: cation exchange capacity, bsK: base saturation of potassium, bsCa: base saturation of calcium, bsMg: base saturation of magnesium, bsNa: base saturation of sodium, TC: total carbon, TOrC: total organic carbon, wextOC: water extractible organic carbon, wextTotalN: water extractible total nitrogen, C: N: carbon-nitrogen ratio, DSW: dry soil weight

AMF Root Colonization

Effect of Tillage Regime on AMF Colonization

Although no significant differences were established between tillage treatments, small differences in mycorrhizal colonization were observed. In general, mean values of hyphal, vesicular, and arbuscular colonization were highest in plots under minimum tillage, and lowest in plots under conventional tillage (Figure 6). However, a significant difference was observed between the total mycorrhizal colonization of plots under minimum tillage and no-tillage management (Table 3). Total colonization was the lowest under no tillage management (13%) and was highest under minimum tillage (19%).

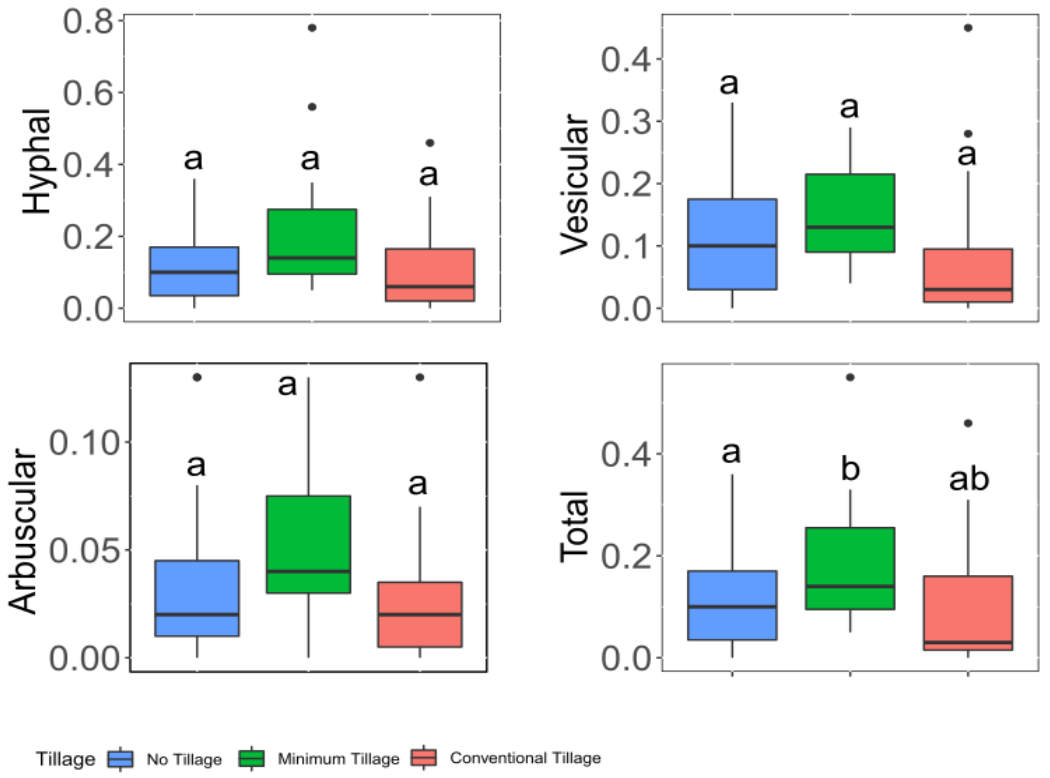

Figure 6: Change in mycorrhizal colonization with respect to three types of tillage management. No tillage ( ), Minimum tillage ( ), and Conventional tillage ( ). Hyphal: hyphal colonization, Vesicular: vesicular colonization, Arbuscular: arbuscular colonization, Total: total colonization. Statistical significance is represented here with two different letters "a" and "b" at ($p < 0.05$) level.

Effect of Fertilizer Levels on AMF Colonization

The results showed that the overall hyphal, vesicular, arbuscular, and total mycorrhizal colonization were at the maximum level in plots with organic fertilizer application and were the lowest for plots where the highest amount inorganic fertilizer was applied. Interestingly, colonization level decreased as the amount of inorganic fertilizer got intensified, such as from 50 lbs to 150 lbs per acre of plots (**Figure 7**). Significant differences were observed between the hyphal, vesicular, arbuscular, and total mycorrhizal colonization in plots treated with five different fertilizer amounts and types. Hyphal colonization decreased under 0 (control) to 150 lbs of inorganic fertilizer (25%, 12%, 9%, 6%, respectively, **Table 3**), and increased under organic fertilizer application (27%). Vesicular colonization decreased under 0 (control) to 150 lbs of inorganic fertilizer (17%, 10%, 9%, 4% respectively), and increased under organic fertilizer application (19%). Arbuscular colonization decreased under 0 (control) to 150 lbs. of inorganic fertilizer (6%, 3%, 3%, 1% respectively), and increased in plots under organic fertilizer application (6%). Total colonization decreased under 0 (control) to 150 lbs. of inorganic fertilizer (19%, 11%, 8%, 6% respectively), but increased in plots under organic fertilizer application (27%). The p-values derived from the post hoc test for both tillage and fertilizer application managements showed that fertilizer treatment was statistically significant in case of hyphal, vesicular, arbuscular, and total mycorrhizal colonization (p-value <0.05) (**Table 4**).

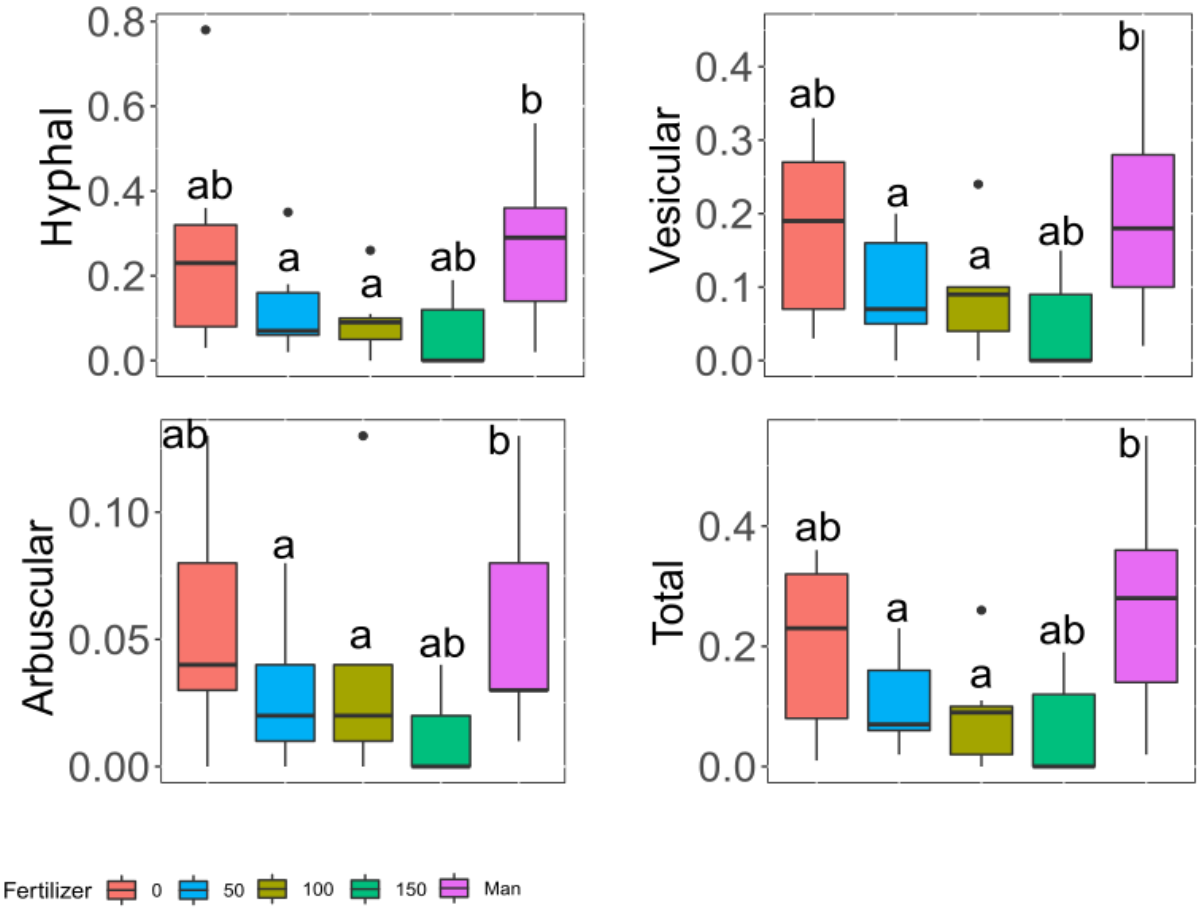

Figure 7: Change in mycorrhizal colonization with respect to five types of fertilizer application. 0 ( ), 50 ( ), 100 ( ), 150 ( ), manure ( ). Hyphal: hyphal colonization, Vesicular: vesicular colonization, Arbuscular: arbuscular colonization, Total: total colonization. Statistical significance is represented here with two different letters "a" and "b" at ($p < 0.05$) level.

The table below (**Table 3**) shows the mean values of AMF colonization for three tillage and five fertilizer treatments. Statistical significance is represented with two different letters "a" and "b" at ($p < 0.05$) level.

Table 3: Mean values of mycorrhizal colonization in three tillage types and five fertilizer regimes. Letters "a" and "b" indicate statistical significance at (p-value < 0.05)

Mycorrhizal Colonization	NT	MT	CT	0	50	100	150	Manure
Hyphal Colonization	13%^{a}	23%^{a}	11%^{a}	25%^{ab}	12%^{a}	9%^{a}	6%^{ab}	27%^{b}
Vesicular Colonization	11%^{a}	15%^{a}	9%^{a}	17%^{ab}	10%^{a}	9%^{a}	4%^{ab}	19%^{b}
Arbuscular Colonization	4%^{a}	5%^{a}	3%^{a}	6%^{ab}	3%^{a}	3%^{a}	1%^{ab}	6%^{b}
Total Colonization	13%^{a}	19%^{b}	10%^{ab}	19%^{ab}	11%^{a}	8%^{a}	6%^{ab}	27%^{b}

Note: NT = no tillage, MT= minimum tillage, CT= conventional tillage

The table below (**Table 4**) contains the p-values derived after post hoc test of AMF colonization for three different tillage and five different fertilizer practices to show the statistical significance for each treatment.

Table 4: Mycorrhizal colonization in three tillage types and five fertilizer regimes with respective p-values. * and ** indicate statistical significance at ($p < 0.05$ and 0.01) correspondingly. NS: non-significant.

Mycorrhizal Colonization	Tillage p-value	Fertilizer p-value
Hyphal Colonization	0.05154 (NS)	0.00994 (**)
Vesicular Colonization	0.06238 (NS)	0.01762 (*)
Arbuscular Colonization	0.08799 (NS)	0.02483 (*)
Total Colonization	0.4467 (*)	0.785 (**)

Discussion

Impact on Soil Biochemistry

In my study, soil pH was decreased at no-tillage compared to minimum-tillage and conventional-tillage. Previous research by Blevins et al. (1983) suggested that tillage treatments affected soil pH in different ways, for example, when tillage intensity gets reduced, crop residues and applied fertilizer get mixed with soil less intensely. In case of no-tillage, soil remains

uninterrupted for a longer period, and when fertilizers are applied on an undisturbed soil surface, soil nutrients and organic matter pile up at the surface. A lower pH in no-tilled soil might be because the soil surface was not disturbed and therefore a bulk density of organic matter accumulated to stop the infiltration of inorganic nitrogenous fertilizer into deeper soil layers (Black et al., 1996; Fageria, 2002; Knorr et al., 2005; Li et al., 2020; López-Fando and Pardo, 2009; Malik et al., 2018; Sithole et al., 2016). My observation was consistent with the findings from previous research studies (Blevins et al., 1983; Dick, 1983; Li et al., 2020) for corn and a corn/oats/meadow rotation respectively. My study also showed a reduced soil pH following the application of inorganic fertilizers and this was in agreement with previous studies (Geisseler and Scow, 2014; Heinze et al., 2010; Guo et al., 2010; Pierre, 1928). My study revealed a significant increase in soil pH for organic fertilizer application. Generally, soil with more organic matter have a higher amount of positively charged molecule which is also known as the cation exchange capacity (CEC) (www.waltsorganic.com). According to a previous study by (Heinze et al., 2010), application of organic fertilizer, and therefore, the increased CEC could be a possible reason for such observation under organic fertilizer application. Furthermore, this study found a higher nitrate content in no-tilled soil comparing with minimum- and conventional-tillage which was consistent with the findings from (Franzluebbers and Hons, 1996) for sorghum, wheat and soybean. However, my study was in contradiction with the findings of (Nyborg and Malhi, 1989; Roldán et al., 2005; Halvorson et al., 2001; Angle et al., 1993). According to a three-year study by Angle et al. (1993), lower soil nitrate under no-tillage compared to conventional tillage was probably because of the different rate of denitrification. In my study, rate of soil nitrate increased as inorganic fertilization intensified as found previously (Angle et al., 1993; Roth and Fox, 1990; Liang et al., 1991). The

present study showed a significanty lower concentration of nitrate for organic fertilizer application in agreement with previous studies (Angle et al., 1993).

The current study revealed an increase in phosphorus concentration for no-tillage which was likely because of a high phosphorus level on soil surface due to a limited incorporation of phosphorus from applied fertilizer and therefore, a decreased phosphorus fixation (Verhulst et al., 2010). My results were in agreement with the findings from a number of previous studies who reported about a high level of phosphorus in no-tilled soil rather than a conventionally-tilled soil for corn and wheat respectively (Duiker and Beegle, 2006; Ismail et al., 1994; Thomas et al., 2007; Unger et al., 1991). The researchers suggested surface application of fertilizer, and therefore, a reduced mixing of fertilizer with soil as a probable reason of a higher phosphorus accumulation in no-tilled soil. The present study showed an incresae in soil phosphorus for inorganic fertilizer application which went in agreement with the findings from Shen et al. (2014) for corn and wheat and the researchers suggested diffrences in amounts of applied phosphorus and phosphorus storage by crop grains and stubbles as a possible explanation. However, my study also revealed a decrease in soil phosphorus for application of 150 lbs inorganic fertilizer. A likely reason for that could be phosphorus leaching by an outwash from irrigation (McDowell and Condron, 2012). A significant increase in soil phosphorus content was noted in case of organic manure which was in consistence with the previous findings from (Campbell et al., 1986; Gichangi et al., 2009; Song et al., 2017). According to Adler and Sikora (2003), production of humic matter and organic acid following application of organic fertilizer might increase solubility of phosphorus in soil and could be responsible for an increased concentration of soil phosphorus.

The results exhibited an increase in soil potassium in no-tillage compared to conventional-tillage, and the lowest at minimum-tillage which was in agreement with (López-Fando and Pardo,

2009; Lal et al., 1990) for grey pea/barley and corn/soybean respectively. According to Tan et al. (2015), soil potassium level was higher under no-tilled soil probably because the soil surface stays unperturbed and a substantial amount of organic matter accumulates on the soil surface. I observed an increased amount of soil potassium from my control to 100 lbs of inorganic fertilizer, and then the potassium content decreased for 150 lbs which was in agreement with the findings from Zhao et al., (2014) for a wheat-corn rotation. The decrease in soil potassium I found for 150 lbs inoragnic fertilizer was probably for a large depletion of available potassium from soil (Zhao et al., 2014). My study showed a significant increase in soil potassium level for organic manure application which was consistent with the previous studies from (Aziz et al., 2014; Gil et al., 2008; Soumare et al., 2002) for corn. According to Odlare et al. (2008), organic fertilizers rich in potassium were most likely able to increase plant-available soil potassium content.

The present study found an increment in C/N ratio from no-tilled soil to conventionally-tilled soil which probably could be explained from a higher accumulation of nitrogen on soil surface (Nascente et al., 2013). This observation was in consistence with the findings from Xue et al. (2015). Lou et al. (2012) studied the effects of tillage on soil carbon and nitrogen dynamics for corn and suggested that a higher amount of residue accumulated on no-tilled soil surface might be a reason for the higher C/N ratio and reveals disintegration of organic matter in a smaller amount on the unperturbed soil surface. The current study showed a significant increase in C/N ratio following organic fertilizer application which was in agreement with the finding from Liu and Zhou (2017). I detected a decrease in C/N ratio from control to 150 lbs of inorganic fertilizer application from my study which might be due to intense application of inorganic nitrogenous fertilizers and the rapid disintegration of soil organic matter by inorganic fertilizers (Liu and Zhou, 2017; Wu et al., 2004).

Impact on Abundance of AMF Colonization

The total colonization of AMF was the highest under minimum-tillage and a decrease from no-tillage to conventional-tillage. It could be suggested from a similar study on how external mycelial network affects the vesicular-arbuscular mycorrhizal colonization in disturbed soil for corn conducted by Evans and Miller (1990) that such difference in colonization was likely because the extraradical hyphal network got disrupted from conventional-tillage. This decrease was consistent with the previous studies (Bilalis and Karamanos, 2010; Borie et al., 2006; Brito et al., 2012; Kabir et al., 1998, 1997). My study found a decrease in mycorrhizal hyphal colonization as well from no-tillage to conventional-tillage which was consistent with the findings from (Borie et al., 2006; Kabir et al., 1998, 1997). Kabir et al. (1998) suggested such difference in hyphal colonization based on tillage treatment was probably because the adverse effects of conventional tillage on mycorrhizal hyphal density. It can be suggested that the intensity of conventional tillage in soil can harshly impact on plant roots and physically disrupt the AMF hyphal network, therefore, can disrupt the abundance of AMF colonization in soil (Patanita et al., 2020). I found a decrease in the total colonization rate for inorganic fertilizer application which was in agreement with the previous findings from (Gryndler et al., 2006; Hayman, 1982; Olsson et al., 1997; Treseder and Allen, 2002). We also found a decrease in hyphal colonization for chemical fertilizer application which went in agreement from the study of Kabir et al. (1997) for corn. It can be suggested that since inorganic fertilizers provide an instant nutrition to plants, therefore, plants get dependent on them. As a result, plants start relying less on their AMF symbionts, and the AMF colonization gets less abundant as the intensity of inorganic fertilizer increase in soil. That can be a probable reason of a lower abundance of AMF colonization under inorganic fertilizer application, and a decrease in AMF colonization was apparent as the amount of applied inorganic fertilizer got intense.

However, I did observe a significant rise in the total AMF colonization following organic fertilizer application which was in consistence with the previous studies from (Gryndler et al., 2006, 2002; Joner, 2000; Joner and Jakobsen, 1995; Ravnskov et al., 1999). My study also showed an increase in hyphal colonization following organic manure application which went in agreement with the studies from Kabir et al. (1998, 1997) who found a similar result for corn, and suggested that it might be because organic manure enriched the biological properties of soil and therefore, supported the arbuscular mycorrhizal colonization.

Conclusion

The study revealed that intensive management practices such as tillage and different rates of chemical fertilizer application can affect the biochemical properties of soil and the arbuscular mycorrhizal fungal colonization of roots. Nitrogen fertilizer applied in high amount could lower soil pH and rise the amount of mineral nitrogen in soil which might lead to soil acidification and water eutrophication. While application of organic fertilizer can improve soil phosphorus and potassium level and can improve soil fertility, organic carbon in soil can also be improved from organic fertilizer and thus can promote the crop production and soil fertility. My study shows the adverse effect of conventional tillage on the hyphal network of AMF colonization in plant roots and the dependency of plants on inorganic fertilizers instead of the symbiotic AMF in their roots. It is evident that intensive farming practices and excessive inorganic fertilizer applications reduce the AMF colonization of roots, while organic manure treatment and conservation tillage (no-tillage and minimum-tillage) increased the colonization. Overall, the results obtained from this study provide insights into the effects on intensive farming practices on soil health indicators and beneficial mycorrhizal fungi, which are two critically important aspects of cropping systems. Based on the observations of this study, minimum tillage in combination with organic fertilizer

can be recommended to retain soil health and improve crop production. However, since this study was based on a single time point and one crop, further observations would be required to formulate more robust recommendations.

EFFECTS OF INTENSIVE AGRICULTURAL TREATMENTS ON SOIL MICROBIOME STRUCTURE, COMPOSITION, AND NETWORK CONNECTIVITY

Introduction

There is a widespread variety of niches in soil environment which nurtures a huge diversity of microorganisms. Soil microbes play a significant role in maintaining soil function and maneuvering soil structure, nutrient cycling, and decomposition of organic matter (Guo et al., 2022; Navarro-Noya et al., 2013). Several research on soil microorganismal community discovered that agricultural practices such as tillage practice and fertilizer application have significant effects on soil microbial community and composition (Liao et al., 2018). Tillage works as one major factor to alter soil physical properties, biochemistry and subsequently, the community structure and functions of soil microorganisms (Sun et al., 2016). Fertilizer application works as a popular agricultural management practice to sustain soil fertility and crop production, and can have significant influence on soil microbial community composition and activities (Eo and Park, 2016; Shen et al., 2010). Although inorganic fertilizer application could significantly increase crop yield, however, previous researchers found that overdoing inorganic fertilizer directed soil degradation such as increased soil salinity, soil acidification, heavy metal pollution (Lin et al., 2019). Previous research has suggested that organic fertilization can improve the amount of organic carbon in soil and lead to a high microbial diversity (Dang et al., 2022; Liao et al., 2018).

Alpha and beta diversities are broadly used to evaluate the dynamics of soil microorganisms. Alpha diversity refers to the species diversity within a particular area or community or ecosystem while beta diversity is a comparison of species diversity between two communities or ecosystems (Whittaker, 1972). Fertilization can modify the alpha diversity and phyla of soil bacterial community significantly (Dang et al., 2022). According to (Geisseler and

Scow, 2014), soil physiochemical properties are directly connected with the alpha diversity of soil microbes. Since alpha diversity sum up the composition of an ecological community by its species richness, evenness or both, it is important to evaluate the alpha diversity of the amplicon sequencing data in order to assess the variances between environments (Willis, 2019). Some of the common alpha diversity indices are species richness, ACE, Shannon Index. Species richness is the number of species within a particular area or ecosystem (https://bio.libretexts.org). Abundance-based Coverage Estimator or ACE indicates species richness which are responsive to rare OTUs. The higher the value of ACE, the greater the species diversity is (Chao and Lee, 1992). ACE is a nonparametric method to assess the number of species using the total of the probabilities of observed species. ACE calculate expected OTUs based on observed OTUs. Observed OTUs are parted into abundant groups and rare groups in case of ACE method. Technically, species with more than ten individuals in a sample are referred as the abundant species and the species with less than ten individuals are referred as the rare species. With ACE, only the presence or absence of abundant species are counted. The exact occurrences for rare species are required in ACE method because estimating the number of missing species fully relies on the rare species (Kim et al., 2017). The Shannon index is also a popular diversity index in microbial ecology. Shannon index assesses the diversity of microbial community and examine the relative abundance of various species. For this, Shannon index considers the species richness. Moreover, Shannon index evaluates the average degree of improbability to calculate where the randomly chosen individual species will fit in a dataset. As the number of species increases and the distribution of individuals among the species gets even, the value of Shannon index increases (Kim et al., 2017).

Sequencing data can unravel the microbial diversity, composition, and how the communities might change spatiotemporally across agricultural practices. Detailing with

associations between microbial taxa across varied communities help to determine the functional roles of environment on those microorganisms. The associations between soil microorganisms can be explored by employing a microbial network analysis. In a network, microbial OTUs or ASVs are referred as nodes and the statistically significant associations between the nodes are referred as edges (Barberán et al., 2012; Ma et al., 2020). Agricultural management practices might alter the community structure of soil microorganisms (Price et al., 2021), with subsequent implications for microbe-microbe associations. The interrelations between microbial taxa help in revealing the niche shared by community members, such as bacteria and archaea. Such information is particularly valuable for soil environments where many microbial taxa are still unknown (Barberán et al., 2012; Janssen, 2006; Proulx et al., 2005).

Keystone taxa are the highly associated microbes which have vital roles in the microbiome composition and functioning (Banerjee et al., 2018). These taxa can also exert a considerable influence on ecosystems, therefore, are often called as the ecosystem engineers (Dunne et al., 2002)). Keystone taxa that are highly connected in a microbiome, can be statistically identified using network analysis (Banerjee et al., 2016a; Vick-Majors et al., 2014). Members of Alphaproteobacteria and Actinobacteria are bacterial keystone species, and members of Pezizomycotina are often the fungal keystone taxa. Previous research shows that agricultural intensification reduces the network complexity and abundance of keystone taxa in plant root microbiome (Banerjee et al., 2019). Investigation on keystone taxa is ongoing and cutting-edge analytical techniques are needed for a better understanding of the involvement of keystone taxa in microbiomes of different environments.

Research Questions

Intensive farming practices such as tillage and intense application of inorganic fertilizer may affect soil microbial communities and their associations, which motivated me to explore the following questions:

1. How do intensive agricultural treatments such as tillage and inorganic fertilizer application influence the diversity, community composition and network complexity of microorganisms in the bulk soil, rhizosphere soil and plant roots?
2. Which soil microorganisms are the indicator species and keystone taxa in bulk soil, rhizosphere soil and plant roots?

Hypotheses

1. The assembly and recruitment of soil microorganisms in bulk soil, rhizosphere soil and plant roots vary between intensive inorganic fertilizers compared to organic manure applications.
2. The microbial network complexity in bulk soil, rhizosphere soil and plant roots will differ following intensive inorganic fertilizer application compared to organic fertilizer application.

Materials and Methods

Molecular Work

DNA Extraction

Soil and plant samples were collected from agricultural plots located at the Carrington Research Extension Center in Foster County, North Dakota. The sampling design is previously described in Chapter I. Soil and root samples were stored in a freezer at -20°C until the DNA is extracted. For each bulk soil sample, 200 mg of soil and for each rhizosphere soil sample, 350 mg

of soil was measured and used for DNA extraction. For each root sample, 350 mg of root pieces were measured and used for DNA extraction. Soil and plant root DNA was extracted using the Qiagen DNeasy Power Soil Pro kit and Qiagen DNeasy Plant Pro kit (Qiagen Inc., Valencia, CA), and the extraction was followed according to the manufacturer's instructions. Two technical replicates were made from each soil and root sample and the replicates were combined to obtain approximately 50 µl DNA for further molecular analyses. Quality, quantity, and yield of DNA were determined by a NanoDrop ND-1000 spectrophotometer. After the quality and yield were determined, each DNA sample was standardized using nuclease-free water to attain 100 µl at a concentration of five ng/µl.

Amplicon Sequencing

DNA concentrations was determined with a Qubit fluorometer (Life Technologies, Paisley, UK). A barcoded high-throughput sequencing approach was be employed to assess the diversity and composition of bacterial and fungal communities. Bacterial communities were examined by amplifying the V3-V4 region of the bacterial 16S rRNA gene using the primers 515FB (GTGYCAGCMGCCGCGGTAA) and 806RB (GGACTACNVGGGTWTCTAAT) (Walters et al., 2015). Fungal communities were examined by amplifying the ITS 1 region using the primers ITS1F (CTTGGTCATTTAGAGGAAGTAA) (Gardes and Bruns, 1993) and ITS2 (GCTGCGTTCTTCATCGATGC) (White et al., 2014). Each primer was tagged with a 5-nucleotide-long padding sequence and an 8-nucleotide-long Golay barcode. For all amplicons, Illumina 300 bp pared-end sequencing was performed at the University of Minnesota Genomic Center (https://genomics.umn.edu). After sequencing, demultiplexing were performed and adapters were removed using Cutadapt (Martin, 2011). Bacterial sequences were analyzed following the DADA2 pipeline (Callahan et al., 2016). The whole pipeline started with quality

filtering and trimming of the reads. Before filtering and trimming the reads, the correct paths for forward and reverse reads had to be determined. In the quality profile plots for both forward and reverse reads, the bases are along the x-axis and the quality score is on the y-axis. Forward reads were cut at 250 bp and reverse reads at 200 bp to get rid of the lower quality base pairs. Next, error models were created for both forward and reverse reads by understanding the specific errors in the dataset, as each sequencing run will vary slightly from its error profile. On the error plot, consensus quality score lies along the x-axis, and error frequency lies along the y-axis. The red line in the plot denotes what is anticipated based on the quality score, the black line represents the estimation, and the black dots signify the observed (Callahan et al., 2016). Next, dereplication was performed. DADA2 dereplicates sequences and creates a new quality score profile for each novel sequence based on the average quality scores of each base of all sequences those were its replicates. Then a count table was made by using the *makeSequenceTable()* function. This is one of the primary products after processing an amplicon dataset. This can also be described as a biome table or an OTU matrix. I made three different sequence tables from the amplicon sequencing datasets of bulk soil, rhizosphere soil, and plant root samples. Incomplete extensions in PCR permit the consequent PCR cycles to use a partly extended strand to attach with the template of a different but analogous sequence. This partly extended strand then acts as a primer to extend and form a chimeric sequence. Once formed, the chimeric sequence further amplifies in successive cycles. Basically, chimeras are artifact sequences formed by two or more biological sequences inappropriately joined together. This commonly takes place during PCR reactions using unrefined environmental samples. Several aspects can prompt chimera formation, such as, pairwise sequence identity between 16S rRNA genes, number of PCR cycles, and relative abundance of gene specific PCR templates. During PCR amplification of 16S gene, chimeric sequences can form and work as

a mutual font for 16S sequence artifacts. It is important to distinguish chimeric sequences while using 16S sequences to summarize microbial communities (Haas et al., 2011). DADA2 classifies the chimeras by lining up each sequence with those that were salvaged in plenty and then checking if there are any scarce sequences that can be made just by uniting left and right pieces of two of the plentiful ones. These are then eliminated. I have three different sequence tables of three different amplicon datasets from three different samples. Expected chimeras were removed from the sequence tables by detecting them through DADA2. The final stage is to define the microbial taxonomy. Typically, sequence reads are first put together or binned into taxonomic units, and then, the microbial composition in test samples is analyzed and compared. A reference taxonomy is needed for taxonomic analyses. Binning of sequence reads are usually done by alignment of reads against the reference sequences. SILVA is the largest reference database for 16S rRNA based taxonomy and offers databases on phylogenies for both small subunit (SSU) and large subunit (LSU) rRNA genes. SILVA datasets include ample sets of background and sequence related information, which are: taxonomic classifications from several taxonomic sources, multiple sequence orientation, information about types and strains, and recent legitimate nomenclatures. Quality was checked for all sequences. Corresponding data are available as ARB files, FASTA, and comma-separated value (CSV) formats. In the end, those can be looked through straight from the SILVA website. SILVA database contains taxonomic information for bacteria, archaea, and eukaryote domains (Balvočiute and Huson, 2017; Quast et al., 2013). To delineate the taxonomy based on the 16S rRNA gene, I used DECIPHER package and SILVA as the reference taxonomic database. I generated a FASTA file, a count table, and a taxonomy table from our amplicon sequencing datasets of bulk soil, rhizosphere soil, and plant roots (Callahan et al., 2016). I performed the entire DADA2 pipeline on R (R version 4.0.2).

PIPITS is a user-friendly analytical tool for a programmed processing for large sequence datasets of fungal ITS sequences. PIPITS is mostly devised to analyze demultiplexed paired-end reads from Illumina MiSeq sequencing, whose quality have been verified as well. Fungal ITS sequences were processed using the PIPITS pipeline (Gweon et al., 2015). Bacterial ASVs and fungal OTUs were classified according to SILVA (Pruesse et al., 2007) and UNITE (Abarenkov et al., 2010) databases, respectively. The PIPITS pipeline is comprised of three parts: PIPITS_PREP, PIPITS_FUNITS and PIPITS_PROCESS. The raw sequence reads of fungal ITS are prepared by PIPITS_PREP from an Illumina MiSeq sequencer, fungal ITS subregions (ITS1 or ITS2) from the reads are extracted by PIPITS_FUNITS, and OTU (operational taxonomic unit) abundance tables and taxonomy tables are produced after analyzing the reads by PIPITS_PROCESS for further analyses. Generally, UNITE fungal ITS reference training dataset is needed to consign taxonomy and UNITE UCHIME reference dataset helps to subtract chimera. Raw ITS sequences are commonly demultiplexed FASTQ files which are divided into separate files by the Illumina MiSeq sequencer so that one file is allotted for one barcode. The directory with raw sequence files, the output directory, and the tab-delimited file listing pairs of filenames for forward and reverse reads and sample IDs are selected by PIPITS_PREP. Next, PIPITS_PREP joins the read pairs on the overlapping areas of sequences. After quality filtering of the subsequent reads, headers of each read are tagged with an index number and a sample ID, and finally converted into a FASTA format to merge into a single file. Then, PIPITS_FUNITS jumps into action and takes the output from PIPITS_PREP. The redundant sequences are deleted and dereplication begins in this stage. It is important to identify which ITS subregion needs to be removed. The elimination from the sequences is done with the help of an extraction tool ITSx. Next, the subsequent sequences are magnified again to manifest their actual abundances. Further,

VSEARCH pipeline is utilized by PIPITS_PROCESS for grouping sequences into OTUs. This pipeline entails dereplication of input sequences before clustering the sequences at a user defined level. UNITE UCHIME reference dataset is used to detect and remove chimera from the following sequences. Next, the entered ITS sequences are plotted onto the chimera-removed sequences at the defined threshold and UNITE fungal ITS reference dataset is used for taxonomic assignment of the chimera-free sequences. Finally, the results are deciphered into an OTU abundance table and a phylotype abundance table. By default, PIPITS uses the canonical 97% sequence identity to cluster sequences into OTUs (Gweon et al., 2015).

Statistical Analyses

All statistical analysis were performed in R (R version 4.0.2). The R packages used were: *dplyr* (Wickham et al., 2022), *ggplot2* ((Wickham et al., 2016) (Wickham H (2016). *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York. ISBN 978-3-319-24277-4), *tidyverse* (Hadley Wickham et al., 2019), *readxl* (Wickham et al., 2019), *phyloseq* (McMurdie and Holmes, 2013), *devtools* (Wickham et al., 2021), *FSA* (Ogle et al., 2022), *indicspecies* (De Cáceres et al., 2020), *permute* (Simpson et al., 2022), *VennDiagram* (Chen, 2021), *vegan* (Oksanen et al., 2020), *Desctools* (Signorell, 2018), *PMCMR* (Pohlert, 2016), and *ggpubr* (Kassambara, 2020). Alpha diversity indices such as species richness, ACE, and Shannon-Weaver Index were calculated using the *phyloseq* package in R (McMurdie and Holmes, 2013). ASV count tables and taxonomy tables were formed from the bacterial 16S and fungal ITS sequencing data of soil and root samples using the DADA2 pipeline. After removing the singletons from the phyloseq objects, the archaeal domains were removed from the ASV table, so that only bacterial domain remains to work with. Rarecurves from both tillage and fertilizer treatment were plotted after rarefying the bacterial 16S data. Boxplots were created to visualize the measurement of the calculated alpha

diversity indices. Finally, Kruskal-Wallis and Dunn post hoc tests were performed for significance. Beta diversity patterns were performed in R to check the effects of tillage and fertilizer application treatments on microbial community and fungal community structure in the test soil and root samples. Consistency of the multivariate distributions for both tillage and fertilizer treatments were checked with PCoA test (Principal Coordinate Analysis) using the Bray-Curtis dissimilarity matrix (Adonis2 test, 999 permutations) (Bray and Curtis, 1957). To find out the effects of tillage and fertilizer treatments on soil microbial community, PERMANOVA testing (Permutational multivariate analysis of variance) and CAP (Canonical Analysis of Principal coordinates) were performed with 99 permutations. Each statistical analyses for alpha and beta diversity prototypes were measured for fungal ITS data obtained from bulk soil, rhizosphere soil, and plant root samples as well. We performed Spearman's correlation and regression analysis to check how different soil properties are correlated with tillage and fertilizer regimes for each calculated alpha diversity index from bulk soil, rhizosphere soil, and plant root samples on R. Indicator species for both bacterial 16S and fungal ITS dataset for each tillage and fertilizer treatment were identified in R using the *indicspecies* package including the *multipatt* function (De Cáceres et al., 2020). The relative abundance of soil microbial community for three different tillage regimes and five fertilizer applications were assessed creating stacked bar charts on R (R version 4.0.2) using the *forplotting* function for both bacterial 16S and fungal ITS dataset all test soil and root samples.

Network Analysis

Bacterial 16S ASV datasets and fungal ITS datasets from the bulk soil, rhizosphere soil and root samples were used. A metadata was created for each treatment containing both bacterial ASVs and fungal OTUs. After removing the singletons using R, ASVs and OTUs were filtered out which were present in 50% of all samples for each dataset. To reduce pairwise comparisons and

manage the false discovery rate (FDR) (Benjamini and Hochberg, 1995) of 0.01, the network analysis was performed on the ASVs and OTUs that were contributing to 90% difference in bacterial and fungal communities between treatments (Banerjee et al., 2016c, 2016b). Spearman's correlation was performed based on a highly significant correlation (p-value<0.001) to detect the pattern of cooccurrence (Junker and Schreiber, 2008). A correlation table was formed for each network afterwards to identify the positive and negative correlations. Networks were created for each of the three tillage and five fertilizer application regimes. Three holistic meta-networks were also created from the test bulk soil, rhizosphere soil, and root samples. There are at least 2 edges per node in each network. The *Gephi* software (Gephi version 0.9.2) was used to create the associations between microbial communities and treatments which were significant at the FDR of 1% (Banerjee et al., 2016c; Bastian et al., 2009). The nodes were colored by taxonomy. Each network contains both bacterial ASVs and fungal OTUs, therefore, the nodes denoting the bacterial ASVs were given a spherical shape, and the nodes representing the fungal OTUs were given a triangular shape. The edges showing positive correlations were colored as solid black lines, and the edges showing negative correlations were colored as solid red lines. Keystone taxa were identified for each compartment using node scores. High degree and high closeness centrality were considered for a statistical identification of keystone taxa (Zheng et al., 2021).

Results

Microbial Diversity

Species richness, ACE, and Shannon Index were determined from bacterial 16S sequences of bulk soil, rhizosphere soil, and plant root samples based on the rarefied data (**Figure 8**). No statistical significance was found under both tillage and fertilizer treatments in bulk soil samples and only under the tillage treatments for species richness, ACE, and Shannon index in rhizosphere

soil samples (**Table 5**). However, significant differences were found for rhizosphere soil samples under fertilization for species richness and ACE (**Table 7**). The 0 lbs of inorganic fertilization (control) was significantly greater than 50 lbs and significantly lower than 150 lbs of inorganic fertilization for species richness and ACE in rhizosphere soil samples, however, organic fertilization and 100 kg of inorganic fertilization were not significantly different from 0 lbs, 50 lbs and 150 lbs of inorganic fertilization. Species diversity, ACE and Shannon Index in rhizosphere soil samples were the greatest under organic fertilizer application. No statistical significance was found under the tillage treatments for plant root samples as well. However, significant differences were found for plant root samples under fertilization for species richness and ACE. 100 lbs of inorganic fertilization was significantly greater than organic fertilization for species richness ACE and Shannon index in plant root samples, however, 0 lbs, 50 lbs and 150 lbs of inorganic fertilization were not significantly different from organic fertilization and 100 lbs of inorganic fertilization. Species diversity, ACE and Shannon Index in plant root samples were the greatest under 0 lbs of inorganic fertilizer application.

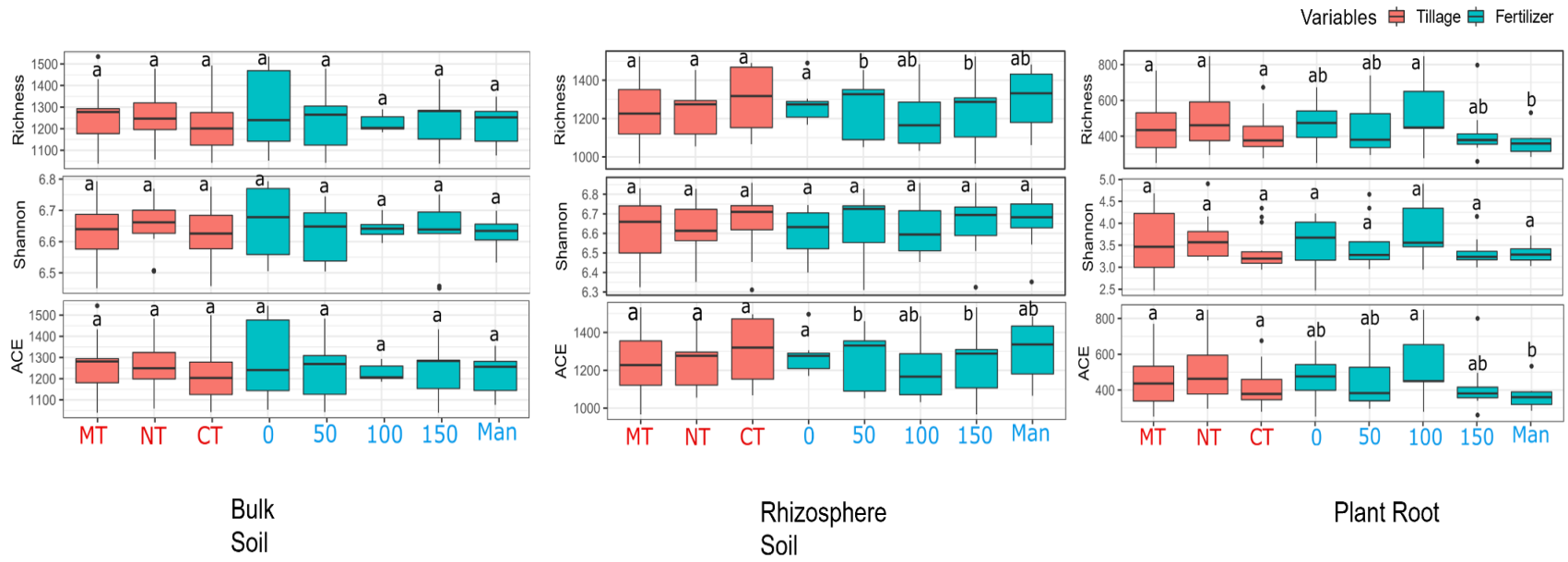

Figure 8: Alpha diversity indices (Richness, ACE, Shannon Index) plotted together based on three tillage and five fertilizer treatments from bacterial 16S sequences of soil and root samples. Red boxes determine three tillage treatments (NT, MT, CT) and blue boxes determine five fertilizer treatments (0, 50, 100, 150, Man). NT: No Tillage, MT: Minimum Tillage, CT: Conventional Tillage, Man: manure. Statistical significance is denoted with the letters 'a' and 'b' at ($p < 0.05$) level

Species richness, ACE, and Shannon Index were determined from fungal ITS OTU tables of bulk soil, rhizosphere soil, and plant root samples based on the rarefied data (**Figure 9**). No statistical significance was found under the tillage treatments for bulk soil samples (**Table 6**). However, significant differences were found for bulk soil samples under fertilization (**Table 8**). 0 lbs of inorganic fertilization (control) was significantly lower than 100 lbs of inorganic fertilization for species richness in bulk soil samples. 0 lbs of inorganic fertilization (control) was significantly greater than organic fertilization and significantly lower than 100 lbs of inorganic fertilization for species richness and ACE in bulk soil samples. Organic fertilization was greater but not significantly different from 50 lbs and 150 lbs of inorganic fertilization for species richness; however, 50 lbs of inorganic fertilization is significantly lower than 0 lbs of inorganic fertilization for species richness. 50 lbs and 150 lbs of inorganic fertilization are not significantly different from each other and organic fertilization and 0 lbs are significantly lower than 100 lbs of inorganic fertilization in ACE. 50 lbs and 100 lbs of inorganic fertilization were significantly different from 150 lbs of inorganic fertilization for Shannon index; however, organic fertilization and 0 lbs of inorganic fertilization were not significantly different from either. No statistical significance was found under the fertilizer treatments for rhizosphere soil samples. However, significant differences were found for rhizosphere soil samples under tillage treatments for species richness and ACE. Species richness under conventional tillage was significantly greater than minimum tillage, however, no tillage is showing the highest species richness. In case of ACE, minimum tillage is significantly lower than no tillage and conventional tillage however, no tillage is showing the highest number for ACE. Also, no statistical significance was found under the tillage and fertilizer treatments for plant root samples.

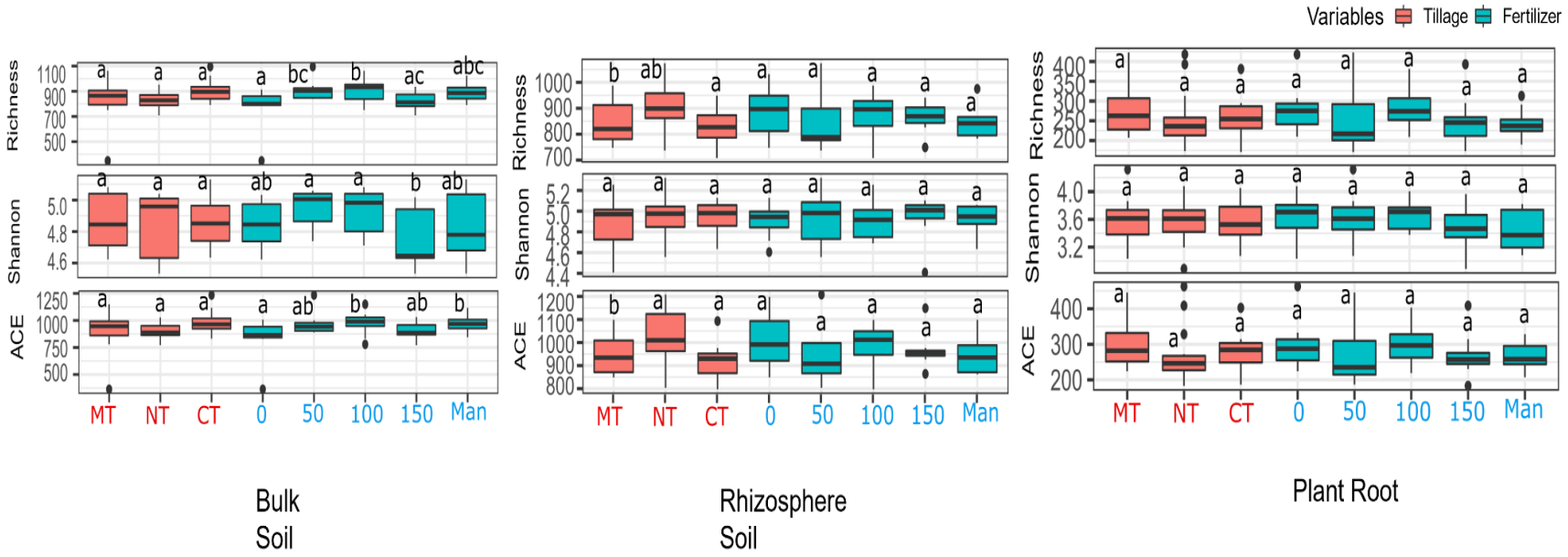

Figure 9: Alpha diversity indices (Richness, ACE, Shannon Index) plotted together based on three tillage and five fertilizer treatments from fungal ITS sequences of soil and root samples. Red boxes determine three tillage treatments (NT, MT, CT) and blue boxes determine five fertilizer treatments (0, 50, 100, 150, Man). NT: No Tillage, MT: Minimum Tillage, CT: Conventional Tillage, Man: manure. Statistical significance is denoted with the letters 'a', 'b', and 'c' at ($p < 0.05$) level.

The table below represents the mean values and statistical significance for bacterial 16S data from bulk soil, rhizosphere soil and plant root samples under three different tillage treatments. The alpha diversity indices are species richness, ACE, and Shannon index. Statistical significance is denoted with the letters 'a' and 'b' at ($p < 0.05$) level.

Table 5: Mean values and statistical significance for bacterial 16S data from bulk soil, rhizosphere soil and plant root samples under three different tillage treatments. The alpha diversity indices are species richness, ACE, and Shannon index. Statistical significance is denoted with the letters 'a' and 'b' at ($p < 0.05$) level compared across columns for different tillage treatments.

Tillage	**NT_16S**	**MT_16S**	**CT_16S**	**NT_16S**	**MT_16S**	**CT_16S**	**NT_16S**	**MT_16S**	**CT_16S**
Sample	**bulksoil**	**bulksoil**	**bulksoil**	**rhizo**	**rhizo**	**rhizo**	**root**	**root**	**root**
Richness	1128.533[a]	1033.333[a]	1226.333[a]	1099.133[a]	1313.066[a]	1014.333[a]	480.733[a]	375.066[a]	396.333[a]
ACE	1131.133[a]	1036.654[a]	1228.532[a]	1100.616[a]	1316.051[a]	1016.593[a]	483.356[a]	377.032[a]	398.651[a]
Shannon	6.184[a]	5.325[a]	6.630[a]	6.182[a]	6.653[a]	5.305[a]	3.503[a]	2.986[a]	3.143[a]

Note: rhizo: rhizosphere soil, NT: No Tillage, MT: Minimum Tillage, CT: Conventional Tillage, Man: manure

The table below represents the mean values and statistical significance for fungal ITS data from bulk soil, rhizosphere soil and plant root samples under three different tillage treatments. The alpha diversity indices are species richness, ACE, and Shannon index. Statistical significance is denoted with the letters 'a' and 'b' at ($p < 0.05$) level.

Table 6: Mean values and statistical significance for fungal ITS data from bulk soil, rhizosphere soil and plant root samples under three different tillage treatments. The alpha diversity indices are species richness, ACE, and Shannon index. Statistical significance is denoted with the letters 'a' and 'b' at ($p < 0.05$) level compared across columns for different tillage treatments.

Tillage	**NT_ITS**	**MT_ITS**	**CT_ITS**	**NT_ITS**	**MT_ITS**	**CT_ITS**	**NT_ITS**	**MT_ITS**	**CT_ITS**
Sample	**bulksoil**	**bulksoil**	**bulksoil**	**rhizo**	**rhizo**	**rhizo**	**root**	**root**	**root**
Richness	718.466[a]	829.266[a]	799.800[a]	908.666[ab]	684.866[b]	768.400[a]	291.333[a]	253.066[a]	172.866[a]
ACE	768.535[a]	896.492[a]	878.108[a]	1017.629[a]	772.837[b]	864.673[a]	313.080[a]	269.202[a]	187.529[a]
Shannon	3.926[a]	4.867[a]	4.485[a]	4.956[a]	3.969[a]	4.541[a]	3.746[a]	3.340[a]	2.676[a]

Note: rhizo: rhizosphere soil, NT: No Tillage, MT: Minimum Tillage, CT: Conventional Tillage, Man: manure

The table beneath represents the mean values and statistical significance for bacterial 16S data from bulk soil, rhizosphere soil and plant root samples under five different fertilizer treatments. The alpha diversity indices are species richness, ACE, and Shannon index. Statistical significance is denoted with the letters ‘a’ and ‘b’ at ($p < 0.05$) level.

Table 7: Mean values and statistical significance for bacterial 16S data from bulk soil, rhizosphere soil and plant root samples under five different fertilizer treatments. The alpha diversity indices are species richness, ACE, and Shannon index. Statistical significance is denoted with the letters 'a', 'b', and 'c' at ($p < 0.05$) level compared across columns for different fertilizer treatments.

Fertilizer	**0_16S**	**50_16S**	**100_16S**	**150_16S**	**Man_16S**	**0_16S**	**50_16S**	**100_16S**	**150_16S**	**Man_16S**	**0_16S**	**50_16S**	**100_16S**	**150_16S**	**Man_16S**
Sample	**bulk soil**	**bulk soil**	**bulk soil**	**bulk soil**	**bulk soil**	**rhizo**	**rhizo**	**rhizo**	**rhizo**	**rhizo**	**root**	**root**	**root**	**root**	**root**
Richness	1301.111[a]	1204.222[a]	1179.777[a]	1187.333[a]	774.555[a]	957.222[a]	913.444[b]	1235.666[ab]	1239.555[b]	1365.000[ab]	495.444[ab]	431.333[ab]	434.222[a]	407.888[ab]	334.666[b]
ACE	1304.982[a]	1206.785[a]	1183.442[a]	1189.856[a]	775.466[a]	958.644[a]	914.567[b]	1238.077[ab]	1242.715[b]	1368.098[ab]	497.666[ab]	434.104[ab]	435.863[a]	410.511[ab]	336.921[b]
Shannon	6.663[a]	6.618[a]	5.942[a]	6.598[a]	4.411[a]	5.167[a]	5.125[a]	6.593[a]	6.579[a]	6.770[a]	3.654[a]	3.428[a]	3.418[a]	19.880[a]	2.340[a]

Note: rhizo: rhizosphere soil, Man: manure

The table beneath represents the mean values and statistical significance for fungal ITS data from bulk soil, rhizosphere soil and plant root samples under five different fertilizer treatments. The alpha diversity indices are species richness, ACE, and Shannon index. Statistical significance is denoted with the letters ‘a’ and ‘b’ at ($p < 0.05$) level.

Table 8: Mean values and statistical significance for fungal ITS data from bulk soil, rhizosphere soil and plant root samples under five different fertilizer treatments. The alpha diversity indices are species richness, ACE, and Shannon index. Statistical significance is denoted with the letters 'a', 'b', and 'c' at ($p < 0.05$) level compared across columns for different fertilizer treatments.

Fertilizer	**0_ITS**	**50_ITS**	**100_ITS**	**150_ITS**	**Man_ITS**	**0_ITS**	**50_ITS**	**100_ITS**	**150_ITS**	**Man_ITS**	**0_ITS**	**50_ITS**	**100_ITS**	**150_ITS**	**Man_ITS**
Sample	**bulk soil**	**bulk soil**	**bulk soil**	**bulk soil**	**bulk soil**	**rhizo**	**rhizo**	**rhizo**	**rhizo**	**rhizo**	**root**	**root**	**root**	**root**	**root**
Richness	799.000a	696.222bc	863.222b	776.888ac	777.222abc	920.333a	890.555a	752.777a	728.333a	644.555a	267.777a	199.888a	257.111a	238.555a	232.111a
ACE	855.198a	748.452ab	936.451b	860.336ab	838.122b	1038.532a	999.230a	849.156a	823.408a	714.906a	287.429a	212.104a	275.778a	254.470a	253.239a
Shannon	4.898ab	3.837a	4.871a	4.288b	4.238ab	5.016a	4.920a	4.391a	4.397a	3.718a	3.317a	2.779a	3.521a	3.246a	3.406a

Table note: rhizo: rhizosphere soil, Man: manure

Microbial Community Structure

In case of bacterial communities, bulk soil, rhizosphere soil and root samples explained 50%, 51% and 33% of the total variance respectively. In case of bacterial communities, bulk soil, rhizosphere soil and root samples explained 38%, 31% and 19% of the total variance respectively. Bacterial and fungal communities formed their separate clusters and grouped within each cluster. (**Figure 10**) represents the PCoA plots for bacterial 16S datasets and (**Figure 11**) represents the PCoA plots for fungal ITS datasets of bulk soil, rhizosphere soil, and plant root samples based on both tillage and fertilizer treatments.

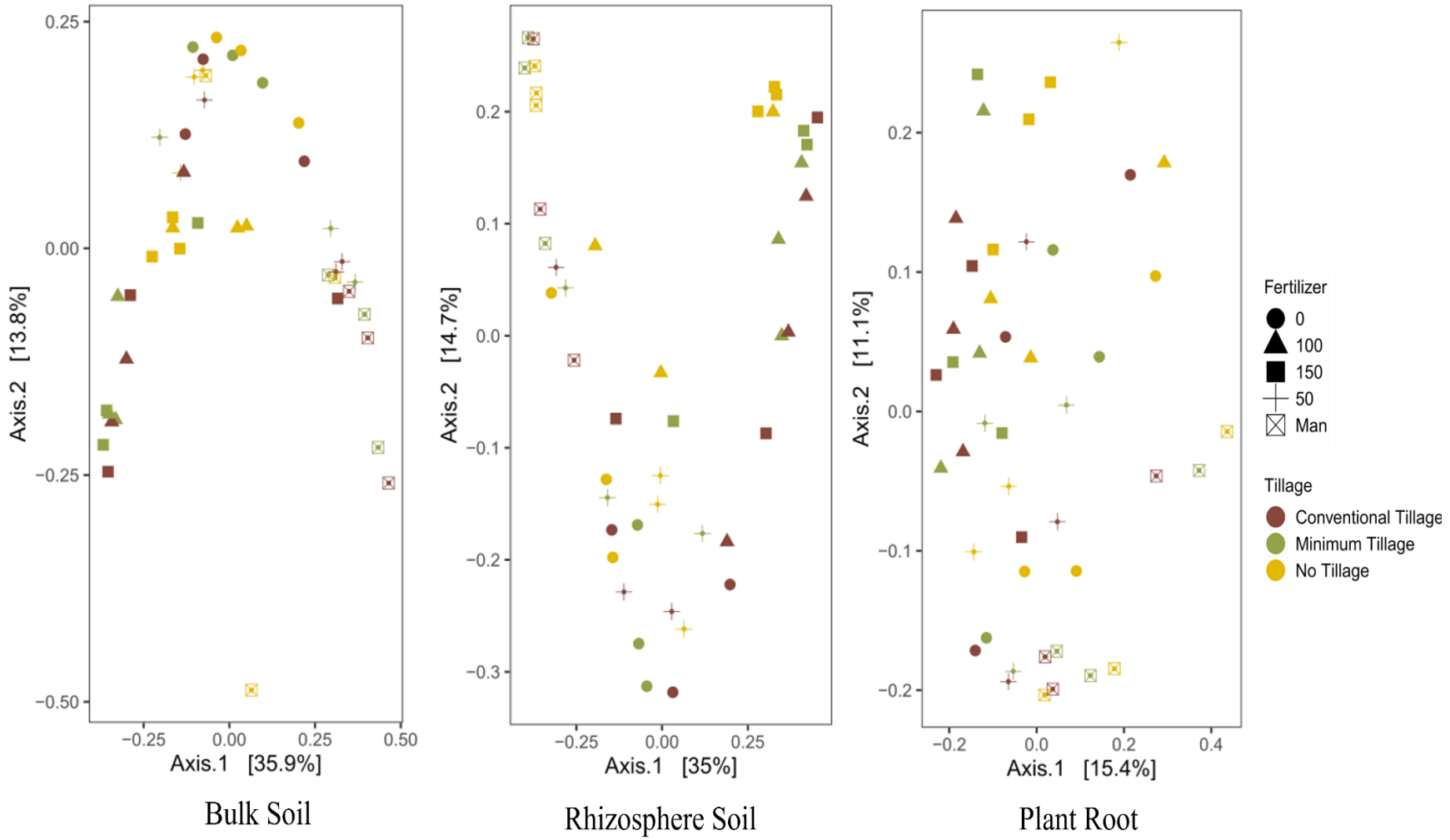

Figure 10: PCoA plots for bacterial 16S datasets of bulk soil, rhizosphere soil, and plant root samples with both tillage and fertilizer treatments. ● denotes no tillage, ● denotes minimum tillage, ● denotes conventional tillage. ● denotes 0 lbs, + denotes 50 lbs, ▲ denotes 100 lbs, ■ denotes 150 lbs of inorganic fertilizer, and ⊠ denotes organic manure application.

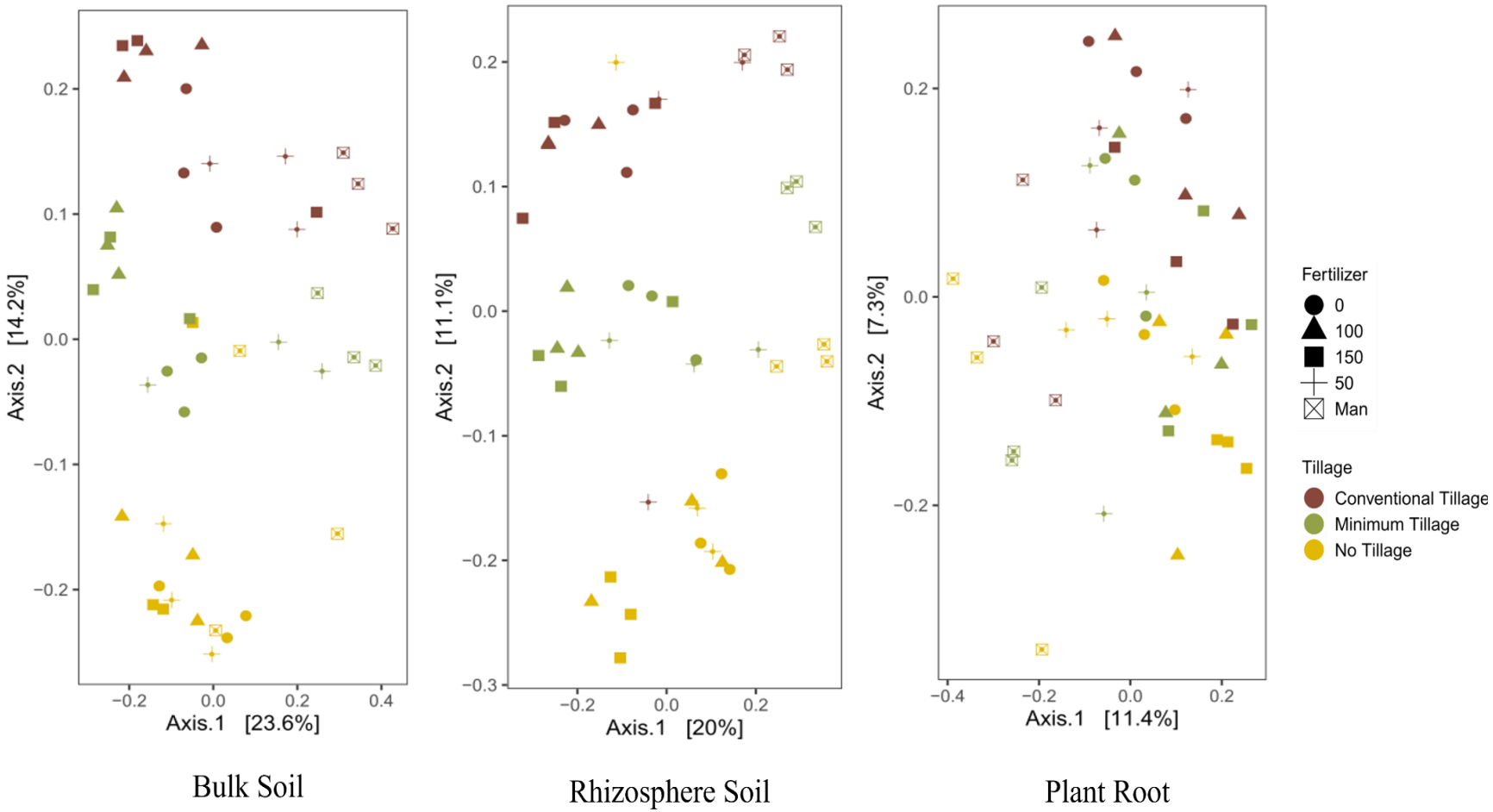

Figure 11: PCoA plots for fungal ITS datasets of bulk soil, rhizosphere soil, and plant root samples with both tillage and fertilizer treatments. ● denotes no tillage, ● denotes minimum tillage, ● denotes conventional tillage. ● denotes 0 lbs, + denotes 50 lbs, ▲ denotes 100 lbs, ■ denotes 150 lbs of inorganic fertilizer, and ⊠ denotes organic manure application.

PERMANOVA and CAP Analysis

The marked differences between bacterial and fungal communities in bulk soil, rhizosphere soil and plant root samples for tillage and fertilizer treatments were confirmed by PERMANOVA (**Table 9**). For bacterial communities, no significance was found for tillage treatment in bulk soil, rhizosphere soil and plant roots. However, fertilizer treatment was statistically significant for bulk soil and rhizosphere soil, and plant root samples. For fungal communities, tillage and fertilizer treatment was statistically significant for bulk soil, but not significant for rhizosphere soil and plant root samples.

Table 9: PERMANOVA test for both bacterial 16S and fungal ITS datasets for bulk soil, rhizosphere soil, and plant root samples under tillage and fertilizer treatments. Degree of freedom, p-value and statistical significance have been shown. df: degree of freedom, NS: No Significance. * and ** indicates statistical significance for (p-values < 0.05 and 0.01) respectively.

Sample	Treatment	df	R^2	p-value	Statistical significance
Bulk Soil (16S)	**Tillage**	2	0.05975	0.14	NS
	Fertilizer	4	0.21773	0.01	**
Rhizosphere Soil (16S)	**Tillage**	2	0.04793	0.31	NS
	Fertilizer	4	0.15764	0.04	*
Plant Root (16S)	**Tillage**	2	0.05292	0.18	NS
	Fertilizer	4	0.12698	0.01	**
Bulk Soil (ITS)	**Tillage**	2	0.06593	0.04	*
	Fertilizer	4	0.24137	0.01	**
Rhizosphere Soil (ITS)	**Tillage**	2	0.03506	0.70	NS
	Fertilizer	4	0.10371	0.08	NS
Plant Root (ITS)	**Tillage**	2	0.04224	0.51	NS
	Fertilizer	4	0.09718	0.16	NS

CAP plots of bacterial and fungal communities showed clear clustering in soil and root samples treated with fertilizer. (**Figure 12**) represents the CAP plots for bacterial 16S datasets; and (**Figure 13**) and (**Figure 14**) represents the CAP plots for fungal ITS datasets of bulk soil, rhizosphere soil, and plant root samples based on tillage and fertilizer treatments.

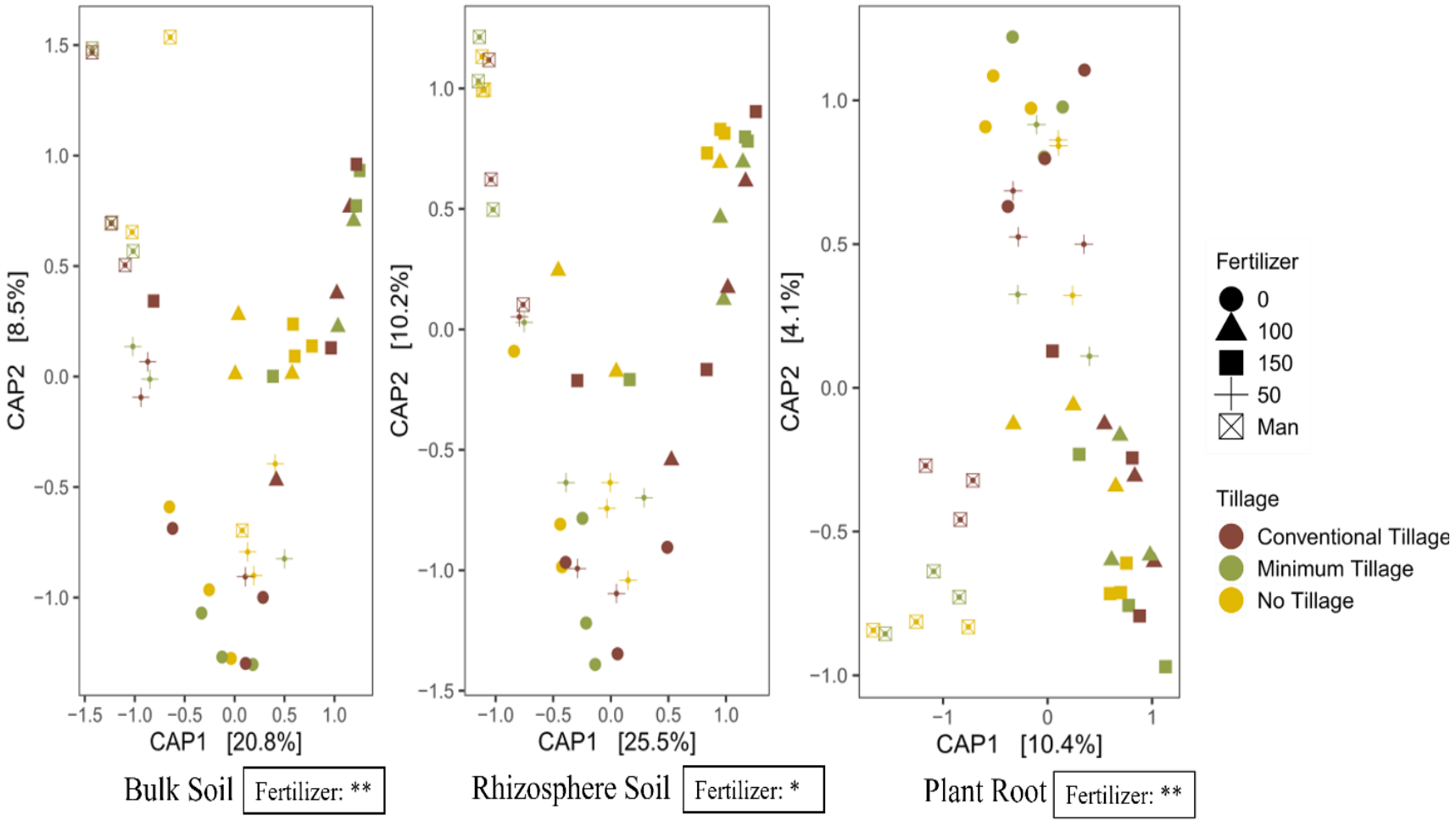

Figure 12: CAP plots for bacterial 16S datasets of bulk soil, rhizosphere soil, and plant root samples with both tillage and fertilizer treatments. ● denotes no tillage, ● denotes minimum tillage, ● denotes conventional tillage. ● denotes 0 lbs, + denotes 50 lbs, ▲ denotes 100 lbs, ■ denotes 150 lbs of inorganic fertilizer, and ⊠ denotes organic manure application.

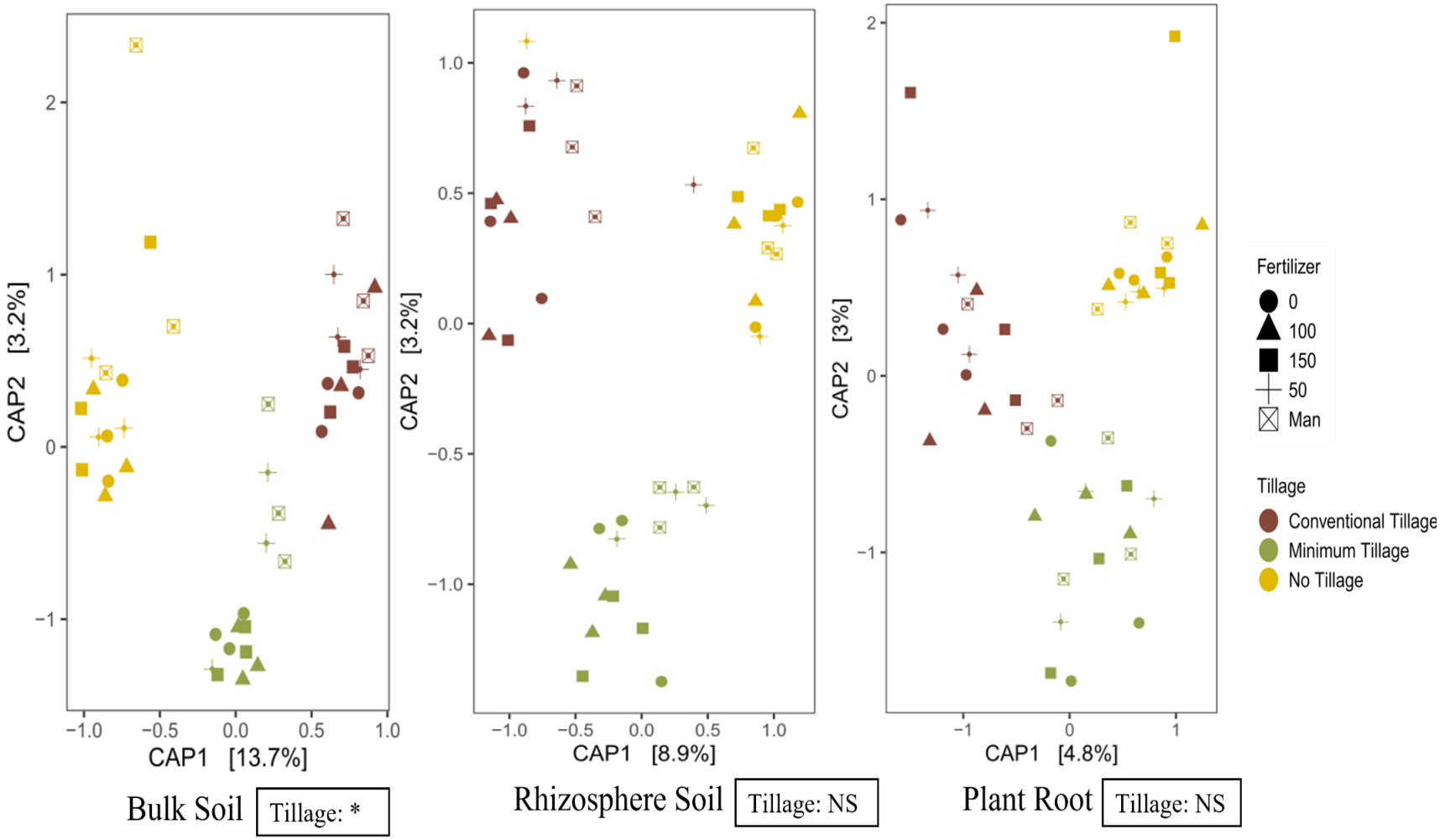

Figure 13: CAP plots for fungal ITS datasets of bulk soil, rhizosphere soil, and plant root samples with both tillage and fertilizer treatments. ● denotes no tillage, ● denotes minimum tillage, ● denotes conventional tillage. ● denotes 0 lbs, + denotes 50 lbs, ▲ denotes 100 lbs, ■ denotes 150 lbs of inorganic fertilizer, and ⊠ denotes organic manure application.

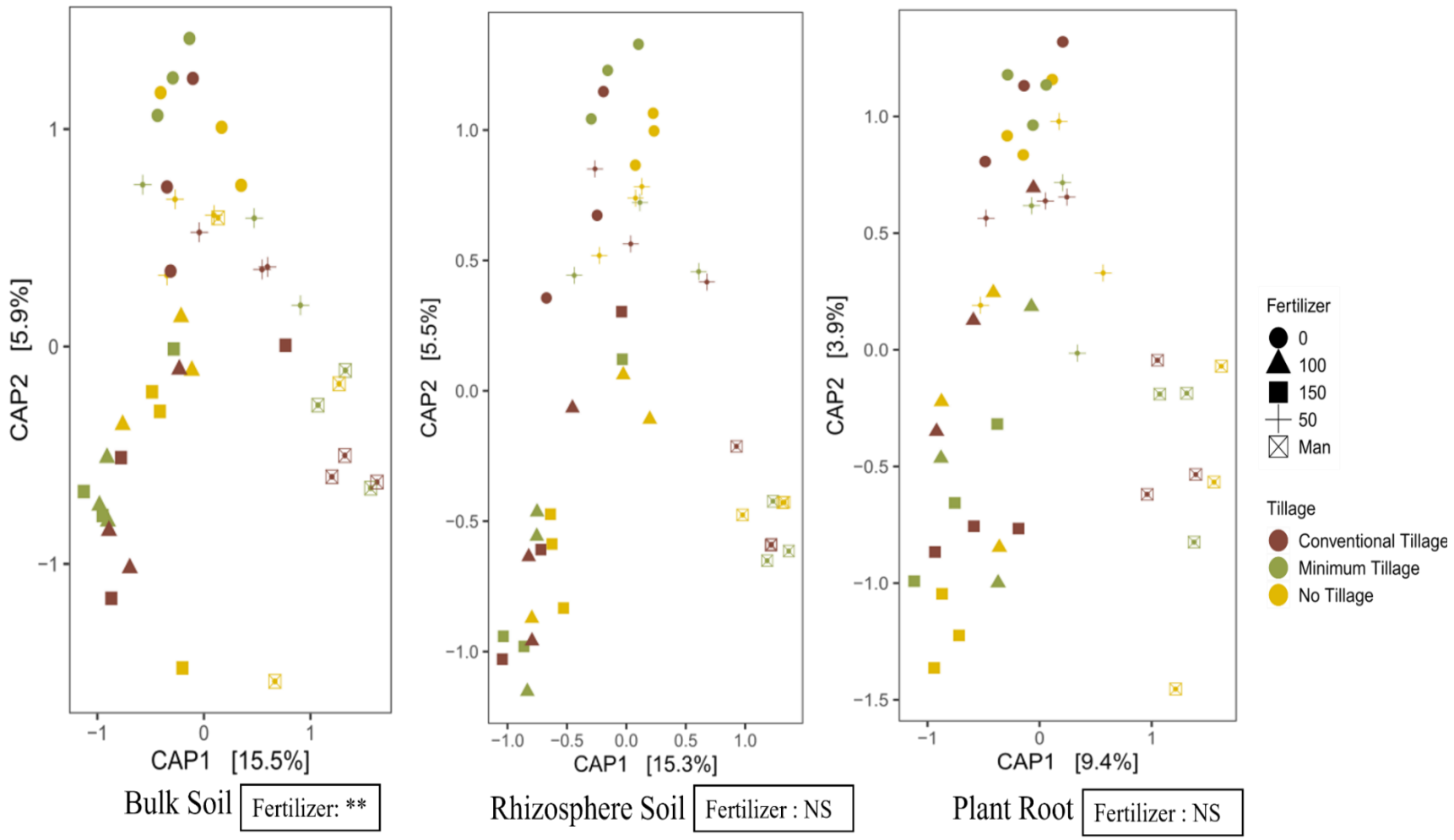

Figure 14: CAP plots for fungal ITS datasets of bulk soil, rhizosphere soil, and plant root samples with both tillage and fertilizer treatments. ● denotes no tillage, ● denotes minimum tillage, ● denotes conventional tillage. ● denotes 0 lbs, + denotes 50 lbs, ▲ denotes 100 lbs, ■ denotes 150 lbs of inorganic fertilizer, and ⊠ denotes organic manure application.

Community Composition of Microorganisms

Figure 15 represents the community composition of the top ten bacterial phyla for bulk soil, rhizosphere soil, and plant root samples based on three different tillage treatments. Statistical significance was denoted in red. NS signified no significance, and * and ** indicated statistical significance based on the p-values (p-value<0.05 and 0.01) respectively (**Table 10**). For bulk soil samples, phylum Actinobacteriota (p= 0.01649), Planctomycetota (p= 0.00699), Proteobacteria (p= 0.00551) and Verrucomicrobiota (p= 0.00431) were statistically significant. However, no statistical significance was found for any bacterial phyla in case of rhizosphere soil and plant root samples for three different tillage treatments. The observations suggest that the bacterial community composition in bulk soil and rhizosphere soil samples under three different tillage treatments are similar, however, the bacterial community composition in plant root samples differed from the communities found in the soil samples. In case of plant root samples, phyla Cyanobcteria and Patescibacteria were found in the top ten bacterial communities, which were not present in the bacterial communities obtained from the soil samples. Additionally, phylum Acidobacteriota was found in the bacterial communities obtained from the soil samples, which was absent in the bacterial community composition of plant root samples.

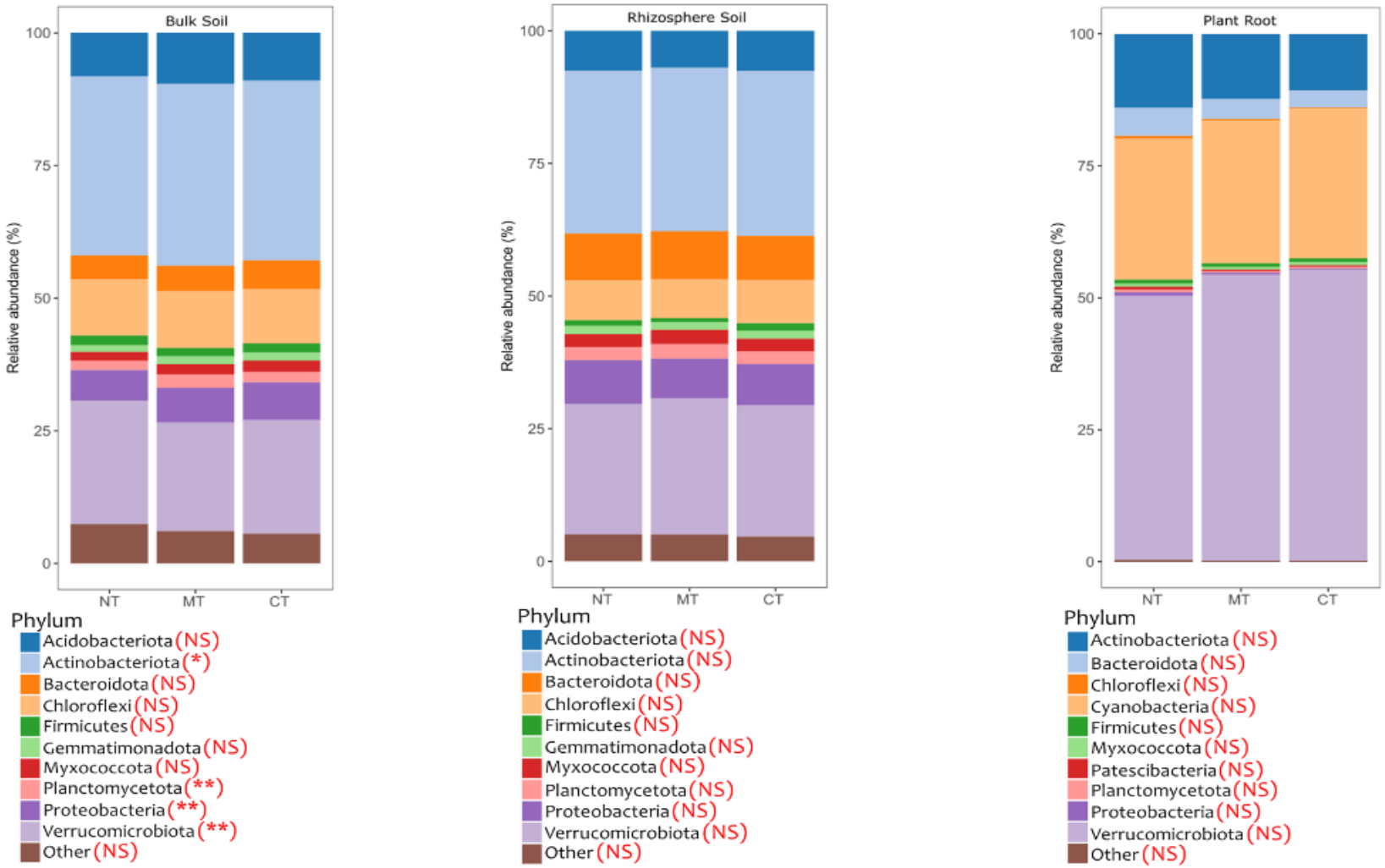

Figure 15: Community composition plots for bacterial 16S datasets of bulk soil, rhizosphere soil, and plant root samples based on three tillage treatments. Taxonomic legends are beneath the stacked bar plots. NT: No Tillage, MT: Minimum Tillage, CT: Conventional Tillage, NS: Not Significant. * and ** indicate statistical significance based on p-values (p-values<0.05, and 0.01) respectively

Figure 16 represents the community composition for bacterial 16S datasets of bulk soil, rhizosphere soil, and plant root samples for five different fertilizer treatments. Statistical significance was denoted in red. NS signified no significance, and *, **, *** indicated statistical significance based on the p-values (p-value<0.05, 0.01, 0.001) respectively (**Table 10**). For bulk soil samples, phylum Acidobacteriota (p= 0.0241), Bacteroidota (p= 0.00536), Chloroflexi (p= 0.02129), Firmicutes (p= 0.01109), Gemmatimonadota (p= 0.009112), Myxococcota (p= 0.00003), Planctomycetota (p= 0.02394), Proteobacteria (p= 0.01094) and Verrucomicrobiota (p= 0.01861) were statistically significant. For rhizosphere soil samples, phylum Actinobacteriota (p= 0.00858), Bacteroidota (p= 0.00174), Firmicutes (p= 0.000981), Gemmatimonadota (p= 0.00296), Myxococcota (p= 0.00000), Planctomycetota (p= 0.00028), Proteobacteria (p= 0.00024) and Verrucomicrobiota (p= 0.00000) were statistically significant. Bacterial phyla other than the top ten phyla were also showing statistical significance for bulk soil (p= 0.00392) and rhizosphere soil samples (p= 0.00029). For plant root samples, phylum Myxococcota (p= 0.02929) was statistically significant. Our observations tell us that the bacterial community composition in bulk soil and rhizosphere soil samples under three different tillage treatments are similar, however, the bacterial community composition in plant root samples differed from the communities found in the soil samples. In case of plant root samples, phyla Cyanobacteria and Patescibacteria were found in the top ten bacterial communities, which were not present in the bacterial communities obtained from the soil samples. Additionally, phylum Acidobacteriota was found in the bacterial communities obtained from the soil samples, which was absent in the bacterial community composition of plant root samples.

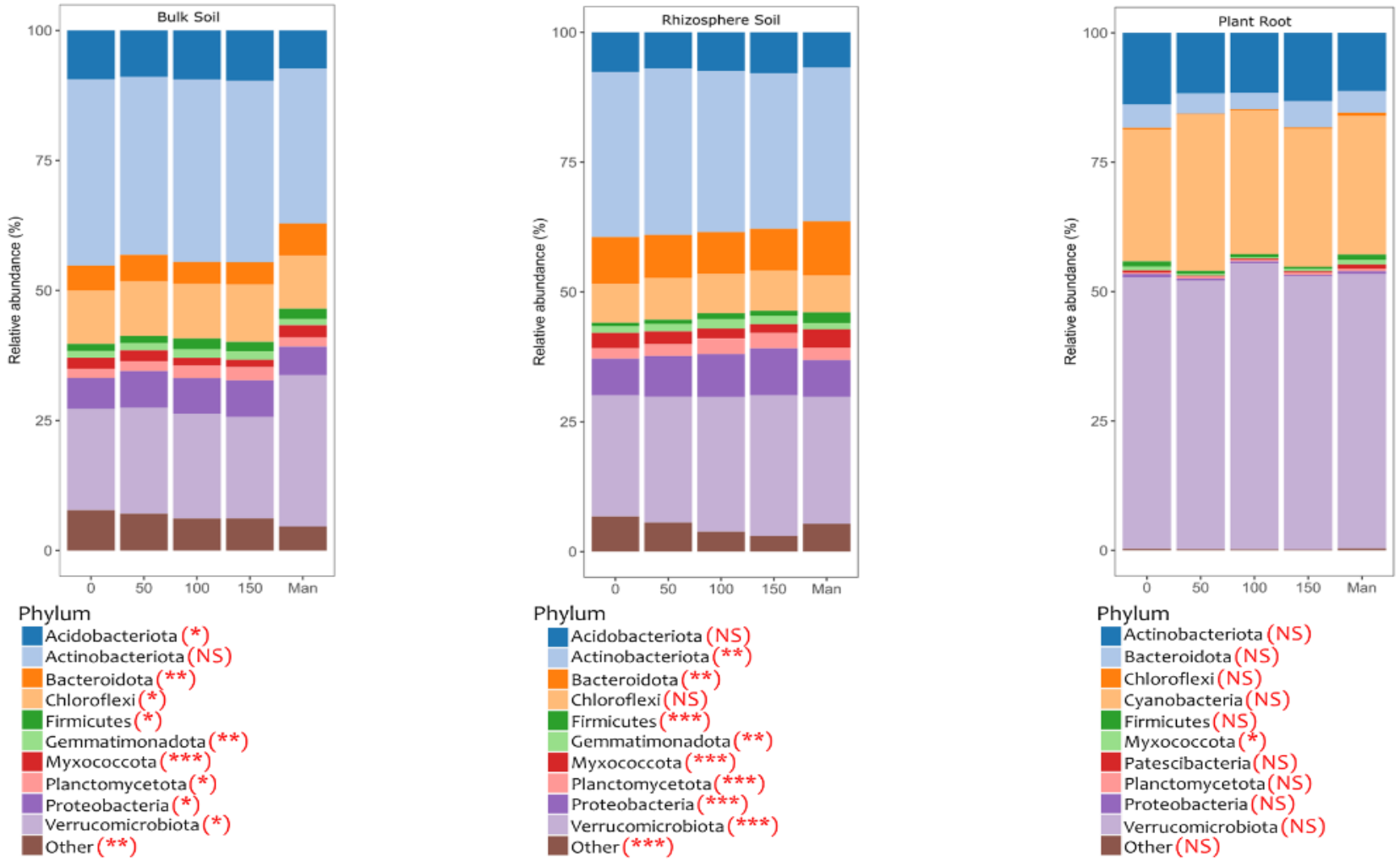

Figure 16: Community composition plots for bacterial 16S datasets of bulk soil, rhizosphere soil, and plant root samples based on five fertilizer treatments. Taxonomic legends are beneath the stacked bar plots. Man: manure, NS: Not Significant. * and ** indicate statistical significance based on p-values (p-values<0.05, and 0.01) respectively.

The table beneath represents the community composition for bacterial 16S datasets of bulk soil, rhizosphere soil, and plant root samples respectively. Top ten bacterial phyla and the p-values for both tillage and fertilizer treatments for soil and root samples are mentioned. Statistical significance has been indicated with *, ** and *** for (p-values<0.05, 0.01, and 0.001) respectively.

Table 10: Community composition for bacterial 16S datasets for bulk soil, rhizosphere soil, and plant root samples. Top ten bacterial phyla, p-values for both tillage and fertilizer treatments, and statistical significance have been shown. NS: No Significance. *, **, and *** indicate statistical significance for (p-values < 0.05, 0.01, and 0.001) respectively.

Sample	Bacterial Phylum	p-value for tillage	p-value for fertilizer
Bulk Soil	Acidobacteriota	0.05357 (NS)	0.02410 (*)
Bulk Soil	Actinobacteriota	0.01649 (*)	0.33260 (NS)
Bulk Soil	Bacteroidota	0.23194 (NS)	0.00536 (**)
Bulk Soil	Chloroflexi	0.57231 (NS)	0.02129 (*)
Bulk Soil	Firmicutes	0.22821 (NS)	0.01109 (*)
Bulk Soil	Gemmatimonadota	0.16616 (NS)	0.00911 (**)
Bulk Soil	Myxococcota	0.13723 (NS)	0.00003 (***)
Bulk Soil	Planctomycetota	0.00699 (**)	0.02394 (*)
Bulk Soil	Proteobacteria	0.00551 (**)	0.01094 (*)
Bulk Soil	Verrucomicrobiota	0.00431 (**)	0.01861 (*)
Bulk Soil	Other	0.05087 (NS)	0.00392 (**)
Rhizosphere Soil	Acidobacteriota	0.33887 (NS)	0.24216 (NS)
Rhizosphere Soil	Actinobacteriota	0.78178 (NS)	0.00858 (**)
Rhizosphere Soil	Bacteroidota	0.50789 (NS)	0.00174 (**)
Rhizosphere Soil	Chloroflexi	0.12235 (NS)	0.53477 (NS)
Rhizosphere Soil	Firmicutes	0.22645 (NS)	0.00098 (***)
Rhizosphere Soil	Gemmatimonadota	0.49740 (NS)	0.00296 (**)
Rhizosphere Soil	Myxococcota	0.53696 (NS)	0.00000 (***)
Rhizosphere Soil	Planctomycetota	0.23960 (NS)	0.00028 (***)
Rhizosphere Soil	Proteobacteria	0.66567 (NS)	0.00024 (***)
Rhizosphere Soil	Verrucomicrobiota	0.70540 (NS)	0.00000 (***)
Rhizosphere Soil	Other	0.55596 (NS)	0.00029 (***)
Plant Root	Actinobacteriota	0.27793 (NS)	0.68931 (NS)
Plant Root	Bacteroidota	0.09346 (NS)	0.21627 (NS)
Plant Root	Chloroflexi	0.35455 (NS)	0.29766 (NS)
Plant Root	Cyanobacteria	0.64891 (NS)	0.24933 (NS)
Plant Root	Firmicutes	0.95764 (NS)	0.11896 (NS)
Plant Root	Myxococcota	0.90896 (NS)	0.02929 (*)
Plant Root	Patescibacteria	0.08342 (NS)	0.50682 (NS)
Plant Root	Planctomycetota	0.24865 (NS)	0.86402 (NS)
Plant Root	Proteobacteria	0.10390 (NS)	0.72379 (NS)
Plant Root	Verrucomicrobiota	0.14486 (NS)	0.49430 (NS)
Plant Root	Other	0.24962 (NS)	0.28169 (NS)

Note: For bulk soil and rhizosphere soil samples, the top ten bacterial phyla were: Acidobacteriota, Actinobacteriota, Bacteroidota, Chloroflexi, Firmicutes, Gemmatimonadota, Myxococcota, Planctomycetota, Proteobacteria, and Verrucomicrobiota. For plant root samples, the top ten bacterial phyla were: Actinobacteriota, Bacteroidota, Chloroflexi, Cyanobacteria, Firmicutes, Myxococcota, Patescibacteria, Planctomycetota, Proteobacteria, and Verrucomicrobiota. The rest of the phyla were collectively referred to as 'Other' in the three plots for all soil and root samples.

Figure 17 represents the community composition for fungal ITS datasets of bulk soil, rhizosphere soil, and plant root samples based on three different tillage treatments. Statistical significance was denoted in red. NS signified no significance, and *, **, and *** indicated statistical significance based on the p-values (p-value<0.05, 0.01, and 0.001) respectively (**Table 11**). For bulk soil samples, class Dothideomycetes (p= 0.00034), Leotiomycetes (p= 0.00096), Pezizomycetes (p= 0.00443) and Sordariomycetes (p= 0.03187) were statistically significant. Fungal class other than the top ten class was also statistically significant for bulk soil samples (p= 0.04265). For rhizosphere soil samples, class Agaricomycetes (p= 0.01968) and Dothideomycetes (p= 0.0006) were statistically significant. For plant root samples, class Agaricomycetes (p= 0.03796), Glomeromycetes (p= 0.0074), and Leotiomycetes (p= 0.00476) were statistically significant. Class Chytridiomycetes was found in the fungal community composition obtained only from bulk soil samples, class Glomeromycetes was found in the fungal community composition obtained only from rhizosphere soil samples, and class Orbilliomycetes was found in the fungal community composition obtained only from plant root samples. The fungal class Glomeromycetes represents the arbuscular mycorrhizal fungi. Trends of class Glomeromycetes in rhizosphere and plant root samples matched with our observations in Chapter I. Under conventional tillage, the abundance of this fungal class was the lowest in rhizosphere soil samples, and, under no tillage, the abundance of this class was observed the highest in plant root samples.

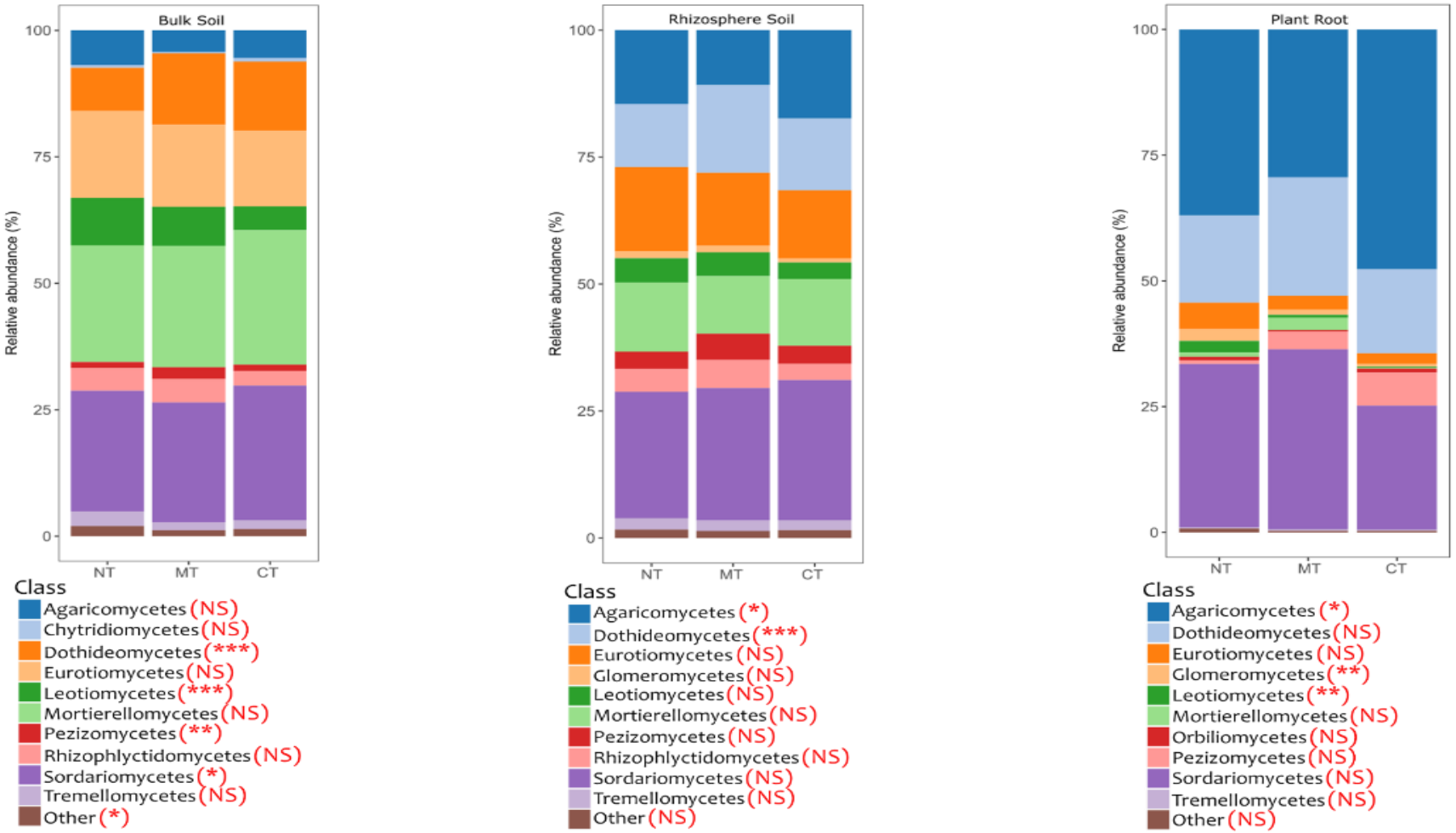

Figure 17: Community composition plots for fungal ITS datasets of bulk soil, rhizosphere soil, and plant root samples based on three tillage treatments. Taxonomic legends are beneath the stacked bar plots. NT: No Tillage, MT: Minimum Tillage, CT: Conventional Tillage, NS: Not Significant. *, **, and ** indicate statistical significance based on p-values (p-values<0.05, 0.01, and 0,001) respectively.

Figure 18 represents the community composition for fungal ITS datasets of bulk soil, rhizosphere soil, and plant root samples based on five different fertilizer treatments. Statistical significance was denoted in red. NS signified no significance, and *, **, and *** indicated statistical significance based on the p-values (p-value<0.05, 0.01, and 0.001) respectively (**Table 11**). For bulk soil samples, class Eurotiomycetes (p=0.00084), Leotiomycetes (p=0.00266), Mortierellomycetes (p= 0.0007) and Tremellomycetes (p=0.02414) were statistically significant. For rhizosphere soil samples, class Eurotiomycetes (p=0.00007), Glomeromycetes (p=0.0005), and Mortierellomycetes (p=0.00016) were statistically significant. Fungal class other than the top ten class was also statistically significant for rhizosphere soil samples (p=0.01912). For plant root samples, class Dothideomycetes (p=0.00372), Eurotiomycetes (p=0.00093), Glomeromycetes (p=0.00213), and Orbiliomycetes (p=0.01209) were statistically significant. Class Chytridiomycetes was found in the fungal community composition obtained only from bulk soil samples, class Glomeromycetes was found in the fungal community composition obtained only from rhizosphere soil samples, and class Orbilliomycetes was found in the fungal community composition obtained only from plant root samples. The fungal class Glomeromycetes represents the arbuscular mycorrhizal fungi. The abundance was lower under organic fertilizer application in rhizosphere and plant root samples. We are speculating that the interactive effect of tillage and fertilizer application is a probable reason for such observation.

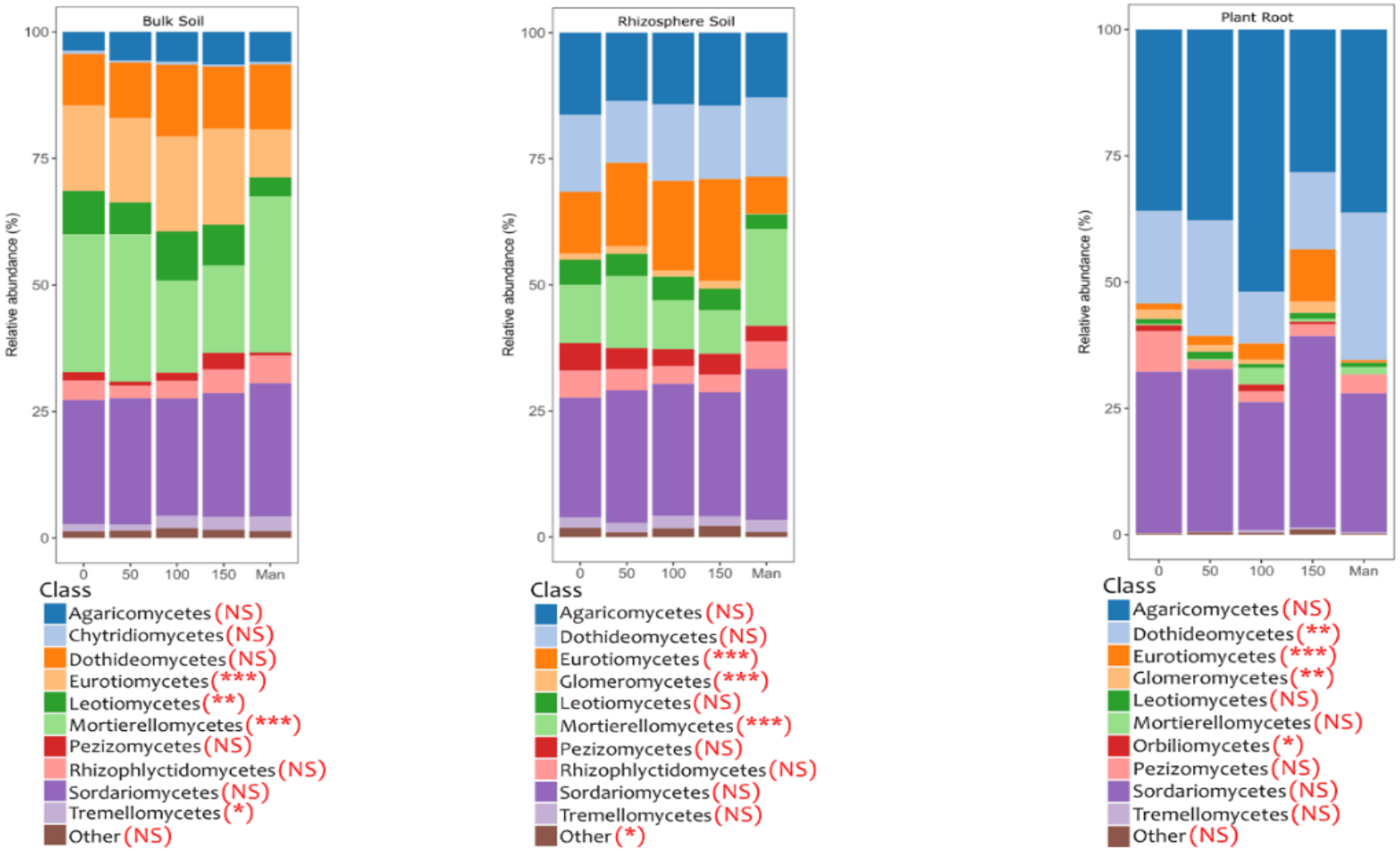

Figure 18: Community composition plots for fungal ITS datasets of bulk soil, rhizosphere soil, and plant root samples based on five fertilizer treatments. Taxonomic legends are beneath the stacked bar plots. Man: manure, NS: Not Significant. *, **, and *** indicate statistical significance based on p-values (p-values<0.05, 0.01, and 0.001) respectively.

The table beneath represents the community composition for fungal ITS datasets of bulk soil, rhizosphere soil, and plant root samples respectively. Top ten fungal class and the p-values for both tillage and fertilizer treatments for soil and root samples are mentioned. Statistical significance has been indicated with *, ** and *** for (p-values<0.05, 0.01, and 0.001) respectively.

Table 11: Community composition for fungal ITS datasets for bulk soil, rhizosphere soil, and plant root samples. Top ten fungal class, p-values for both tillage and fertilizer treatments, and statistical significance have been shown. NS: No Significance. *, **, and *** indicate statistical significance for (p-values < 0.05, 0.01, and 0.001) respectively.

Sample	Fungal Class	p-value for tillage	p-value for fertilizer
Bulk Soil	Agaricomycetes	0.22768 (NS)	0.13085 (NS)
Bulk Soil	Chytridiomycetes	0.37280 (NS)	0.30719 (NS)
Bulk Soil	Dothideomycetes	0.00034 (***)	0.40845 (NS)
Bulk Soil	Eurotiomycetes	0.44881 (NS)	0.00084 (***)
Bulk Soil	Leotiomycetes	0.00096 (***)	0.00266 (**)
Bulk Soil	Mortierellomycetes	0.45825 (NS)	0.00070 (***)
Bulk Soil	Pezizomycetes	0.00443 (**)	0.05775 (NS)
Bulk Soil	Rhizophlyctidomycetes	0.13844 (NS)	0.94991 (NS)
Bulk Soil	Sordariomycetes	0.03187 (*)	0.46543 (NS)
Bulk Soil	Tremellomycetes	0.18520 (NS)	0.02414 (*)
Bulk Soil	Other	0.04265 (*)	0.41853 (NS)
Rhizosphere Soil	Agaricomycetes	0.01968 (*)	0.95234 (NS)
Rhizosphere Soil	Dothideomycetes	0.00060 (***)	0.25874 (NS)
Rhizosphere Soil	Eurotiomycetes	0.63772 (NS)	0.00007 (***)
Rhizosphere Soil	Glomeromycetes	0.12935 (NS)	0.0005 (***)
Rhizosphere Soil	Leotiomycetes	0.44812 (NS)	0.33126 (NS)
Rhizosphere Soil	Mortierellomycetes	0.53634 (NS)	0.00016 (***)
Rhizosphere Soil	Pezizomycetes	0.19949 (NS)	0.42878 (NS)
Rhizosphere Soil	Rhizophlyctidomycetes	0.13419 (NS)	0.59350 (NS)
Rhizosphere Soil	Sordariomycetes	0.31806 (NS)	0.13807 (NS)
Rhizosphere Soil	Tremellomycetes	0.61141 (NS)	0.38885 (NS)
Rhizosphere Soil	Other	0.40231 (NS)	0.01912 (*)
Plant Root	Agaricomycetes	0.03796 (*)	0.16606 (NS)
Plant Root	Dothideomycetes	0.17848 (NS)	0.00372 (**)
Plant Root	Eurotiomycetes	0.07695 (NS)	0.00093 (***)
Plant Root	Glomeromycetes	0.00740 (**)	0.00213 (**)
Plant Root	Leotiomycetes	0.00476 (**)	0.28614 (NS)
Plant Root	Mortierellomycetes	0.10195 (NS)	0.87180 (NS)
Plant Root	Orbiliomycetes	0.78974 (NS)	0.01209 (*)
Plant Root	Pezizomycetes	0.11710 (NS)	0.10456 (NS)
Plant Root	Sordariomycetes	0.07507 (NS)	0.32874 (NS)
Plant Root	Tremellomycetes	0.45247 (NS)	0.09508 (NS)
Plant Root	Other	0.12033 (NS)	0.9215 (NS)

Note: For bulk soil samples, the top ten fungal class were: Agaricomycetes, Chytridiomycetes, Dothideomycetes, Eurotiomycetes, Leotiomycetes, Mortierellomycetes, Pezizomycetes, Rhizophlyctidomycetes, Sordariomycetes, and Tremellomycetes. For rhizosphere soil samples, the top ten fungal class were: Agaricomycetes, Dothideomycetes, Eurotiomycetes, Glomeromycetes, Leotiomycetes, Mortierellomycetes, Pezizomycetes, Rhizophlyctidomycetes, Sordariomycetes, and Tremellomycetes. For plant root samples, the top ten fungal class were: Agaricomycetes, Dothideomycetes, Eurotiomycetes, Glomeromycetes, Leotiomycetes, Mortierellomycetes, Orbiliomycetes, Pezizomycetes, Sordariomycetes, and Tremellomycetes. The rest of the class were collectively referred to as 'Other' in the three plots for all soil and root samples.

Indicator Species Analysis

For bulk soil samples the selected bacterial indicator species were: phylum Crenarchaeota under no tillage; phyla Actinobacteriota, Acidobacteriota, and Planctomycetota under minimum tillage; phyla Actinobacteriota, Alphaproteobacteria, Chloroflexi and Bacteroidota under conventional tillage; phyla Alphaproteobacteria, Gammaproteobacteria, and Actinobacteriota, under 0 lbs; phyla Acidobacteriota, Alphaproteobacteria, and Gammaproteobacteria under 50 lbs; phyla Actinobacteriota, Alphaproteobacteria, and Gammaproteobacteria under 100 lbs; phyla Bacteroidota and Proteobacteria under 150 lbs; and phyla Actinobacteriota, Alphaproteobacteria and Gammaproteobacteria under organic fertilizer. For rhizosphere soil samples the selected bacterial indicator species were: phyla Bacteroidota and Planctomycetota under no tillage; phylum Bacteroidota under minimum tillage; phyla Alphaproteobacteria, Gammaproteobacteria and Chloroflexi under conventional tillage; phyla Alphaproteobacteria, Gammaproteobacteria and Actinobacteriota under 0 lbs; phyla Actinobacteriota, Acidobacteriota and Alphaproteobacteria under 50 lbs; phyla Alphaproteobacteria, Gammaproteobacteria and Actinobacteriota under 100 lbs; phyla Verrucomicrobiota, Myxococcota and Bacteroidota under 150 lbs; and phyla Chloroflexi, Alphaproteobacteria and Gammaproteobacteria under organic fertilizer. For plant root samples the selected bacterial indicator species were: phyla Alphaproteobacteria, Gammaproteobacteria and Actinobacteriota under no tillage; phylum Alphaproteobacteria and Gammaproteobacteria under conventional tillage; phyla Actinobacteriota and Alphaproteobacteria under 0 lbs; phyla Actinobacteriota, Gammaproteobacteria, and Bacteroidota under 50 lbs; phyla Alphaproteobacteria, Gammaproteobacteria and Actinobacteriota under 100 lbs; and phylum Gammaproteobacteria under organic fertilizer. For bulk soil samples the selected fungal indicator species were: order Pezizales and Eurotiales under no tillage; order Pleosporales, Phallales,

Sordariales and Hypocreales under minimum tillage; order Hypocreales, Sordariales, Pezizales and Glomerales under conventional tillage; order Sordariales, Pleosporales, Hypocreales and Glomerales under 0 lbs; order Hypocreales, Agaricales, Pleosporales and Sordariales under 50 lbs; order Glomerales, Pleosporales, Sordariales and Hypocreales under 100 lbs; order Entomophthorales, Cantharellales, Boletales and Tubeufiales under 150 lbs; and order Sordariales, Agaricales, Hypocreales and Pleosporales under organic fertilizer. For rhizosphere soil samples the selected fungal indicator species were: order Pleosporales and Glomerales under no tillage; order Chaetothyriales and Spizellomycetales under minimum tillage; order Phallales, Glomerellales, Pleosporales and Auriculariales under conventional tillage; order Coniochaetales, Agaricales, Paraglomerales and Chaetothyriales under 0 lbs; order Mortierellales under 50 lbs; order Hypocreales, Glomerellales, Pleosporales and Cantharellales under 100 lbs; order Pleosporales and Agaricales under 150 lbs; and order Pleosporales, Hypocreales, Agaricales, and Polyporales under organic fertilizer. For plant root samples the selected fungal indicator species were order Pleosporales and Pezizales under no tillage; order Pleosporales under 0 lbs; order Auriculariales under 50 lbs; and order Pleosporales under 100 lbs of inorganic fertilization.

Network Connectivity Among Soil Microorganisms

Network Among Soil Microorganisms Based on Tillage

Figure 19 represents the networks from both bacterial 16S and fungal ITS sequences of bulk soil, rhizosphere soil, and plant root samples based on three different tillage treatments. Microbial taxa which are unidentified have been collectively mentioned as ‘others’. For bulk soil samples, the ‘others’ microbial taxa were 24.91%, and the top 15 microbial taxa were: Actinobacteriota (16.46%), Sordariomycetes (11.24%), Alphaproteobacteria (6.45%), Chloroflexi (5.14%), Dothideomycetes (4.09%), Acidobacteriota (3.75%), Gammaproteobacteria (3.48%),

Eurotiomycetes (3.48%), Agaricomycetes (2.87%), Verrucomicrobiota (2.7%), Bacteroidota (2.61%), Planctomycetota (2.18%), Mortierellomycetes (2.18%), Leotiomycetes (1.57%), and Firmicutes (1.05%). For rhizosphere soil samples, the 'others' microbial taxa were 30.97%, and the top 15 microbial taxa were: Actinobacteriota (13.56%), Sordariomycetes (8.49%), Alphaproteobacteria (6.91%), Agaricomycetes (4.9%), Gammaproteobacteria (4.29%), Bacteroidota (4.11%), Dothideomycetes (3.76%), Eurotiomycetes (3.32%), Planctomycetota (2.97%), Chloroflexi (2.62%), Acidobacteriota (2.54%), Verrucomicrobiota (1.75%), Leotiomycetes (1.49%), Mortierellomycetes (1.22%), and Myxococcota (0.87%). For plant root samples, the 'others' microbial taxa were 19.21%, and the top 15 microbial taxa were: Alphaproteobacteria (18.64%), Actinobacteriota (12.99%), Agaricomycetes (10.73%), Gammaproteobacteria (9.6%), Cyanobacteria (8.76%), Bacteroidota (3.39%), Sordariomycetes (3.39%), Dothideomycetes (1.69%), Eurotiomycetes (1.69%), Glomeromycetes (1.41%), Rhizophlyctidomycetes (1.41%), Firmicutes (0.85%), Pezizomycetes (0.85%), Saccharomycetes (0.85%), and Spizellomycetes (0.56%).

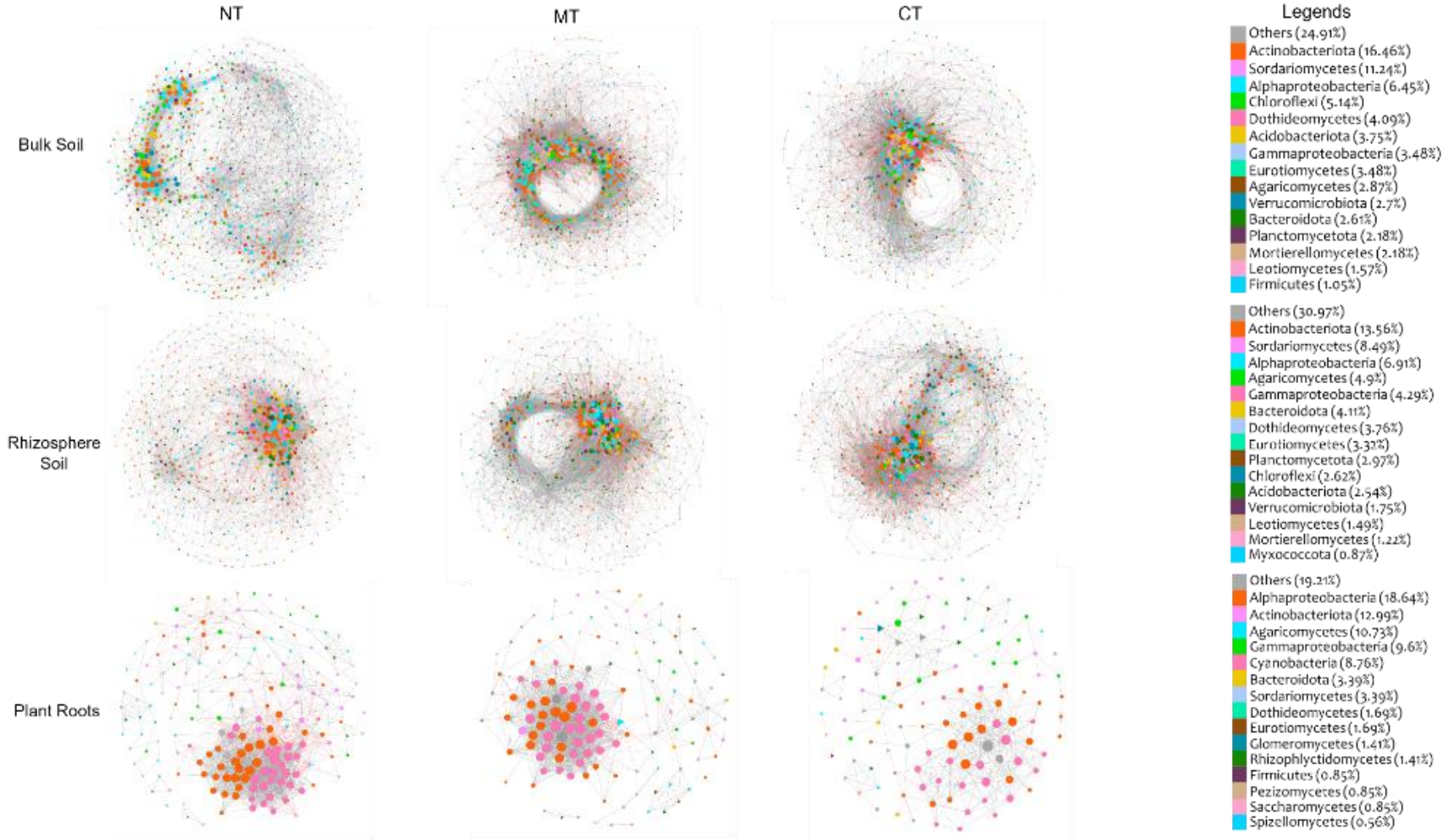

Figure 19: Networks from both bacterial 16S and fungal ITS datasets of bulk soil, rhizosphere soil, and plant root samples based on three tillage treatments. Taxonomic legends for the top 15 microbial taxa with their abundance percentage values are mentioned. NT: No Tillage, MT: Minimum Tillage, CT: Conventional Tillage. Bacterial ASV nodes are spherical and fungal OTU nodes are triangular. Grey lines represent positive interactions between nodes and red lines represent negative interactions.

Table 12 shows how various structural properties differ among network graphs across three different tillage practices. In bulk soil samples, number of nodes decrease from no tillage to conventional tillage and number of edges increase from no tillage to conventional tillage. Percentages of positive edges decrease from no tillage to conventional tillage and percentages of negative edges increase from no tillage to conventional tillage in bulk soil samples. For rhizosphere soil samples, the greatest number of nodes and edges are present under minimum tillage. Although the percentage of positive edges is maximum under no tillage in rhizosphere soil samples, however, the percentage of negative edges is maximum under minimum tillage. In case of root samples, the number of nodes and edges decreased from no tillage to conventional tillage. Similar to rhizosphere soil samples, the percentage of positive edges in root samples are greatest under minimum tillage, however, the percentage of negative edges are lowest under minimum tillage and greatest under no tillage.

Table 12: Difference among structural properties in network graphs across three different tillage treatments.

Structural Properties	BS_ NT	BS_ MT	BS_ CT	RS_ NT	RS_ MT	RS_ CT	Root_ NT	Root _MT	Root_ CT
Number of Nodes	887	848	811	761	863	758	192	140	122
Number of Edges	5767	23484	23832	9369	19183	14145	1491	1169	365
Maximum Degree	91	268	319	181	271	231	57	53	26
Positive Edges	99.52 %	71.25 %	70.74%	67.09%	65.04 %	65.44%	83.19 %	93.87 %	89.39 %
Negative Edges	0.48 %	28.75 %	29.26%	32.91%	34.96 %	34.56%	16.81 %	6.13 %	10.61 %

Note: NT: No Tillage, MT: Minimum Tillage, CT: Conventional Tillage. BS: Bulk Soil, RS: Rhizosphere Soil

Network Among Soil Microorganisms Based on Fertilizer Application

Figure 20 represents the networks from both bacterial 16S and fungal ITS sequences of bulk soil, rhizosphere soil, and plant root samples based on five different fertilizer regimes.

Microbial taxa which are unidentified have been collectively mentioned as ‘others’. For bulk soil samples, the ‘others’ microbial taxa were 24.91%, and the top 15 microbial taxa were: Actinobacteriota (16.46%), Sordariomycetes (11.24%), Alphaproteobacteria (6.45%), Chloroflexi (5.14%), Dothideomycetes (4.09%), Acidobacteriota (3.75%), Gammaproteobacteria (3.48%), Eurotiomycetes (3.48%), Agaricomycetes (2.87%), Verrucomicrobiota (2.7%), Bacteroidota (2.61%), Planctomycetota (2.18%), Mortierellomycetes (2.18%), Leotiomycetes (1.57%), and Firmicutes (1.05%). For rhizosphere soil samples, the ‘others’ microbial taxa were 30.97%, and the top 15 microbial taxa were: Actinobacteriota (13.56%), Sordariomycetes (8.49%), Alphaproteobacteria (6.91%), Agaricomycetes (4.9%), Gammaproteobacteria (4.29%), Bacteroidota (4.11%), Dothideomycetes (3.76%), Eurotiomycetes (3.32%), Planctomycetota (2.97%), Chloroflexi (2.62%), Acidobacteriota (2.54%), Verrucomicrobiota (1.75%), Leotiomycetes (1.49%), Mortierellomycetes (1.22%), and Myxococcota (0.87%). For plant root samples, the ‘others’ microbial taxa were 19.21%, and the top 15 microbial taxa were: Alphaproteobacteria (18.64%), Actinobacteriota (12.99%), Agaricomycetes (10.73%), Gammaproteobacteria (9.6%), Cyanobacteria (8.76%), Bacteroidota (3.39%), Sordariomycetes (3.39%), Dothideomycetes (1.69%), Eurotiomycetes (1.69%), Glomeromycetes (1.41%), Rhizophlyctidomycetes (1.41%), Firmicutes (0.85%), Pezizomycetes (0.85%), Saccharomycetes (0.85%), and Spizellomycetes (0.56%).

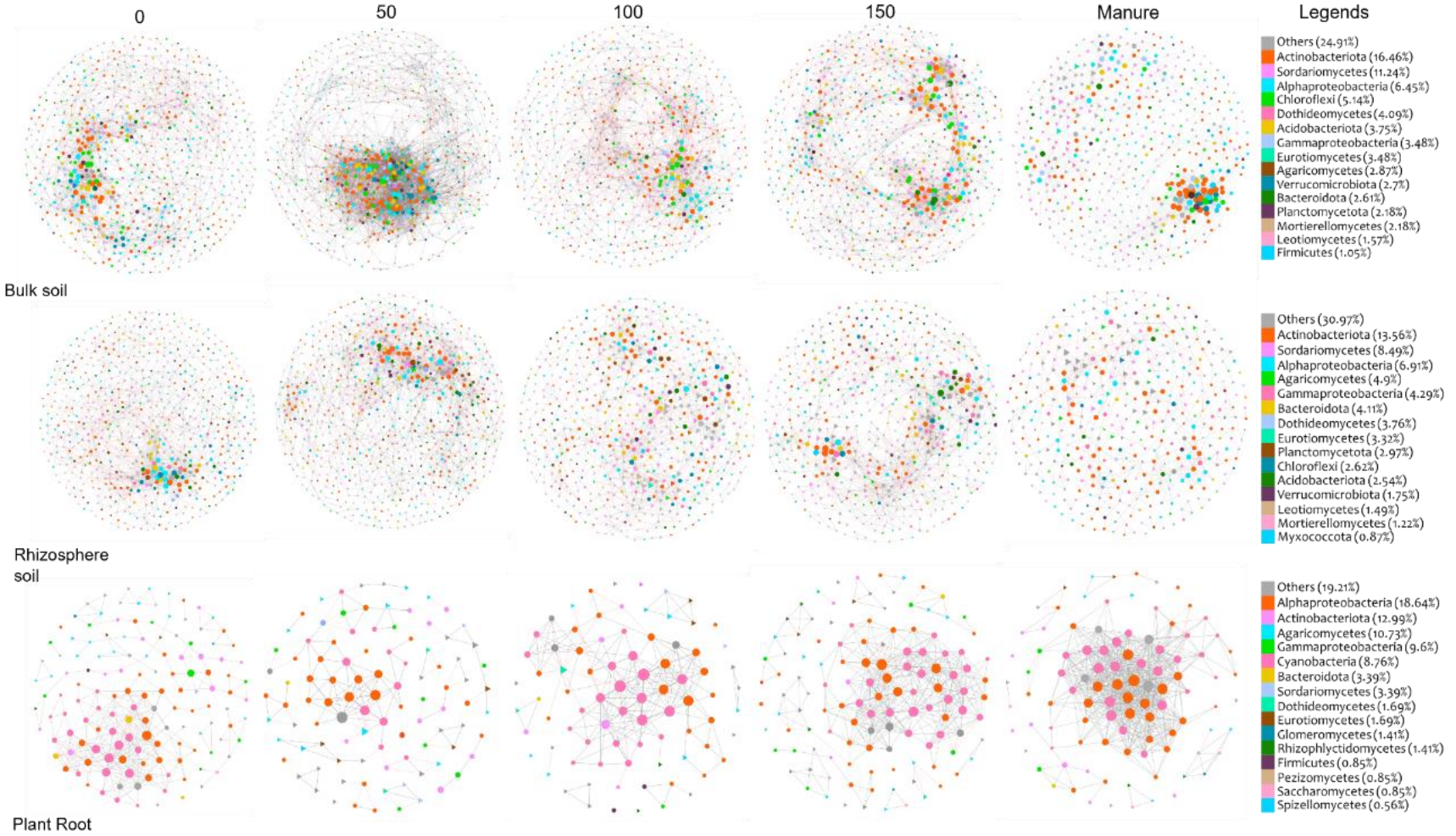

Figure 20: Networks from both bacterial 16S and fungal ITS datasets of bulk soil, rhizosphere soil, and plant root samples based on five fertilizer treatments. Taxonomic legends for the top 15 microbial taxa with their abundance percentage values are mentioned. Bacterial ASV nodes are spherical and fungal OUT nodes are triangular. Grey lines represent positive interactions between nodes and red lines represent negative interactions.

The table below shows how various structural properties differ among network graphs across five different fertilizer practices. In bulk soil samples, number of nodes are greatest under 50 lbs of inorganic fertilizer and lowest under organic fertilizer; and number of edges are greatest under 50 lbs of inorganic fertilizer and lowest under 0 lbs of inorganic fertilizer. Percentages of positive edges are greatest under organic fertilizer and lowest under 100 lbs of inorganic fertilizer; conversely percentages of negative edges are greatest under 100 lbs of inorganic fertilizer and lowest under organic fertilizer in bulk soil samples. For rhizosphere soil samples, the greatest number of nodes and edges are greatest under 50 lbs of inorganic fertilizer and lowest under organic fertilizer. Although the percentage of positive edges is maximum under 0 lbs of inorganic fertilizer in rhizosphere soil samples, the percentage of negative edges is maximum under 100 lbs of inorganic fertilizer. In case of root samples, the number of nodes is greatest under 0 lbs of inorganic fertilizer and lowest under 100 lbs of inorganic fertilizer; and the number of edges is greatest under organic fertilizer and lowest under 50 lbs of inorganic fertilizer. The percentage of positive edges in root samples are greatest under organic fertilizer and lowest under 50 lbs of inorganic fertilizer, however, the percentage of negative edges are lowest under organic fertilizer and greatest under 50 lbs of inorganic fertilizer.

Table 13: Difference among structural properties in network graphs across five different fertilizer treatments.

Structural Properties	BS_0	BS_ 50	BS_100	BS_150	BS_ Man	RS_0	RS_ 50	RS_100	RS_150	RS_ Man	Root _0	Root_ 50	Root _100	Root_ 150	Root_ Man
Number of Nodes	748	780	695	772	555	676	782	643	576	424	137	98	82	121	99
Number of Edges	2376	5907	2618	3483	2700	2029	2936	1865	2039	853	354	145	212	441	643
Maximum Degree	62	121	110	69	57	67	68	47	65	41	22	12	18	27	43
Positive Edges	70.02 %	78.9 7%	56.28%	64.37%	100%	73.92 %	69.3 3%	59.71%	60.89%	60.96 %	82.76 %	70.19 %	87.7 3%	91.41 %	95.61%
Negative Edges	29.98 %	21.0 3%	43.72%	35.63%	0%	26.08 %	30.6 7%	40.29%	39.11%	39.04 %	17.24 %	29.81 %	12.2 7%	8.59%	4.39%

Note: BS: Bulk Soil, RS: Rhizosphere Soil. Man: Manure.

Network Connectivity Across Tillage and Fertilizer Regimes

Figure 21 represents the networks from both bacterial 16S and fungal ITS sequences of bulk soil, rhizosphere soil, and plant root samples based on three different tillage treatments (no tillage, minimum tillage, and conventional tillage) and five different fertilizer regimes (0 kg, 50 kg, 100 kg, 150 kg of inorganic fertilizer and 90.71 kg of organic fertilizer). Top 15 microbial taxa based on their abundance percentages were chosen for legends. Microbial taxa which are unidentified have been collectively mentioned as 'others'. For bulk soil samples, the 'others' microbial taxa were 24.91%, and the top 15 microbial taxa were: Actinobacteriota (16.46%), Sordariomycetes (11.24%), Alphaproteobacteria (6.45%), Chloroflexi (5.14%), Dothideomycetes (4.09%), Acidobacteriota (3.75%), Gammaproteobacteria (3.48%), Eurotiomycetes (3.48%), Agaricomycetes (2.87%), Verrucomicrobiota (2.7%), Bacteroidota (2.61%), Planctomycetota (2.18%), Mortierellomycetes (2.18%), Leotiomycetes (1.57%), and Firmicutes (1.05%). For rhizosphere soil samples, the 'others' microbial taxa were 30.97%, and the top 15 microbial taxa were: Actinobacteriota (13.56%), Sordariomycetes (8.49%), Alphaproteobacteria (6.91%), Agaricomycetes (4.9%), Gammaproteobacteria (4.29%), Bacteroidota (4.11%), Dothideomycetes (3.76%), Eurotiomycetes (3.32%), Planctomycetota (2.97%), Chloroflexi (2.62%), Acidobacteriota (2.54%), Verrucomicrobiota (1.75%), Leotiomycetes (1.49%), Mortierellomycetes (1.22%), and Myxococcota (0.87%). For plant root samples, the 'others' microbial taxa were 19.21%, and the top 15 microbial taxa were: Alphaproteobacteria (18.64%), Actinobacteriota (12.99%), Agaricomycetes (10.73%), Gammaproteobacteria (9.6%), Cyanobacteria (8.76%), Bacteroidota (3.39%), Sordariomycetes (3.39%), Dothideomycetes (1.69%), Eurotiomycetes (1.69%), Glomeromycetes (1.41%), Rhizophlyctidomycetes (1.41%),

Firmicutes (0.85%), Pezizomycetes (0.85%), Saccharomycetes (0.85%), and Spizellomycetes (0.56%).

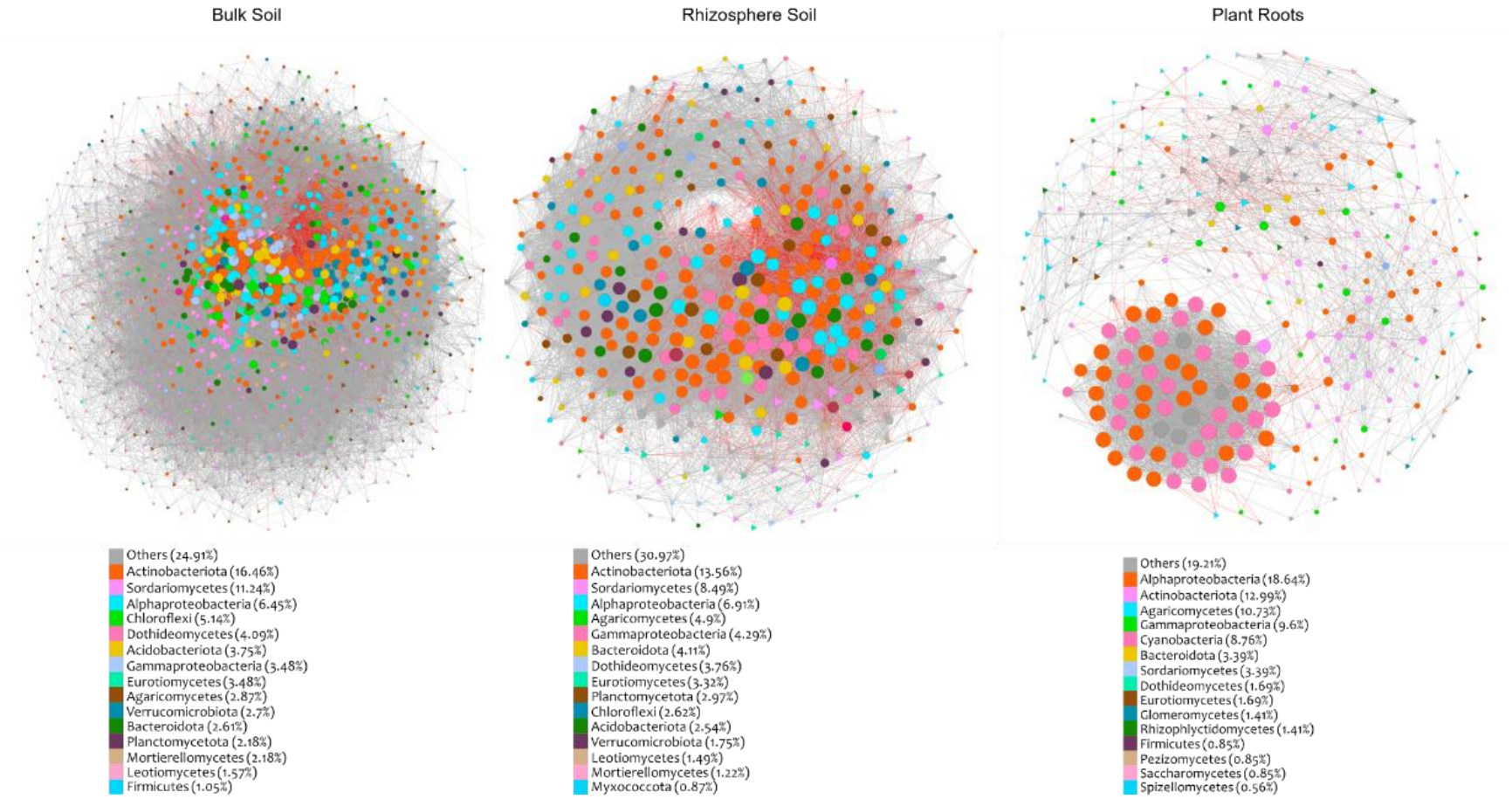

Figure 21: Networks from both bacterial 16S and fungal ITS datasets of bulk soil, rhizosphere soil, and plant root samples based on both three different tillage treatments and five different fertilizer treatments. Taxonomic legends for the top 15 microbial taxa with their abundance percentage values are mentioned. Bacterial ASV nodes are spherical and fungal OUT nodes are triangular. Grey lines represent positive interactions between nodes and red lines represent negative interactions.

The table below shows how various structural properties differ among network graphs across three different compartments for the interactive effects of three different tillage practices and five different fertilizer treatments. The number of nodes and edges decreased from bulk soil samples to root samples. Although the percentage of positive edges are greatest under root samples and lowest under rhizosphere soil samples, conversely, the percentage of negative edges are greatest under rhizosphere soil samples and lowest under root samples. Additionally, the complexity of the network graphs decreased from bulk soil samples to root samples.

Table 14: Difference among structural properties in network graphs across three different compartments on the interactive effects of three different tillage practices and five different fertilizer treatments.

Structural Properties	Bulk Soil	Rhizosphere Soil	Root
Number of Nodes	1133	410	286
Number of Edges	77305	20529	3087
Maximum Degree	460	460	73
Positive Edges	79.22%	78.86%	91.89%
Negative Edges	20.78%	21.14%	8.11%

Discussion

Impact on Microbial Diversity

The results revealed that the bacterial species richness was greatest under conventional tillage. In agreement with my findings, Frey et al., (1999) found a significant increase in bacterial abundance under conventional-tillage compared to no-tillage for soybean/wheat corn and suggested an increased amount of particulate organic carbon in conventionally-tilled soil as a possible reason. The results showed that in rhizosphere soil and plant root samples, fungal species richness was greatest under no tillage My observation was consistent with the study of Yin Wang et al. (2010) and the researchers suggested that a difference in soil temperature and moisture in different regions might be a probable cause for such outcome. According to Frey et al (1999), climatic variance might be responsible for soil water content and hence the fungal community

structure gets affected. According to Souza et al. (2018), no-tilled soil is much more aggregated and Jiang et al. (2011) suggested that highly aggregated soil leads to an increased fungal abundance. Tillage is physically disruptive to fungal hyphae, whether bacteria are minuscule organisms and are relatively less affected by habitat perturbation. That might be a probable reason for fungal communities being affected by tillage compared to bacterial communities. The results exhibited that in rhizosphere soil samples, bacterial species richness was greatest under organic fertilizer. My finding is consistent with the study of Dincă et al. (2022) who suggested about a positive effect of organic fertilization on soil bacteria. A previous research by Jangid et al. (2008) found a greater bacterial species richness and diversity in soil amended with poultry litter compared to the soil treated with inorganic fertilizer which was in consistent with my findings. The researchers suggested that poultry litter changed the soil physicochemical properties by adding labile carbon, microbial biomass, and other elemental nutrients to increase microbial richness and diversity.

Impact on Microbial Community Structure and Assembly

The ordination plots revealed that bacterial and fungal communities of three different compartments (bulk soil, rhizosphere soil, plant root) were affected by agricultural management treatments, with significantly different communities under fertilization. Bacterial communities formed their separate clusters and grouped within each cluster according to fertilization. The plots also showed that fungal communities in bulk soil samples formed their separate clusters and grouped within each cluster according to tillage and fertilizer application. The observations revealed that bacterial and fungal communities and abundance in soil and plant root samples treated with organic fertilizer were distinct from those treated with inorganic fertilizer. However, the fungal communities in bulk soil samples treated with three different tillage practices were also

clearly distinct from each other. According to Yu et al. (2019), a possible reason for such result might be an altered soil biochemistry due to the applied agricultural management treatments which led to a change in microbial community and abundance. The result from bacterial communities in rhizosphere soil was consistent with previous researches from (Cerecetto et al., 2021; Enebe and Babalola, 2020; Sun et al., 2004). The CAP plots of bacterial communities indicated separate clustering in soil and root samples treated with fertilizer. There was clear clustering of fungal communities in bulk soil samples associated with tillage and fertilizer treatments. In case of bacterial and fungal communities, my observation revealed that the soil and plant root samples treated with organic fertilizer were distinct from those treated with inorganic fertilizer. I can suggest that an alteration in soil physicochemical properties such as a shift in soil pH might be a possible reason behind the distinct clustering. My findings indicate that organic fertilizer application might significantly influence the bacterial and fungal community composition in bulk soil, rhizosphere soil and plant roots. Tillage practice might not affect bacterial community and abundance in bulk soil, rhizosphere soil and plant roots, however, fungal community and abundance in bulk soil might be influenced by tillage.

Impact on Microbial Community Composition and Indicator Taxa

The current study showed that the variation in tillage practices and fertilizer regimes influenced the divergence in soil bacterial and fungal communities. Actinobacteriota and Proteobacteria were the dominant bacterial phyla in bulk soil and rhizosphere soil compartments. Previous researches of (Fierer et al., 2007; Lauber et al., 2008) showed that these bacterial phyla are abundant and dominant in most soil environments. On the other hand, this study showed that Eurotiomycetes, Mortierellomycetes and Sordariomycetes were the dominant fungal class in bulk soil samples, and Agaricomycetes and Sordariomycetes were the dominant fungal class in

rhizosphere soil and plant root samples. The findings demonstrate that tillage practice and fertilization modified the soil and root microbial communities differently. According to the observations, bacterial community composition in bulk soil and rhizosphere soil samples under three different tillage treatments are similar, however, the bacterial community composition in plant root samples varied from the communities found in the soil samples. In case of plant root samples, phyla Cyanobacteria and Patescibacteria were found in the top ten bacterial communities, which were not present in the bacterial communities obtained from the soil samples. Additionally, phylum Acidobacteriota was found in the bacterial communities obtained from the soil samples, which was absent in the bacterial community composition of plant root samples. The results also indicate that fungal communities in bulk soil, rhizosphere soil and plant root can be influenced significantly by both tillage and fertilizer application. Class Chytridiomycetes was present only in bulk soil samples, class Glomeromycetes was found in only rhizosphere soil samples, and class Orbilliomycetes was found only from plant root samples. The fungal class Glomeromycetes represents the arbuscular mycorrhizal fungi. Trends of class Glomeromycetes in rhizosphere and plant root samples matched with my observations in Chapter I. Under conventional tillage, the abundance of this fungal class was the lowest in rhizosphere soil samples, and, under no tillage, the abundance of this class was observed the greatest in plant root samples.

Impact on Microbial Network Complexity and Keystone Taxa

Alteration of the physicochemical properties in soil under tillage and fertilization might be a possible reason for such microbial variance. The concentrated area for most of the soil microbial studies related to agriculture were mainly topsoil (Banerjee et al., 2021). In my study, network diameter, average path length, and number of nodes and edges differed between each tillage and fertilizer treatments for all three compartments. Under organic fertilization, the percentage of

negative links within microbial communities decreased noticeably, especially in bulk soil samples. This was consistent with the findings from Banerjee et al. (2016d), suggesting that adding organic nutrients in soil might help enhance resource availability. In the meta networks created from both soil and root microbiome, the complexity of the network graphs decreased from bulk soil to root samples. The complexity and connectivity of the soil microbiome network from bulk soil samples was greater than rhizosphere soil and plant roots was probably due to a large bacterial diversity in bulk soil. In agreement with the previous studies from (Banerjee et al., 2016b; Burke et al., 2011), network graphs from my research showed that bacterial and fungal communities associated with each other despite of the difference in their taxonomic classification- promoting the idea of microbial cooccurrence powered by resource. According to (Faust and Raes, 2012; Fuhrman et al., 2015), the significant cooccurrence of microbes can show the positive or mutualistic associations and simultaneously, negative associations manifest a co-elimination. Peres-Neto et al. (2001) suggested that the reaction of species to environmental components can talk about the pattern of co-occurrences inside one ecosystem. Co-occurrence analysis are important to bring out which species are responding alike to environmental factors, therefore, significant co-occurrences might show a convincing ecological interaction (Freilich et al., 2018). Previous studies from (Hastings et al., 2007; Jones et al., 1994; Stachowicz, 2012; Wright et al., 2002) indicate that the positive interactions develop environmental stress and increase the niche of the included species to make habitats available for other species. However, co-occurrence networks can be limited by ecological and statistical outcomes. According to Freilich et al. (2018),the probability of statistically significant associations increase as the rate of provided species increase; however, such interactions do not necessarily match to the associations. The researchers suggested that a change in environment can have an evident indication in spatial co-occurrence outlines. The positive edges

in network graphs here may imply that the species in a particular ecosystem are associating to influence each other's abundance over treatment and are responding correspondingly to the respective agricultural treatment applied. It might be difficult to separate which associations are related to what interactions and which are related only to common environmental subjectivities.

Keystone taxa were also identified from the network analysis in both bacterial and fungal communities which were associated with tillage practice and fertilization. In agreement with my findings, Banerjee et al. (2016b) found that Gemmatimonadota and Acidobacteriota are highly abundant in agricultural soils and are one of the dominant and most influential groups. The phylum Acidobacteriota has been previously found as a keystone taxon. Harreither et al. (2011) found that Sordariomycetes, a member of phylum Ascomycota, were distinguished for disintegrating organic matter in soil. A previous study from Fierer et al. (2007) revealed that agricultural soils displayed higher abundance of copiotrophic microbes like Bacteroidota, Actinobacteriota and Alphaproteobacteria which are able to decompose soil organic matter and are associated with soil carbon cycle. Fertilizer application is crucial as well to influence soil ecosystem. My study showed abundances of Acidobacteriota and Chloroflexi in bacteria in bulk soil samples under control (0 lbs) and inorganic fertilization. According to Wang et al. (2018), there are many acidogenic bacterial components included in phylum Chloroflexi which can produce methane gas anaerobically. Consistent with the findings of Guo et al. (2010), it is possible that inorganic fertilization led to soil acidification. In contrast to my findings, Ye et al. (2021) found a reduction in the abundance of Chloroflexi under manure application. According to previous studies (Dunfield et al., 2007; Pol et al., 2007; Shen et al., 2017), the phylum Verrucomicrobiota are important in soil carbon cycling as members of this phylum can utilize different carbon compounds in soil. Fungal communities from class Sordariomycetes and Dothideomycetes found in

rhizosphere soil samples are the components of phylum Ascomycota which is a copiotrophic fungal group in soil and play an important role in decomposition of organic matter under organic fertilization. The results were consistent with a study from (Zheng et al., 2021). However, more research is needed to find possible reasons for the presence of bacterial phylum Chloroflexi in rhizosphere soil under organic fertilization. My results presenting a convincing association between the keystone taxa and organic matter decomposition suggest that such taxa can be targeted to improve our understanding of soil health and fertility.

Conclusion

The results of this study show that organic fertilization can create a balance in soil ecosystem and make soil and root microbiota much more resilient against environmental stressors. The overall microbial abundance, community composition and networks were different for bulk soil, rhizosphere soil and plant root samples under different treatments. Moreover, fertilizer regime was more influential compared to tillage practice on soil and root bacterial communities, however, both tillage and fertilizer regime impacted on soil fungal communities. Organic farming may contribute to an enhanced soil health, fertility, and crop productivity Nevertheless, further studies are required for a robust understanding of the association between soil and root microbiota.

STUDY IMPLICATIONS

My study shows that intensive agricultural management practices, such as tillage and excessive inorganic fertilizer application influence the abundance, community composition and network association of bacterial and fungal communities in three different areas- bulk soil, rhizosphere soil, and plant roots. Moreover, my research reveals that intensive agricultural management practices impact the arbuscular mycorrhizal fungal (AMF) colonization in root as well as the soil biochemistry. However, fertilizer application showed a significant impact on soil physicochemical properties and arbuscular mycorrhizal colonization compared to tillage practices.

Abundance of AMF colonization in root was found significantly higher under organic fertilizer applications which was consistent with previous research. Additionally, in agreement with other previous studies, my research acquired that abundance of AMF colonization decreased as the intensity of tillage and inorganic fertilization increased. Therefore, I could accept my hypothesis that intensive agricultural practices have a negative effect on AMF colonization in plant root system. From my research, I also realized that applying organic fertilizer can improve soil fertility and crop productivity by increasing levels of nutrients such as phosphorus, potassium, and organic carbon in soil which agreed with my hypothesis that organic fertilization can be beneficial for soil health and fertility.

My research shows that fertilizer application affects the overall bacterial species richness and diversity in rhizosphere soil and plant root compared to tillage. Conversely, tillage practice affected the overall fungal species richness and diversity in rhizosphere soil, fertilization affected the overall fungal species richness and diversity in bulk soil. In agreement with previous research, it is suggested that intensive agricultural treatments might alter soil biochemistry and lead to a difference in microbial diversity and community structure.

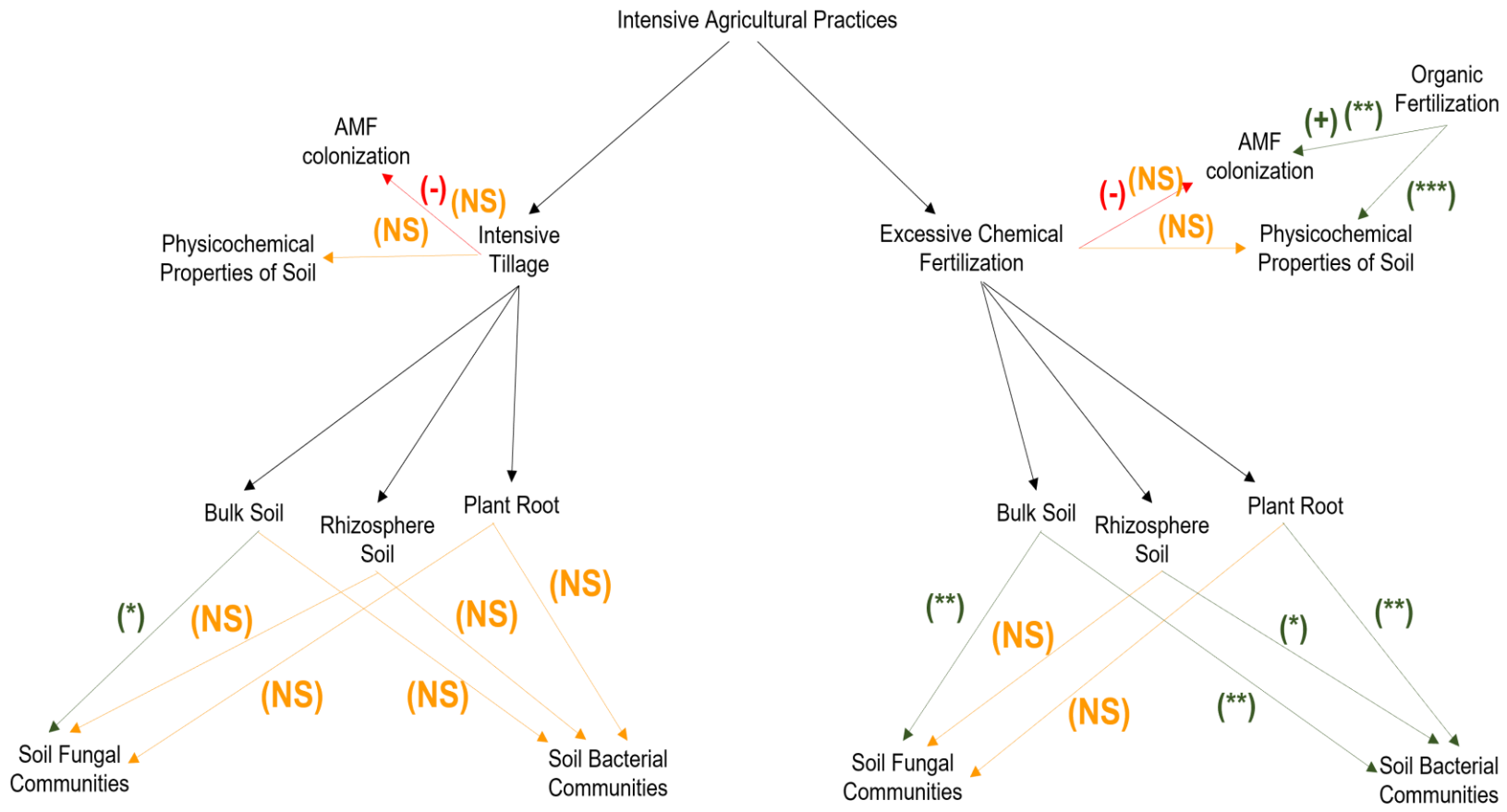

Figure 1: A synoptic diagram of major findings

Ordination analyses showed that bacterial and fungal communities were significantly affected by fertilization, however, fungal communities were also significantly influenced by tillage as well. A separate group of organic fertilizer-treated samples for both bacterial and fungal communities were clearly visible, which made the samples amended with organic fertilizer distinct from those treated with inorganic fertilizer. Since soil and plant sampling was done in June, it can be said that the environmental conditions might have altered microbial abundance and activity than other seasons. Taken together, I accept my hypothesis that the assembly and recruitment of soil microorganisms were different in case of bulk soil, rhizosphere soil and plant roots under organic fertilization compared to intensive agricultural treatment.

To my knowledge, this is one of the first studies investigating the effects of intensive agricultural management treatments and organic fertilization on soil microbiota comparatively in three different compartments- bulk soil, rhizosphere soil and plant roots. The findings from this study will help understand the impact of conventional tillage and excessive inorganic fertilizer as well as organic fertilization on soil health, fertility, and plant productivity in agricultural field soils. This investigation will help improve farming techniques and practices to reduce soil and groundwater acidification and nitrogen losses through leaching and incorporating organic farming practices for a sustainable agriculture. Nonetheless, more research is needed including soil and root samples from different time points and regions to obtain a robust understanding of how intensive agricultural practices influence soil and root microbiota.

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