

THE IMPACTS OF INTEGRATED SOIL HEALTH MANAGEMENT PRACTICES
ON SOIL MICROBIAL COMMUNITY IN A CORN SYSTEM

by

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(Under the Direction of Mussie Y. Habteselassie)

ABSTRACT

Despite the growing adoption of soil health practices, the integrated effects of these practices on key soil microbial composition and activity remain unknown. This study evaluated the impacts of reduced tillage (RT) and combinations of RT with cover crop (RTC), RTC and poultry litter (RTCA), and RTCA and biochar (RTCAB) against a conventional tillage (CT) on soil microbial community in a corn system in a two-year field study. Changes in microbial communities were characterized by measuring activity, abundance, and composition indicators. Shifts in microbial activity depended on corn growth stage and showed an increasing trend in response to integrating the practices. Similarly, bacterial alpha diversity showed an increasing trend over time under the integrated practices, while beta diversity revealed distinct microbial communities between RTCAB vs the rest. Overall, there was a clear trend towards a shift in soil microorganisms in response to integrating the various practices vs RT or CT.

INDEX WORDS: Conventional tillage, Reduced tillage, Cover crop, Biochar, Poultry litter, Microbial activity, Microbial diversity, Ammonia oxidizers, Soil enzyme

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BS, Obafemi Awolowo University, Nigeria, 2020

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial
Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2025

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August 2025

DEDICATION

I dedicate this thesis to God, whose grace and strength have sustained me throughout this journey. To my loving parents, thank you for your unwavering support, sacrifices, and belief in my dreams. To my siblings and wonderful nephews and niece, thank you for your constant encouragement, laughter, and love that brightened even the toughest days.

ACKNOWLEDGEMENTS

I express my deepest gratitude to my major professor, Dr. Mussie Habteselassie, for his invaluable support, guidance, and encouragement throughout this academic journey. His mentorship has shaped both my professional and personal growth. I am also sincerely grateful to my committee members, Dr. Henry Sintim and Dr. Miguel Cabrera, for their insightful feedback, which significantly enhanced the quality of this work.

My heartfelt appreciation goes to my lab mates, Chiara and Benedicta, whose help made this process more enjoyable. I am also grateful to Godfred, Millicent, and Ariful, for their assistance during field sampling and to Samuel Wright for providing essential weather data. Your help was instrumental to the success of this research. I want to express my appreciation to Dr. Jamal for his assistance with bioinformatics tools and statistical analysis. Thanks to Colton and Jalen for their assistance in the lab.

Finally, I am grateful to Dr. Oliver, Perpetual, Ebunade, Teniade, IBK, Olaide, Jumoke, Deji, Dara, Jerry, Bunmi, Munah, Akhila, Lois, Tosin, and Odemairo of blessed memory for their presence along the way. Your support meant more than words can express.

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CHAPTER 1

INTRODUCTION

Corn (*Zea mays*) is one of the most-grown cereals worldwide, cultivated in over 170 geographical areas all over the world (Wang & Hu, 2021). Reports by the United States Department of Agriculture (USDA, 2023) put the United States among the top nations in corn production, dominating the international corn market. Despite the United States being known as a prominent foreign corn trader, most corn produced is channeled towards internal use, which serves as crucial feed for livestock and biofuels as an alternative to energy sources (USDA, 2023).

In 2022, corn production in Georgia experienced a reduction after a steady rise between 2018 and 2021. There was a notable decline of 11.5% in the area allocated for corn cultivation in the state of Georgia in 2022 when compared to the preceding year of 2021, also surpassing that of 2019, the year preceding the onset of the COVID-19 pandemic (Harris & Sintim, 2023). Georgia's corn production had a remarkable milestone in the year 2023, surpassing the past decade, and the average output reached an unprecedented height at 183 bushels per acre, edging over the former record of 182 bushels per acre in 2021 (Tubbs et al., 2024).

This increase in yield is achieved through extensive use of agricultural inputs, including synthetic fertilizers, pesticides, and herbicides. Conventional farming systems also use some tillage practices to address other soil limitations, such as impermeable subsoil, which is common in most GA soils (Tubbs et al., 2024). Despite its success in boosting yield, the conventional tillage system has many drawbacks. The extensive use of synthetic fertilizers can lead to nutrient

loss and water quality problems (Habteselassie et al., 2022). Also, the frequent plowing of the land exacerbates erosion problems and can lead to the depletion of soil organic matter (Devkota et al., 2022; Sithole et al., 2016). All these can result in a decline in soil health (Blevins & Frye, 1993). The above obstacles emphasize the necessity for sustainable measures to tackle these difficulties while conserving productivity.

To address some of these problems, adopting soil health management practices is increasingly being promoted (Yue et al., 2016). Studies have shown that adopting soil health management practices can offer significant environmental benefits and present a promising financial opportunity in the long run (Carlisle, 2016). For instance, using cover crops in a tillage system can release valuable carbon and nitrogen into the soil, enhancing soil health and productivity (Abdalla et al., 2019). Additionally, farmers who embraced cover crops experienced reduced runoff and improved soil health, leading to potential cost savings (Roesch-Mcnally et al., 2018). Other soil health management practices, such as the adoption of reduced tillage and the use of organic amendments, can also help in addressing the undesirable impacts of conventional tillage by controlling erosion and cutting down the need to apply synthetic fertilizers (Holland et al., 2004; Bertgtold & Marty sailus, 2020).

Adopting soil health management practices, such as using cover crops, animal waste, biochar, and reduced tillage, can help improve soil health in GA, where the soil is highly degraded and lacks nutrients. It is also vital to examine if integrating these different practices can maximize their benefits while minimizing their disadvantages. While evaluating their impacts, an emphasis on soil health is important, especially the soil biological component, which reflects the composition and functions of soil microorganisms.

Microorganisms are essential to soil health, and they play a central role in the success of such soil health management practices as they mediate nutrient cycling and organic matter decomposition. The effectiveness of cover crops often requires their symbiotic relationship with microorganisms (Adetunji et al., 2017). The release of nutrients from organic amendments depends on soil microorganisms mediating the mineralization process (Norton & Ouyang, 2019).

Research indicates that integrating soil health management practices such as the use of cover crops (Huang et al., 2020; Wulanningtyas et al., 2021), poultry litter (Dai et al., 2024), biochar (Shahzad et al., 2019) enhances the benefits of reduced tillage, thereby improving soil health. Although limited research exists on reduced tillage, the use of reduced tillage under management practices could have outstanding benefits and improve soil health (Crystal-Ornelas et al., 2021; Tully & McAskill, 2020).

As such, understanding these changes in soil microbial community function and composition in response to these practices can help us predict changes in soil processes and identify combinations of management practices that offer the best possible outcome (Fierer et al., 2007).

Objectives and Hypothesis

The main goal of this study is to assess how integrating various soil health management practices impacts the function and composition of soil microorganisms in a corn system in a field study in Georgia. The specific objectives are to:

- Assess the impact of integrated soil health management practices on microbial activity.
- Determine how integrated soil health management practices affect microbial abundance and composition.

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CHAPTER 2

LITERATURE REVIEW

Corn System

The corn production system is indispensable to global food security, as it is a vital source of nutrition and raw material for industrial and biofuel purposes. According to Ranum et al. (2014), corn provides an essential caloric and nutrient intake for billions of people globally. However, the productivity of corn is intricately tied to external factors such as climate and soil health. Research has shown that climatic variability can significantly impact yields, with drought stress alone reducing corn production by up to 40% in certain regions (Lobell et al., 2011). Similarly, soil fertility plays a crucial role, as the depletion of essential nutrients like nitrogen and phosphorus directly hinders plant growth and yield. This underscores the importance of sustainable soil management practices, such as cover cropping and organic amendments, to maintain soil health and productivity over time (Yaghoubi Khanghahi et al., 2020).

While effective in boosting yields, conventional corn production practices are not without environmental consequences. Foley et al. (2011) highlight that heavy reliance on synthetic fertilizers and pesticides can lead to severe ecological issues, including waterway pollution, biodiversity loss, and greenhouse gas emissions. For instance, nitrate runoff from cornfields is a major contributor to hypoxic zones in aquatic ecosystems, such as the Gulf of Mexico, which severely endangers marine life (Rabalais et al., 2002). Additionally, the mechanical tillage associated with conventional corn production exacerbates soil degradation and contributes to the release of stored carbon, further amplifying environmental challenges. Transitioning to more

sustainable practices can mitigate these negative effects and promote ecological balance. Practices such as incorporating reduced tillage with cover crops, poultry litter and biochar (Alliaume et al., 2014; Dai et al., 2024; Shahzad et al., 2019) have demonstrated success in improving system resilience. For instance, cover crops incorporation reduces the risk of soil erosion and increases the soil's carbon content (Alliaume et al., 2013). In addition, agroecological methods, including intercropping and reduced chemical inputs, align productivity with environmental sustainability (Altieri et al., 2015). The corn production system can achieve long-term sustainability by adopting these strategies while safeguarding food security and minimizing its ecological footprint.

Corn Yield and Productivity

The corn system has experienced notable improvements in yield and productivity in recent years, driven by advancements in genetic breeding, better farming practices, and new technologies. One of the most significant innovations in this area has been biotechnology, which has led to the development of genetically modified (GM) corn varieties. These GM crops have been engineered for specific traits, such as insect resistance, herbicide tolerance, and drought tolerance, which not only enhance yield potential but also minimize crop losses due to environmental stressors and pest damage (Abdul Aziz et al., 2022). According to Brookes and Barfoot (2018), adopting GM insect-resistant and herbicide-tolerant corn varieties led to a substantial global yield increase of 27.1 million tons in 2016, representing a 6.1% improvement in yield. Biotechnology's role in enhancing the resilience and productivity of corn has been pivotal, especially in regions where pest pressure and environmental challenges are prevalent.

In addition to genetic innovations, modern farming techniques, particularly precision agriculture, have revolutionized corn production. Precision agriculture, including site-specific

nutrient management and variable rate technology, has proven highly effective in optimizing inputs and improving yield outcomes. Research by Grisso et al. (2015) found that variable rate technology, a component of precision agriculture, can potentially increase corn yields by 5-10% while reducing fertilizer inputs by 10-20%. This efficiency boosts productivity and promotes environmental sustainability by minimizing the overuse of fertilizers, which can contribute to soil degradation and water contamination. Furthermore, these technological advancements allow farmers to better manage resources, reducing input costs and increasing the overall profitability of corn production. However, while technological advances have greatly enhanced productivity, the agronomic practices associated with traditional corn systems have been intensive and, at times, unsustainable. Intensive practices such as plowing, disking, and harrowing, which are commonly used to prepare the soil before planting, can lead to soil erosion and degradation (Lal et al., 2007). The heavy reliance on synthetic fertilizers, particularly to supply nitrogen (N), phosphorus (P), and potassium, further exacerbates these environmental issues, as excess fertilizer use can result in nutrient runoff, contributing to water pollution and eutrophication (Hatfield & Prueger, 2004).

Moreover, the over-reliance on synthetic pesticides in conventional systems has contributed to pest resistance, which harms beneficial organisms and may ultimately decrease the long-term health of corn crops (FAO & WHO 2019; National Research Council, 1986). Additionally, monoculture cultivation, where corn is grown sequentially on the same land year after year, depletes soil nutrients and leaves crops more vulnerable to pests and diseases (Crews & Peoples, 2004). Therefore, while advancements in genetics and technology have greatly improved yield and productivity, it is crucial to address these sustainability challenges to ensure the long-term viability of the corn system.

Challenges of Conventional Tillage

Conventional tillage practices have long been central to corn production, but they pose significant challenges to soil health, biodiversity, and environmental sustainability. Intensive tillage disrupts soil structure, accelerates erosion, and depletes organic matter, essential for maintaining soil fertility (Lal, 2015). Repeated soil disturbance reduces the activity and diversity of microbial communities, which are critical for nutrient cycling and disease suppression (Lipiec et al., 2006). This degradation of soil health ultimately undermines the long-term productivity of agricultural systems. Furthermore, conventional tillage systems are linked to significant greenhouse gas (GHG) emissions. These include nitrous oxide (N_2O) from microbial nitrification and denitrification, as well as carbon dioxide (CO_2) from the use of fossil fuels in farming machinery (Yuan et al., 2024; Mantoam et al., 2020). The release of these GHGs contributes to climate change, creating a feedback loop that exacerbates agricultural challenges, such as increased drought frequency and reduced crop yields (West & Marland, 2002).

In addition to harming soil health, conventional tillage negatively impacts biodiversity above and below the soil surface. Intensive tillage disrupts soil habitats, leading to declines in populations of earthworms, arthropods, and other beneficial organisms vital for maintaining soil structure and fertility (Tomer et al., 2022). These organisms improve soil porosity, water infiltration, and organic matter decomposition. Aboveground tillage often results in habitat fragmentation that reduces the abundance of pollinators and natural pest predators, which are essential for crop production and ecological balance (Kremen & Miles, 2012). This biodiversity loss reduces farming systems' overall resilience, making them more vulnerable to pests, diseases, and climate variability. By transitioning to conservation tillage or no-till systems, alongside practices like cover cropping and organic amendments, farming systems can restore biodiversity

and foster a more stable ecosystem capable of self-regulating pest populations and supporting crop health.

Conventional tillage also drives environmental pollution through the overuse of synthetic fertilizers and pesticides, which often accompany such systems. The heavy reliance on synthetic fertilizers contributes to nutrient runoff into water bodies, leading to eutrophication and hypoxic zones, such as in the Gulf of Mexico (West & Marland, 2002). Pesticides used in conventional systems can accumulate in soil and water, disrupting ecological balances and posing risks to human health due to chemical residues in food and water (Aktar et al., 2009). Furthermore, the widespread adoption of transgenic crops in conventional systems has introduced new challenges, such as the emergence of herbicide-resistant weeds and insecticide-resistant pests (Heap, 2014). These issues can compromise the long-term viability of conventional farming and highlight the need for more sustainable and integrated approaches.

Sustainable farming practices such as reduced tillage, use of organic amendments, and cover cropping offer viable solutions to the challenges posed by conventional tillage. Reduced tillage minimizes soil disturbance, promoting the sequestration of carbon and improving soil aggregation, which helps retain nutrients and water (Li et al., 2024; Schipanski et al., 2014). Organic amendments, such as compost and manure, replenish soil organic matter and enhance microbial diversity, contributing to nutrient availability and soil resilience (Das et al., 2022). Cover cropping provides multiple benefits, including erosion control, nitrogen fixation, and improved water infiltration, collectively supporting long-term soil health and crop productivity (Six et al., 2002). These practices enhance the sustainability of farming systems and mitigate the environmental and health risks associated with conventional tillage. Transitioning away from

conventional tillage is thus not only a necessity for addressing current environmental challenges but also an opportunity to create more sustainable, climate-smart agricultural landscapes.

Soil Health

The concept of soil health, focusing on certain soil qualities and its capacity to sustain a range of biological activities within its designated habitat, emerged in the 1990s. It plays a crucial role in promoting the development of sustainable agricultural systems (Yang et al., 2020). Soil health is defined as the ability of soil to function as an essential living ecosystem that sustains plants, animals, and humans (Doran, 2002). The primary reason for human impacts on soil health is the need to satisfy an expanding population's fuel, fiber, and food requirements.

Major attempts have been undertaken over the past few decades to boost agricultural output via extensive use of input and land expansion (Tilman et al., 2001). However, there is a growing apprehension that intensive farming practices are exerting immense demand on our soil's ability to sustain its various roles, potentially leading to the degradation of our ecosystem and a significant decline in productivity (Foley et al., 2005; Tilman et al., 2001; Vitousek et al., 2009). The transformation of the ecosystem into farming lands, for instance, harms the environment, such as diminished soil organic matter, reduced biodiversity, and changes to the biochemical and hydrologic cycles (Balmford et al., 2005). As such, there is an immediate requirement to identify early indications of the deterioration in soil health as a result of agricultural management systems (Cardoso et al., 2013; Grime et al., 1997).

Assessing soil health involves measuring the indicators of chemical, physical, and biological parameters. Biological indicators focus on the role of microorganisms in mediating various biogeochemical soil processes. Soil Biological health integrates biogeochemical processes that are not fully captured by solely measuring physical and chemical indicators, as

such it is often a preferable over the others (Kibblewhite, Jones et al., 2008; Mueller et al., 2010; Nielsen & Winding, 2002; Sudarta, 2022).

Biological Soil Health Indicators

Soil enzymes are highly responsive to changes in soil and have been used as indicators of soil biological health (Nannipieri et al., 2012; Schmidt et al., 2011) as they are mainly produced by microorganisms. These indicators reflect the overall status of microbial community, including abundance, composition, and soil enzyme activities. Employing readily quantifiable parameters such as microbial abundance, soil respiration, urease activity, phosphatase, fluorescein diacetate (FDA) hydrolysis, and beta-glucosidase is a prevalent and pragmatic method for assessing soil biological health (Wienhold et al., 2004). As such, their activity can be used as a proxy indicator of microbial activity.

Microbial abundance and composition are significantly influenced by land use changes and vegetation types (Doran & Zeiss, 2000; Yao et al., 2000). For example, it has been demonstrated that extensive tillage, like mechanized farming, has a detrimental effect on soil microbial communities as it disrupts fungi networks and inhibits enzymatic activity (Holland & Coleman, 1987; Lützow et al., 2006). Thus, measuring larger microbial groups, including bacteria, fungi, and ammonia-oxidizers, is useful for assessing biological soil health since functional redundancy is anticipated from numerous soil microbial taxa. (Barrios, 2007). The two key microorganisms essential for autotrophic nitrification are ammonia oxidizers, which play a critical role in the global nitrogen cycle. The enzyme ammonia monooxygenase first catalyzes the oxidation of ammonia (NH_3) to hydroxylamine (NH_2OH), which is then converted to nitrite (NO_2^-) by nitrite-producing prokaryotes (Hatzenpichler, 2012; Norton & Stark, 2011; Norton, 2011). In most soils, the nitrite (NO_2^-) produced by ammonia oxidizers is rapidly

converted to nitrate (NO_3^-) by specialized nitrite-oxidizing bacteria, yielding a highly mobile, water-soluble nitrogen form (Robertson & Groffman, 2007). Ammonia-oxidizing archaea (AOA) exhibit oligotrophic characteristics and more robust cell envelopes, enabling them to persist under low-nutrient or oxygen-limited conditions and to tolerate temperatures up to 30 °C, near the upper survival limit for many prokaryotes as compared to AOB (Hatzenpichler, 2012). Although generally less abundant, certain AOB species have also been isolated from extreme habitats, underscoring their physiological versatility (Norton, 2011). Competition for ammonium (NH_4^+) and habitat preferences ultimately govern the relative abundance and spatial distribution of AOA and AOB within a given ecosystem (Norton & Stark, 2011; Wessén & Hallin, 2011). Because both groups are globally ubiquitous and closely tied to nitrogen-cycling rates, their community structure and activity serve as valuable bioindicators of soil nitrification dynamics (Hatzenpichler, 2012; Wyngaard et al., 2016).

Among the indicators of soil health, soil respiration shows the most significant response to soil management. Soil respiration is widely used as a generic indicator of microbial activity and organic matter decomposition (Schloter et al., 2003). It is the process by which carbon dioxide is released into the atmosphere from decomposition of soil organic matter (Yang et al., 2022). Temperature, moisture content, amount and quality of substrate are factors that influence microbial respiration (Ryan & Law, 2005). Soil respiration is comprised of heterotrophic respiration, which emits carbon dioxide from the decomposition of organic matter present in the soil, and autotrophic respiration accounts for CO_2 released by living roots (Van Der Heijden et al., 2008). However, there is a positive correlation between these two respiratory components, and numerous studies indicate that the rising pace of nitrogen addition has led to a significant reduction in respiration rates and an augmentation of terrestrial carbon (Boetius, 2019; Niu et al.,

2016; Singh et al., 2010; Treseder, 2008). For instance, Gao et al. (2014) reported a rise in subtropical forest following nitrogen supplementation, attributed to a rise in autotrophic respiration. This explains that microbial respiration rates are typically interpreted as enhanced soil organic matter mineralization activity, which is accompanied by the release of nitrogen, phosphorus, and other nutrients in plant-accessible forms. Consequently, higher soil respiration values are considered indicative of improved soil health.

Thus, understanding how soil respiration reacts to management practices is important because it captures biological responses and reflects microbial activity in relation to these practices (Firth et al., 2022).

Phosphatases are a class of enzymes that facilitate the hydrolysis of phosphoric acid esters and anhydrides (Eivazi & Tabatabai, 1977). They release Phosphorus (P) for plant absorption by detaching the phosphate group from organic molecules, especially from Organic P, which is dissolved (Brady & Weil, 2008). The soil acid and alkaline phosphatases are widely found in a broad spectrum of soil pH conditions (Dick et al., 1988; Drouillon & Merckx, 2005; Eivazi & Tabatabai, 1977). There is evidence that soil health management practices like cover crops increase phosphatase activity (Feng et al., 2021). (Chatterjee et al., 2021; Choudhary et al., 2021) also reported that phosphatase activity responds positively to organic and inorganic fertilizers when applied to soils.

Urea is a nitrogen fertilizer extensively utilized in agriculture. The application of urea to soil often leads to immediate hydrolysis into ammonia/ammonium, and this process is mediated by the urease enzyme, which is mainly produced by soil microorganisms (Wessén et al., 2010; Yadav et al., 1987; Krajewska, 2009). In soils, the rate at which urea is hydrolyzed is determined by the activity of the extracellular (Fisher et al., 2016). The primary sources of urease in soils are

ureolytic microorganisms, with bacteria as the main contributor among these organisms, and are greatly affected by environmental factors (Zhao et al., 2022). Furthermore, urease activity is influenced by numerous factors, including temperature and soil pH, with optimal activity often observed in the 6.5-8.0 range (Kandeler & Gerber, 1988; Moyo et al., 1989). Heavy metals can strongly inhibit urease activity (Krajewska, 2009). The management of urease activity, often through synthetic urease inhibitors co-formulated with fertilizer, is a key strategy for improving nitrogen use efficiency and mitigating environmental impacts (Me-Trenkel, 2021).

Another example of soil enzyme often used as an indicator of microbial activity is fluorescein diacetate hydrolysis (FDA). The quantity of fluorescein generated through hydrolysis was seen to be equivalent to the growth of microbial populations (Swisher & Carroll, 1980). As such, the fluorescein diacetate hydrolysis assay has been considered a preferred method in estimating generic microbial activity (Jiang et al., 2016). The technique does not require much soil and is fast (Adam & Duncan, 2001; Green et al., 2006).

Beta-glucosidase activity is also used to characterize microbial activity. It is involved in breakdown of cellulose, a primary component of plant polysaccharides. Cellulose is made up of polymer chains consisting of β -1,4-linked glucose molecules. The enzymatic breakdown of cellulose happens when endo- β -1,4-glucanase (EC 3.1.2.4) splits cellulose into fragments and cellobiohydrolase (EC 3.1.2.91), which separates the dimer cellobiose (two β -1,4 linked glucose units) from the molecules' reducing ends. This hydrolysis is completed by the enzyme Beta-glucosidase, which triggers the splitting of glycosidic bonds, thereby releasing two moles of glucose for every one mole of cellobiose released, which consequently influences the availability of a crucial energy source for microbes that cannot directly assimilate cellobiose (Turner et al., 2002). Soil heterotrophs produce most beta-glucosidase, specifically those in the fungal

community (Hayano & Tubaki, 1985). Cellobiose, glucose, and their metabolites (byproducts of cellulose degradation), stimulate the production of the enzyme by these organisms (Stewart & Leatherwood, 1976). Presently, extracellular enzymes are commonly being used as biological markers in accessing soil health due to their responsiveness to environmental changes and their quick response to changes in land management (Dick, 1997; Yakovchenko et al., 1996).

Chemical Soil Health Indicators

In addition to the biological indicators, chemical indicators, most notably soil nutrient concentrations, are essential for tracking changes in soil health management and are indispensable to any comprehensive evaluation of soil health. pH acts as a primary ecological filter for the microbial community. Broad-scale metagenomic and phylogenetic studies have consistently demonstrated that soil pH is the strongest predictor of bacterial community structure and diversity at both local and global scales, often exerting more influence than factors like climate or soil type (Fierer & Jackson, 2006; Lauber et al., 2009). The composition of key functional groups is also highly pH-sensitive; for example, the balance between ammonia-oxidizing archaea and bacteria, which perform the first step of nitrification, is strongly dictated by soil pH (Prosser & Nicol, 2012). Additionally, acidic conditions generally favor fungal growth over bacterial growth, leading to a lower bacterial-to-fungal biomass ratio, which has significant implications for carbon sequestration and nutrient cycling dynamics, as fungi often exhibit higher carbon use efficiency (Rousk et al., 2010). Thus, maintaining an optimal pH range is fundamental for supporting a diverse, active, and functionally stable soil microbial community.

These chemical indicators also extend to exchangeable cations and micronutrients. The major exchangeable cations calcium (Ca^{2+}), potassium (K^{+}), and magnesium (Mg^{2+}) are vital not

just for plants, but for microbial physiology and soil structure. Calcium is a primary agent in the formation of stable soil aggregates, acting as a bridging cation that binds negatively charged clay surfaces to the extracellular polymeric substances produced by microbial communities, effectively cementing particles together (Oades, 1984; Czarnes et al., 2000). These aggregates protect organic matter and create diverse microhabitats that promote microbial diversity (Six et al., 2004). Potassium, the most abundant cation within microbial cells, is critical for managing osmotic stress and serves as a required cofactor for numerous enzymes essential for protein synthesis (Epstein, 2003; Hazelton & Murphy, 2016). Magnesium is similarly vital as a structural component of ribosomes and a bridging element for ATP in nearly all energy transfer reactions (Cowan, 2002). This precise regulation by soil life continues with micronutrients like zinc (Zn) and manganese (Mn), which are essential components of enzymes and proteins. Microbes acquire these metals by producing siderophores, high-affinity chelating agents that increase their solubility and availability (Neubauer, Furrer, & Schulin, 2000).

The cycling of manganese provides a compelling example of biological control, as the redox transformation between its soluble and insoluble forms is almost exclusively mediated by bacteria and fungi (Tebo et al., 2004). The resulting manganese oxides are potent natural oxidants that are crucial in the degradation of recalcitrant organic matter like lignin (Keiluweit et al., 2015). Therefore, the chemical availability of macro and micronutrients directly reflects the complex metabolic capabilities present in the soil's microbial community. Just as pH is a critical property, the soil's ability to resist changes in pH its lime buffering capacity (LBC) is essential for creating a stable biological environment. Soils with high LBC, often due to high clay or organic matter content, protect microbial communities from abrupt pH fluctuations caused by external inputs. This ensures that biological processes remain undisrupted (Weil & Brady, 2017).

This reciprocal relationship is bidirectional: chemical conditions dictate the feasibility and rates of biological processes, while biological activities, such as organic matter decomposition, nitrogen fixation, and nutrient solubilization, substantially alter soil chemical properties (van der Heijden et al., 2008).

Soil Health Management Practices

Even though conventional farming is not a sustainable paradigm, it presently contributes to a significant portion of world crop output (Cerilli et al., 2024). Soil degradation is a critical issue for the foreseeable future of global farming systems (Tilman et al., 2002). Due to concerns associated with conventional agricultural management systems, there is a movement towards adopting soil health management systems that counteract the undesirable effects of conventional farming (Roldán et al., 2007). Many soil conservation strategies have been suggested and tried in field crop systems to stop or minimize soil degradation. These techniques include reduced tillage, use of cover crops, and organic amendments such as poultry litter and biochar.

Reduced tillage

As defined by FAO (1993), reduced tillage involves less soil disturbance, leaving the subsoil essentially unaltered and thereby retaining soil structure and aggregates. Reduced tillage has many merits, including runoff mitigation, soil fertility enhancement, and soil and water preservation (Sharma & Abrol, 2012). Reduced tillage has been proven to improve microbial population and composition by reducing soil disruption and preserving organic matter (Kurm et al., 2023). Even though this tillage practice offers some benefits to the soil, a recent study found that these benefits may be less extensive than thought (Pearsons et al., 2023). For example, according to Ye et al., (2021), in a 40-year study of soil health assessment using reduced tillage, it was found to have no effects on soil biological and chemical indicators. However,

incorporating cover crops under reduced tillage enhances soil organic carbon and might improve soil biological health (Wulanningtyas et al., 2021). Alliaume et al. (2014) also reported that the integration of reduced tillage with the cover crop is an effective method that can reduce soil loss, enhance water intake, minimize nitrogen leaching, and decrease carbon dioxide emissions (Abdalla et al., 2013; Boeckx et al., 2011; Jokela & Nair, 2016; Zhang et al., 2020).

Additionally, the integration of reduced tillage with cover crops impact the entire agricultural system, influencing the availability of nutrients, soil temperature, and organic matter (Alliaume et al., 2014; Cook et al., 2006; Jokela & Nair, 2016; Peigné et al., 2007; Tittarelli et al., 2018).

As such, reduced tillage systems with cover crops must be customized to the site's unique conditions to address the yield disparity compared to conventional tillage systems. These management practices depend on using herbicides to remove cover crops and suppress weed germination. Even though the research on reduced tillage is limited, its benefits may be strongly apparent when combined with other soil health management practices (Crystal-Ornelas et al., 2021; Tully & McAskill, 2020).

Cover crops

Cover crops are planted to protect the soil from erosion and avert loss of nutrients due to leaching and surface runoff and are often used in organic systems due to their health-enhancing attributes (Butler et al., 2016; Kaye & Quemada, 2017). These crops are grown between the main crops and are not only planted based on their marketable commodity but also on their role to serve as a mechanism to contribute to the well-being of the environment by enhancing productivity and yield (Blanco-Canqui et al., 2015). Cover crops mainly comprise legumes planted to preserve the soil surface and to improve soil chemical and biophysical properties. The best cover crop should be one that is quick to sprout and appear on time, able to fix nitrogen

from the air, build greater biomass in a shorter period, not compete with the main crop, be resistant to pests, insects, and diseases, also be able to suppress weed and economical to grow (Lepoint et al., 2017). Cover crops further function as a nitrogen supply for soil via fixation and mineralization through residue input (Lu et al., 2000; Sharma et al., 2018). Even though cover crops have numerous advantages, selecting the right one is crucial since there may be a trade-off between their capacity to reduce weeds and improve soil and nitrogen availability. For instance, crimson clover and hairy vetch are leguminous cover crops and, while rich in nitrogen, may not effectively reduce weeds (Teasdale & Abdul-Baki, 1998). These compromises could be attributable to the carbon-to-nitrogen ratio, edaphic soil characteristics, environmental circumstances, and tillage methods (Teasdale & Abdul-Baki, 1998; O'Connell et al., 2015).

Additionally, cover crops have proven to be an essential part of sustainability in agriculture due to their effect on improving soil health and conserving the moisture in the soil for upcoming crops as reported by Sharma et al. (2018). However, Nielsen et al. (2015) found cover crops to reduce soil moisture for the following crops, where water scarcity and irrigation are not made available. Overall, adopting cover crops in management practices promotes biological soil health, providing a source of carbon and nutrients for microorganisms. As such, integrating cover crops benefits the farmers as it enhances long-term farm output while simultaneously reducing the expenses on tillage and fertilization, improving farm profitability (Shackelford et al., 2019; Montgomery, 2017).

Poultry litter

Poultry litter, a combination of poultry droppings, feathers, feed residues, and wood shavings, is a nutrient-rich organic amendment widely used to enhance soil fertility and crop productivity. It contains essential macronutrients such as N, P, and potassium (K) and

micronutrients necessary for plant growth (Ashworth et al., 2020). Applying Poultry litter to croplands improves soil structure, increases microbial activity, and enhances nutrient cycling, making it a valuable alternative to synthetic fertilizers (Adeli et al., 2023). Research by Yin and Sykes (2024) found that integrating poultry litter with crop rotations, such as corn-soybean and soybean-cotton, significantly improves soil health indicators. Also, Hoover et al (2019) reported an increase in soil health over a long-term application of poultry litter under no-till in corn, cotton, and soybean. Additionally, poultry litter contributes to improved soil aggregation, leading to better water retention and reduced erosion risks. One of the primary benefits of poultry litter application is its contribution to soil organic matter (SOM) accumulation, which enhances water-holding capacity and soil microbial diversity (Chandra, 2024). The decomposition of organic material from poultry litter supports the formation of stable soil aggregates, reducing erosion and improving soil aeration (Ashworth et al., 2018). Furthermore, poultry litter fosters beneficial microbial communities that promote nutrient cycling and suppress soilborne pathogens, and long-term studies suggest that continuous poultry litter application increases soil carbon sequestration, an important factor in climate change mitigation (Ashworth et al., 2020). When combined with crop rotations, poultry litter further optimizes soil chemical and microbial properties (Ashworth et al., 2018).

Despite its benefits, poultry litter applications must be managed carefully to prevent nutrient runoff and water contamination. Excessive phosphorus accumulation from repeated poultry litter applications can lead to eutrophication in nearby water bodies (Chandra, 2024). Strategies such as incorporating poultry litter into the soil can minimize these risks while ensuring efficient nutrient use (Everts et al., 2006). Additionally, integrating poultry litter with cover crops has been found to enhance soil stability and mitigate erosion, particularly in no-till

systems (Dai et al., 2024), and Woodruff et al (2019) stated that poultry litter and cover crops could have synergistic effects, and integrating both can increase sustainability in agricultural systems and promote nutrient cycling. Cover crops act as a buffer, absorbing excess nutrients and reducing leaching losses (Pokhrel et al., 2021). The combination of poultry litter and cover crops presents an opportunity to improve the sustainability of conventional cropping systems. Cover crops, such as legumes and grasses, provide ground cover that prevents soil erosion, enhances soil organic matter, and contributes to nitrogen fixation (Bilenky, 2021). Studies show that cover crops and poultry litter integration can improve soil health indicators, such as microbial biomass, aggregate stability, and water infiltration (Mirsky et al., 2023). This combination has also been linked to increased yields in no-till corn and soybean rotations, demonstrating its potential for improving both productivity and environmental resilience (Nyakatawa et al., 2001). Therefore, there is a need to assess how integrating poultry litter alongside other soil health management practices can help improve soil health.

Biochar

Biochar is an organic amendment formed through pyrolysis under limited oxygen conditions, and it is frequently used to enhance soil health and alleviate greenhouse gas emissions (Qian et al., 2015). The specific thermal processing biochar undergoes facilitates its surface area increment, which makes it endure in soil with minimal biological decay. Relative to other soil amendments, it can absorb water and nutrients due to its large surface area (Hunt et al., 2010). Adding biochar also increases soil pH and fosters microbial populations; it has also been observed to impact the presence of essential plant nutrients (Sharma et al., 2025). Research has indicated an increase in cation exchange capacity in biochar amended soils, considerably higher than the bulk of soil or soil organic matter (Sohi et al., 2009; Yuan et al., 2011). Consequently,

incorporating biochar in soil increases the soil's cation exchange capacity (Manyà, 2012).

Furthermore, the addition of biochar to soils improves the emergence of seed, crop productivity, yield and vegetative growth (Glaser et al., 2002). However, incorporating biochar with organic fertilizers can significantly boost agricultural output and improve microbial function (Lehmann et al., 2011; Verheijen et al., 2010).

Integrating soil health management practices can be crucial for maintaining productive and sustainable agricultural systems. Numerous studies have highlighted the benefits of adopting a holistic approach to soil management, combining various practices to address multiple aspects of soil health (Baveye et al., 2016; Lal, 2015). For example, the integrated use of minimum tillage with organic amendments and cover crops can enhance soil structure and nutrient cycling (Baveye et al., 2018). Despite the advocacy for adopting integrated soil health management practices, there have not been enough studies that evaluate their impact on soil microbial communities that drive key ecological processes, such as nutrient cycling, organic matter, and disease suppression. Such studies are particularly needed in states like GA, where the soil is highly weathered and low in organic matter and nutrients.

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CHAPTER 3

THE IMPACT OF INTEGRATED SOIL HEALTH MANAGEMENT PRACTICES ON
MICROBIAL ACTIVITY¹

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Abstract

Soil microorganisms play an essential role in nutrient cycling and maintaining soil health. However, the prolonged dependence on conventional farming practices has contributed to decline in soil health. In response, sustainable soil health management practices are being adopted. While previous research has largely focused on individual management practices, the integrated effects of these practices on microbial communities remain insufficiently explored. This study evaluated the impact of integrated soil health management practices on microbial activity by measuring soil respiration and enzyme activities. Five treatments were evaluated: conventional tillage (CT), reduced tillage (RT), reduced tillage with cover crops (RC), reduced tillage with cover crops and poultry litter (RCA), and reduced tillage with cover crops, poultry litter, and biochar as an organic amendment (RCAB). Soil samples were collected from a 15 cm depth at four corn growth stages (preseason, V6-V8, VTR1, and R6 harvest). RCAB produced the highest soil respiration at V7/V8 in 2023 ($48.0 \text{ mg CO}_2 \text{ kg}^{-1} \text{ soil h}^{-1}$; $p = 0.01$) and maintained elevated rates across all growth stages. In 2024, RCA led in respiration at V6/V7 and R6. RCA and RCAB showed peak urease, β -glucosidase, and fluorescein diacetate hydrolysis activities at the R6 stage ($p \leq 0.01$), indicating increased nutrient-cycling potential. Correlation analyses underscored strong positive relationships between β -glucosidase and base cations and P ($r \approx 0.93$) and between soil respiration and K, Mn, Zn, and P ($r > 0.97$). These findings highlight the potential benefits of integrating soil health management practices over time.

Introduction

Conventional farming systems, with intensive tillage practices and heavy reliance on agricultural inputs like pesticides, herbicides, and synthetic fertilizers, can be detrimental to soil health. These practices impair soil structure, disrupt microbial communities, and reduce soil organic carbon, cumulatively undermining soil fertility and productivity (Melero et al., 2006). Negative outcomes include reduced crop yields, increased susceptibility to diseases, and diminished nutrient bioavailability (Nielsen & Winding, 2002).

To address these challenges, sustainable soil health management practices such as reduced tillage, the integration of cover crops, and organic amendments have emerged as crucial tools to rebuild soil health and ecological resilience (Farmaha et al., 2022; Galindo et al., 2022; Mbuthia et al., 2015). For instance, reduced tillage has been consistently associated with improved microbial biomass and enzymatic activities, largely due to decreased physical disturbance and increased organic matter retention (Mbuthia et al., 2015; Niewiadomska et al., 2020). When coupled with cover crops, these systems create beneficial microenvironments rich in root exudates that stimulate microbial activity and promote enzyme activities essential to nutrient cycling such as phosphatase, urease, and β -glucosidase activities (Stegarescu et al., 2021; Vieira et al., 2025). Field experiments have shown that applying poultry manure in no-till systems, whether on the soil surface or incorporated subsurface, consistently enhances soil organic matter content and productivity (Pote et al., 2011).

Despite this progress, a knowledge gap exists in understanding how these practices interact when integrated. While the individual effects of tillage reduction, cover cropping, and organic amendments are well-documented, their interactive or combined impacts on microbial ecology and activity alongside varying chemical indicators remain underexplored. Recent studies

emphasize that the combinations of sustainable soil health management practices can significantly boost enzyme activities and can outperform singular approaches (Erdel et al., 2023; Brennan et al., 2019). Thus, a holistic approach incorporating soil health management practices can be important for designing resilient agroecosystems that maintain long-term soil productivity and ecological integrity.

Reliable indicators of soil health are crucial for effectively guiding these management strategies. Doran et al., (1996) defined soil health as a crucial concept that incorporates soil's biological, physical, and chemical qualities, indicating its ability to sustain productivity in farming, environmental quality, and biodiversity. Since microbial communities are the primary facilitators in regulating nutrient cycling, organic matter decomposition, and the overall functioning of the ecosystem, assessing the soil's biological parameters provides a better understanding of soil health (Doran et al., 1994). Measuring soil health parameters such as enzymatic activities and soil respiration, offers a careful evaluation of how different management practices influence microbial activities in soil (Burns, 1977). Soil respiration is commonly used to indicate microbial metabolism and to determine the rate at which organic matter decomposes (Schloter et al., 2003). Enzyme activities such as β -glucosidase, urease, phosphatase, and fluorescein diacetate (FDA) hydrolysis, which are mainly secreted by the microorganisms in the soil, mediate carbon (C) and nitrogen (N), phosphorus (P) cycling and are sensitive indicators of microbial activity (Burns et al., 2013; Diera et al., 2020; Green et al., 2006; Turner et al., 2002). For instance, β -glucosidase catalyzes the conversion of cellobiose to produce glucose, while urease facilitates the hydrolysis of urea into ammonia. Phosphatase mediates the conversion of organic phosphorus into inorganic phosphorus through the mineralization process, and FDA

hydrolysis provides a broad index of total microbial activity (Adam & Duncan, 2001; Adetunji et al., 2017; Merino et al., 2016; Ugolini & Edmonds, 1983).

In addition to biological indicators, soil nutrient concentrations provide chemical measures for monitoring changes in soil health management and are indispensable to any comprehensive evaluation of soil health. Exchangeable calcium (Ca^{2+}) and magnesium (Mg^{2+}) are base-forming cations that influence base saturation and pH stabilization, but also directly influence microbial community structure and enzyme kinetics (Anderson et al., 2017). Potassium (K^{+}) is essential for microbial osmotic regulation and the activation of key intracellular enzymes, and field studies have demonstrated that higher soil K availability is positively associated with increased microbial biomass pool and overall metabolic activity (Nair & Ngouajio, 2012). Micronutrients like manganese (Mn) and zinc (Zn) also serve as essential cofactors for key microbial enzymes, thereby modulating carbon and nitrogen cycling processes (Shepherd & Oliverio, 2024). Moreover, lime buffering capacity (LBC) and its equivalent (LBCeq) give us a better insight into the soil's resistance to pH fluctuations, as soils with higher LBCeq tend to have a greater buffering against acidification, maintaining optimal pH for microbial enzyme functions (Kissel et al., 2012).

The main objective of this study was to evaluate how integrating various soil health management practices, including reduced tillage, cover crops, poultry litter, and biochar, impacts microbial activity in a corn system in a field study.

Materials and Methods

Study site and experimental set-up

The study evaluated the impact of five soil health management practices, two baseline treatment that included conventional Tillage (CT), reduced tillage (RT) and three integrated

practices combining reduced tillage with cover crop (RC), reduced tillage with cover crop and poultry litters (RCA), reduced tillage with cover crop, poultry litter and biochar (RCAB) in a field study at the University of Georgia Southeast Georgia Research and Education Centre in Midville, GA (32° 52'46.44N, 82° 12'33.86W) from 2023 -2024. The poultry litter used in the experiment was sourced from Powell Poultry Farms, LLC, in Omega, GA, while the biochar was obtained from Wakefield BioChar, located in Valdosta, GA. The biochar was produced through pyrolysis at a temperature of 600 °C and had an alkaline pH of 10.7. The amendments were broadcast uniformly across the designated plots on the surface prior to tillage operations.

Temperature, rainfall, and soil moisture data were obtained from the nearby Georgia Automated Weather Monitoring Network (Georgia AEMN, 2023, 2024) and are shown in Figures 3.1A and 3.1B. Total rainfall amounts for the 2023 and 2024 corn-growing seasons were 82 mm and 90 mm, respectively. The mean average air temperature were 18.77 °C in 2023 and 22 °C in 2024. Initial soil pH was 6.39_{1:2(soil/water)}, 6.54_{1:2(soil/water)}, and lime buffering capacity (LBC) 235 and 266 mg CaCO₃ kg⁻¹ soil pH⁻¹ in 2023 and 2024, respectively. The study site's soil is a Dothan sandy loam (Fine-loamy, kaolinitic, thermic Plinthic Kandiudults). Texture analysis of the 0-15 cm layer was 93.26% sand, 6.50% clay, and 0.24% silt (Soil Survey Staff, 2022). Organic matter content was determined by using the loss on ignition method and averaged 0.86 % in the 0-15 cm depth.

The five treatments were organized in a randomized complete block design with four replications, and the size of each treatment plot was 7.3 by 9.1 m. The experimental field was laid out in three equal sections, allowing for the rotation of corn, peanut, and cotton over three years (2021-2023) under each management system. The crops were sequenced in the first section as corn (2021), peanut (2022), and cotton (2023). In the second section, peanuts were planted in

2021, cotton in 2022, and corn (planted each April) in 2023. For the third section, cotton was planted in 2021, corn in 2022, and peanuts in 2023. All plots received fertilizers at rates that meet the University of Georgia Agricultural and Environmental Services Laboratories (UGA-AESL) recommendations to achieve the desired corn yield of 15.7 Mg ha^{-1} , as summarized in Table 3.1. A combination of ammonium sulfate, diammonium sulfate, urea and potassium chloride were used to supply required amounts of N, P, K and S. Treatments CT, RT, and RC received the full nutrient rates of 280 kg N ha^{-1} , $135 \text{ kg ha}^{-1} \text{ P}$, $168 \text{ kg ha}^{-1} \text{ K}$, and $11 \text{ kg ha}^{-1} \text{ S}$ from the above-mentioned fertilizers. The nutrient levels expected to be available from the poultry litter and biochar were considered before applying synthetic fertilizers for RCA and RCAB. Table 3.2 shows estimated levels of available nutrients from poultry litter and biochar based on the 2.24 Mg ha^{-1} application rate, assuming 60% of the N and 80% of the other nutrients in poultry litter were available (Bryant et al., 2020). Thus, treatment RCA received only 165 kg N ha^{-1} . The remaining N and all the other nutrients came from poultry litter. Treatment RCAB received 219 kg N ha^{-1} , $53 \text{ kg ha}^{-1} \text{ P}$, $89 \text{ kg ha}^{-1} \text{ K}$ and $26 \text{ kg ha}^{-1} \text{ S}$ from synthetic sulfur fertilizers. The remaining amounts came from a combination of poultry litter and biochar. Nitrogen was applied before the planting of the corn, at the V3-V4 and liquid side dress was applied at the V6 stage to all treatments at $112 \text{ kg ha}^{-1} \text{ N}$ with urea ammonium nitrate (34-0-0) at 14 days after planting (DAP). For the in-season application, the fertilizers in CT plots were surface-broadcast, while all other treatments were broadcast at both pre-plant and in-season applications.

The conventional tillage involves tilling and preparing the soil using a harrow and field cultivator. The reduced tillage is a one-time strip tillage, and cereal rye was used as a cover crop. The cereal rye was planted in the fall, using the John Deere 750 no-till drill with a seed drill at a

rate of 56 kg ha⁻¹. The cover crop was planted in winter preceding the year before planting and were terminated by spraying glyphosate [isopropylamine salt of N-(phosphonomethyl) glycine] in spring. A concise overview of the key field operations, including the dates for amendment applications, corn planting, and soil sampling, is shown in Figure 3.2. The strip tillage implement had a roller-crimper attachment that lays down the rye cover as mulch. Poultry litter was applied at 4.48 Mg ha⁻¹ in the RCA treatments and 2.24 Mg ha⁻¹ in RCAB treatment, respectively. The corn variety planted was different in 2021 and 2022; however, in 2023, the Corn variety planted was Croplan® Genetics 5678, at a rate of 79,074 seeds ha⁻¹.

Soil sampling and processing

Soil sampling was conducted throughout the study at four corn growth stages: Pre-season and the following corn growth stages: V6-V8, VT/R1 and R6 as denoted in Table 3.3. A composite of up to five cores of samples (each 2.5 cm in diameter) was collected from each plot from the top 15 cm. The pre-season soil samples were taken before planting. The V6-V8 samples were taken 4-6 weeks after seed emergence, with leaf collars determining the stages. VT/R1 samples were taken around 6-10 weeks after emergence, and R6 stage was 60 days after silking, determined by the average silking of the whole field. These soil samples were stored in a chest cooler with ice packs and transported to the University of Georgia campus (Griffin, GA) for analysis. Upon arrival at the laboratory, the soil samples were sieved through a 2 mm sieve mesh (USDA standard) to remove debris and stones. The sieved soil was then divided into two portions: one portion was stored at 4 °C for measurements of soil health parameters. At the same time, the other was stored at -20 °C for DNA extraction and downstream molecular microbial analyses. Midseason soil samples were used to assess nutrient content and pH in both years. The

samples were submitted to the University of Georgia Agricultural and Environmental Services Laboratories (UGA-AESL).

Soil respiration

This is a key indicator of microbial activity and soil health and was determined by weighing 20 g of soil (± 0.03) and placing it into 850 ml mason jars. The cover was tightened to ensure a lack of CO₂ exchange with outside environment. Afterward, the jars were incubated for 24 hours, and the temperature was set to 25 °C. The CO₂ released was measured using the CO₂ gas analyzer (EGM-5 from PP systems) per the manufacturer's instructions. Briefly, 10 mL of the jar's headspace was sampled using a syringe and injected slowly into the EGM-5 over 15 seconds. Readings were converted from parts per million ($\mu\text{mol/mol}$) to milligrams of CO₂ per kilogram of soil per hour ($\text{mg CO}_2 \text{ kg}^{-1} \text{ soil h}^{-1}$). The conversion involved using the Ideal Gas Law to calculate the number of moles of gas in the jar's headspace and the corresponding CO₂ concentration for that specific volume. It was then converted to mg CO₂ per dry soil weight equivalent. The blank, which consisted of two empty, air-tight mason jars (jars with no soil sample), had its average CO₂ reading subtracted from each treatment before conversion.

Urease activity

Urease activity was determined based on the protocol described in Tabatabai (1994). Briefly, 1 g of soil (± 0.01) was weighed into a section of a bi-plate. Each sample had two bi-plates, one for treatment and the second for control. Three mL of tris maleate buffer of pH 7.0 was added to part of each bi-plate that had soil, and 3 mL of the boric acid-indicator mixture was added to the other half of the bi-plate that did not contain the soil. The reaction was initiated by pipetting 1000 μL of 6M urea solution (substrate) to the plate labeled treatment, and 1000 μL of water was added to the bi-plate labeled control. The bi-plates were covered and incubated at

room temperature (25 °C) for an hour. The reaction was then terminated in both plates by adding 500 μL of 10 mM silver sulfate to the soil. The ammonia was released on both plates by adding 3M potassium carbonate to the soil. The plates were gently placed and slipped into Ziploc bags, with all treatments in all bags and controls in another Ziploc and left on the benchtop for 24 hours. The boric acid-indicator mixture was titrated with 0.02N HCL until the color changed to that of a fresh solution (we put some boric acid into another plate and use that as a comparison). The amount of each HCL used for each plate of treatment and control was recorded, and urease activity was expressed in μmol of $\text{NH}_3 \text{ g}^{-1}$ of soil h^{-1} .

Phosphatase activity

This is a measurement of aryl phosphatase activity in soil and was done according to the protocol described in Tabatabai (1994). Briefly, 1 g of soil (± 0.01) was weighed into two dark centrifuge tubes labeled tube #1 and tube #2, representing treatment and control, respectively. 400 μL of tris maleate buffer with pH 7.0 was added to both tubes and swirled gently. This is because the enzymes responsible for phosphatase activity are sensitive to pH changes. The reaction was initiated by adding 1000 μL of p-nitrophenyl phosphate to tube #1, which serves as treatment tube. Both tubes were then placed on an aluminum rack orbit shaker (LAB-LINE, Melrose Park, IL) and allowed to shake for 1 hour at 175 rpm. After incubation, 1000 μL of p-nitrophenyl phosphate was immediately added to tube #2, which was the control tube. The reaction was terminated by adding 1000 μL of 0.5M calcium chloride and 4000 μL of 0.5M sodium hydroxide solution to both tubes. Both tubes were centrifuged at 4500 rpm at 4 °C for 10 minutes using Allegra™ X-22R Centrifuge (BECKMAN COULTER, Brea, CA). Sample aliquots were read with a spectrophotometer (EPOCH Microplate Reader, BioTek, Winooski, Vermont) at 400nm along with standards that ranged between 0 and 10 μM in concentration. A

standard curve was generated relating the spectrophotometric reading to the concentration of pNP, which was used to convert the sample's spectrophotometric readings to the corresponding pNP concentration. Phosphatase activity was expressed as $\mu\text{mol pNP g}^{-1}$ of soil h^{-1} .

Fluorescein diacetate hydrolysis assay (FDA)

The FDA was determined based on the protocol described in Adam & Duncan (2001). Briefly, 2 g of soil was weighed and placed in a 50 mL conical flask, followed by the addition of 15 mL of 60 mM potassium phosphate buffer at pH 7.6. Two microliters of a $1000 \mu\text{g mL}^{-1}$ solution of the substrate (fluorescein diacetate) were added to initiate the reaction, and control flasks did not contain the substrate. The content was shaken by hand briefly and then placed in the incubator for 30 minutes, with the temperature set to 30°C . To stop the reaction, 15 mL of chloroform/methanol (2:1) mixture was added immediately after the samples were removed from the incubator. The content was centrifuged at 2000 rpm for 3 min with Allegra™ X-22R Centrifuge. The supernatant of each sample was then filtered (Whatman™, 42Ashless Circles 150mm) into 50 ml conical flasks, and its absorbance was measured with an EPOCH microplate spectrophotometer at 490 nm. A standard curve was generated relating the spectrophotometric reading to the fluorescein concentration and was used to convert sample spectrophotometric readings to concentration. Results were expressed as $\mu\text{g fluorescein g}^{-1}$ of soil h^{-1} .

β -glucosidase activity

β -glucosidase activity was determined according to the protocol described in Deng & Popova (2015). One gram of soil was weighed and transferred to a 50 mL Erlenmeyer flask, one for the treatment set-up and one for the control. Then, 200 μL of toluene was added to each flask, mixed quickly, and allowed to sit for 15 minutes. Following that, 1000 μL of the substrate (p-nitrophenyl glucoside) and 4000 μL of the buffer (MUB, pH 6.0) were added to the treatment

flask, gently stirred, and the soil suspension was placed in an incubator set at 37 °C for 1 hour. Immediately after incubation, 1000 µL of 0.5 M calcium chloride and 4000 µL of 0.1 M Tris(hydroxymethyl)aminomethane (THAM) buffer (pH 12) were added to the treatment tube. The same procedure was followed for the control flask, except that the substrate was added at the end of the incubation time. The contents were mixed, and the soil suspension was filtered using Whatman No. 2 V-folded filter paper. Its absorbance was measured with an EPOCH microplate spectrophotometer at 405 nm. A standard curve was generated relating the spectrophotometric reading to the *p*-nitrophenol concentration released and was used to convert sample spectrophotometric readings to concentrations. The enzyme activity is expressed as µmol pNP kg⁻¹ of soil h⁻¹.

Statistical Analysis

The data were subjected to a one-way analysis of variance at each sampling time and depth at 15 cm with a confidence level of 95% using the R statistical software (v4.3.3. 2024, R. Core Team, Vienna, Austria) to assess the significance of the effect of soil health management practices on microbial activity and selected soil nutrient within each year and growth stage. Post-hoc analysis for mean comparison among the treatments was performed using Tukey's Honestly Significant Difference (HSD) test. Also, the significance of the differences across treatments, year, and growth stages (interaction effect) was analyzed by checking the interaction effect by two-way ANOVA and the post-hoc analysis for mean comparison with Tukey's HSD at the 0.05 significance level to evaluate whether treatment effects differed by year. Pearson correlation analysis was performed on the soil microbial activities measured and selected soil nutrient levels to demonstrate how the activities of these enzymes may be related and the strength of their connection.

Results

Soil pH and nutrient levels

Soil pH across all treatments ranged from 6.17 to 6.81 (Table 3.4). No significant differences in pH were detected among treatments within the same year. Because “year” in our study is effectively a pseudo-replicate (i.e., not a fully crossed, well-replicated factor), when we compared pH across years rather than to an all way, the RCA and RCAB treatments exhibited significantly higher mean pH in 2024 compared to 2023 ($p = 0.01$, $p = 0.05$, respectively). All other treatments did not differ significantly across 2023 and 2024.

Soil Ca was significantly affected by treatment in 2023, with the CT plots exhibiting higher Ca concentrations than RCAB and RT ($p = 0.01$; Table 3.4). In 2024, no differences in Ca among treatments were detected. However, the treatment $\times$ year interaction was highly significant ($p = 0.001$), driven by increases in the RC and RCAB treatments over time, and most pronounced in RCA, where Ca levels in 2024 exceeded those in 2023 (Table 3.4). There were no significant differences in K concentrations in 2023; however, an increase was noted for treatment RCA in 2024, being significantly higher ($p=0.001$) than the other treatments. Considering the treatment and year interaction, treatments RCA and RCAB were significantly higher in 2024 than in 2023, with treatment RCA exhibiting the highest significance ($p=0.001$). There were no significant differences in Mg concentrations among treatments during the 2023 corn growing season, while only RCA was significantly higher within 2024 season (Table 3.4). When compared across years, both RCA and RCAB exhibited significantly higher Mg concentration in 2024 when compared to 2023 (Table 3.4).

During the 2023 growing season, soil-available P did not differ significantly among the treatments (Table 3.4). In 2024, however, the RCA treatment produced a significantly higher

concentration of available P than CT, RT, RC, and RCAB ($p = 0.01$). When data were analyzed across both years (treatment $\times$ year interaction), both RCA and RCAB plots maintained significantly elevated soil-available P relative to the remaining treatments ($p = 0.05$), with RCA exhibiting the most significant level of significance ($p = 0.01$) (Table 3.4).

No significant treatment effects on manganese (Mn) or zinc (Zn) concentrations were observed among treatments in 2023 (Table 3.4). In 2024, however, the RCA treatment yielded significantly higher Mn ($p = 0.01$) and Zn ($p = 0.05$) concentrations than all other treatments. Furthermore, the treatment $\times$ year interaction revealed that RCA maintained significantly elevated Mn levels across both years ($p = 0.01$). At the same time, both RCA and RCAB exhibited significant increases in Zn over the two growing seasons ($p = 0.05$), with RCA displaying the most significant increase.

There were no significant differences in lime buffering capacity (LBC) or its equivalent (LBCeq) observed among treatments during either the 2023 or 2024 growing seasons (Table 4). However, the treatment $\times$ year interaction was significant for the reduced tillage (RT) plots ($p = 0.05$), indicating a temporal shift in LBC and LBCeq values for RT between the two seasons.

Microbial Activities

For the 2023 corn growing season, a significant effect of treatment on soil respiration was observed at the V7/V8 growth stage, where RCAB treatment achieved the highest rate of 48.03 mg CO₂ kg⁻¹ soil hr⁻¹ ($p = 0.01$). In contrast, no significant differences among treatments were detected at either the VTR1 or R6 stages (Table 3.5). Moreover, when respiration rates were compared across all growth stages, RCAB at V7/V8 remained significantly greater ($p = 0.001$) than the other stage measured (Table 3.5). Urease activity did not differ significantly among the various treatments in 2023 (Table 3.5). However, by comparing treatments across growth stages,

urease activity during the R6 growth stage was significantly higher than all other growth stages for all the treatments (Table 3.5). Phosphatase activity ranged from 0.04 to 4.38 $\mu\text{mol pNP g}^{-1}$ soil h^{-1} . The RCAB treatment achieved the highest activity at the R6 stage, significantly outperforming all other treatments both within and across growth stages ($p = 0.001$). β -glucosidase activity did not differ significantly among treatments at any growth stage, except at V7/V8, where the RC and CT treatments exhibited significantly elevated activity ($p = 0.001$) in 2023. When activities were compared across all growth stages, the RCAB treatment at the R6 stage recorded a significantly higher rate than at the V7/V8 and VTR1 growing stages (Table 3.5). In 2023, FDA activity did not differ significantly among treatments at the VT/R1 growth stage. However, the RT treatment measured at the V7/V8 growth stage was significantly higher than all treatments, followed by the RCAB treatment at the R6 growth stage. Across growth stages, only the treatment RT at V7/V8 and the RCAB at R6 maintained significantly higher FDA hydrolysis than other treatment–stage combinations (Table 3.5).

For the 2024 corn growing season, no significant effect among treatments on soil respiration was observed at preseason and VTR1 (Table 3.6). However, the RCA treatment measured at the V6/V7 and R6 growth stages was significantly higher than all other treatments. When compared across stages, treatments RCA and RCAB were significantly higher than the other treatments.

For urease, there was no significant difference among the treatments for V6/V7 and VTR1 growth stages (Table 3.6). However, there was treatments effect measured at the R6 and Preseason growth stages with the RCA treatment significantly higher than all other treatments followed by the RCAB treatment. Phosphatase activity exhibited no significant difference among the treatments within each growth stage (Table 3.6). However, across the growth stages,

RT at R6 was significantly higher than at the other growth stages (Table 3.6). For β -glucosidase, treatments RCAB and RCA were significantly higher than all other treatments at R6 growth stage; the same was true for RCAB at the V6/V7 and RCA at the VT/R1. For pre-season samples, no significant treatment effects were observed (Table 3.6). Treatment RCAB, RCA, and RC were significantly higher than RT and CT when compared across the growth stage. There was no treatment effect on FDA hydrolysis at the VT/R1 stage. However, CT at the pre-season stage, RCA at the V6/V7, and RC were significantly higher than all other treatments when compared within and across sampling times.

Correlation analysis of soil microbial activities, pH, and selected soil nutrient levels

Throughout the 2023 corn growing season, soil β -glucosidase activity demonstrated consistently positive associations with base cations and available phosphorus. Specifically, β -glucosidase correlated positively with Ca, and Mn concentrations ($r=0.93$, $p < 0.05$ for Ca, Mn $r=0.90$ $p < 0.05$), as well as with available P ($r=0.93$, $p < 0.05$). In contrast, FDA hydrolysis exhibited negative correlations with soil pH, Ca, and Mg ($r=-0.89$ to -0.95 , $p < 0.05$). Soil pH also correlated with Ca and Mg concentrations ($r=0.95$ for Ca, $r=0.93$, $p < 0.05$), while others showed no significant correlation (Figure 3A). In 2024, soil respiration exhibited significant positive correlations with macro and micronutrients (Figure 3B). Notably, soil respiration correlated positively with K, Mn, Zn, and P, with correlation coefficients ranging from 0.97 to 0.99 at ($p < 0.01$) (Figure 3B). Similarly, soil respiration was positively correlated with Mg ($r = 0.94$, $p > 0.05$) and Ca ($r = 0.95$, $p > 0.05$).

Temporal Patterns of rainfall, temperature, and soil moisture

The daily weather data for the 2023 growing season showed fluctuating rainfall and relatively consistent air temperatures ranging from 25°C to 35°C. Significant precipitation peaks

occurred around 15, 60, and 95 days after planting, resulting in increased soil moisture. Average soil moisture content was approximately 0.18–0.20 g g⁻¹ following these events, indicating the drained upper limit (field capacity) at the study site. Between rainfall and soil moisture declined gradually (Figure 1A). Throughout the growing season 2024, precipitation was uneven with four intense rainfall events at approximately 10, 30, 50, 80, and 95 days after planting (DAP), each delivering up to 80 mm in a single day. while daily air temperatures remained uniformly warm 20°C to 30°C, sustaining high atmospheric evaporative demand. Based on average volumetric soil moisture readings collected in situ with field sensors, soil moisture increased rapidly within 1 to 2 days of each major storm, rising from baseline levels near 0.14 g g⁻¹ to early-season peaks of 0.18 g g⁻¹ and, following late-season precipitation, to 0.24 g g⁻¹.

Discussion

Soil pH and nutrients

Despite the absence of significant change in soil pH among treatments in each year, there was a significant increase in soil pH in RCA and RCAB over time (2023 vs 2024). The long-term application of poultry litter has been shown to increase soil pH (He et al., 2008; Mitchell & Tu, 2006). The absence of a significant treatment effect likely reflects that the management practices were only in their third and fourth year of implementation, so broader differences may not yet have had to develop. Nevertheless, the observed increase in soil pH under the RCA and RCAB treatments between years three and four indicates that these amendments are beginning to alter the soil chemistry over time, suggesting that more pronounced treatment effects may emerge with continued application. This is beneficial for Georgia soils, which are often acidic. It should be noted that the plots were limed before the 2023 season and again prior to the 2024 season to increase the pH to an optimal range for corn growth; this might have masked the

treatment effect. Similarly, the amendments RCA and RCAB particularly RCA, which received twice the poultry litter rate of RCAB significantly increased the base cations potassium (K) and magnesium (Mg), as well as the micronutrients zinc (Zn), manganese (Mn), and phosphorus (P), compared to the conventional treatment (Table 3.5). This makes sense, since poultry litter is a rich source of these nutrients (Antonangelo et al., 2024; Agbede, 2021). The increase in P following poultry litter application is agronomically beneficial for crop nutrition. However, if poultry litter is applied at very high rates or repeatedly over multiple years such that soil P moves into the high or excessive category Woodruff et al., (2019), there is an increased risk of P loss through surface runoff and subsequent eutrophication of adjacent water bodies (Sauer et al., 1999). Combining poultry litter and biochar can help reduce the amount of poultry litter that needs to be applied to meet N and P requirements, while minimizing soil P accumulation over time and obtaining the benefits of the organic amendments.

Microbial activities

Soil respiration is a key indicator of microbial activity, reflecting microbial biomass and organic matter decomposition rates. Soil respiration was significantly improved by treatment RCAB at the V7/V8 growth stage in 2023, RCA at V6/V7 and R6, suggesting that combining these management practices creates an optimal environment for microbial activity from moderate organic inputs. Deng et al. (2017), also reported that adding organic amendments (poultry litter and biochar) releases mineralizable organic C and helps improve soil structure, thereby stimulating heterotrophic microbes and CO₂ fluxes also linked to the drying and re-wetting of soil. The V7/V8 stage fuels microbial respiration by exuding sugars and organic acids as the corn enters a rapid growth period (De Vries et al., 2019). Non-significant differences could be attributed to the continuous plant growth, potentially reducing the availability of labile carbon

sources for soil microorganisms (Nyamwange et al., 2021). At each sampling date in 2024, measured soil moisture exceeded field capacity. These near-saturated conditions at the time of sampling are likely to limit soil aeration and microbial activity, thereby slowing the mineralization and release of nutrients from poultry litter and cover crops. There were no significant differences in urease activity among treatments in 2023, but a significant treatment effect on urease activity was observed among the treatments in 2024, with higher activities in RCA and RCAB at the pre-season and R6. Poultry litter is a source of organic compounds such as uric acid that hydrolyzes to urea, which is the substrate for the urease enzyme (Bolan et al., 2010). As such, the increase in urease activity because of these two amendments is not surprising. The difference in enzyme activities associated with the sampling time might mainly be associated with changes in microbial turnover and nutrient cycling as root biomass goes through the growth stages that regulate the release of organic substrates. It is also partly associated with the rate of decomposition of the organic amendments. The RCAB treatment, for example, exhibited lower urease activity at early stages in 2024, potentially due to the slow release of N from biochar. Lehmann et al. (2011) also reported that biochar has a high C: N ratio and strong adsorption capacity, which can temporarily immobilize nitrogen, limiting substrate access for urease-producing microbes. In 2024, rainfall at ~80 and 95 DAP led to an increase in soil moisture ($\sim 0.24 \text{ g g}^{-1}$), which might not favor the microorganisms involved in N cycling. Such moisture corresponds to over 70 % water-filled pore space, creating anaerobic microsites and severely restricting oxygen diffusion as such, limited oxygen availability at these moisture levels inhibits aerobic nitrifiers and other oxygen-dependent microbes in N-cycling (Tobert et al., 1992). Conversely, early-season dryness ($\sim 0.14 \text{ g g}^{-1}$) may have limited enzyme activity in some treatments, including RCAB. The timing mismatch between N availability and moisture may

explain the lower urease activity at early stages in RCAB, even under favorable temperatures (20–30 °C).

Phosphatase activity is essential for P cycling (Duhamel, 2024), facilitating the breakdown of organic P compounds into plant-available forms (Ibrahim et al., 2022). Despite poultry litter being a significant source of P, we did not see a consistent increase in phosphatase activity either over the two growing seasons or in any of the sampling times associated with RCA that supplied the largest amount of poultry litter. The only significant increase was associated with RCAB in R6 stage only in 2023. If the increase was associated with the poultry litter and/or biochar, we would expect to see it in 2024 as well. The lack of a consistent impact from poultry litter is not clear but might have do with large variations among replications from hot spots associated with uneven distribution of the organic amendments (Korsaeth et al., 2001). This could be particularly impactful as phosphatase activity is less sensitive to management practices as compared to the other enzyme activities (Diera et al., 2020; Gan & Wickings, 2017), and any small changes that might have been brought about by the treatments could have been masked by the large variability in the plots as a result of the organic amendments.

β -glucosidase is an enzyme associated with carbon cycling, particularly the breakdown of cellulose into simple sugars. The treatments that resulted in higher activity were either those receiving cover crop residue in 2023 (RC) or those that received a combination of cover crop, poultry litter and biochar (e.g., RCAB), indicating the carbon input from these amendments was driving β -glucosidase activity. Stronger impact of the amendments was observed in 2024, indicating the cumulative impact of the treatments over time and their integrative effect. Soils incorporated with cover crops and a low C-to-N ratio benefit the catalytic function of β -glucosidase, leading to rapid degradation of organic matter and nutrient availability (Adetunji et

al., 2017). Furthermore, after cover crop termination, there is an increase in biomass levels, which impacts the metabolism of microbial communities (Bowles et al., 2014; Piotrowska & Wilczewski, 2012; Sinsabaugh et al., 2005).

FDA is similar to soil respiration as being an indicator of the overall microbial activity. The RT treatment at V7/V8 and RCA at R6 resulted in peak enzyme activity in 2023. In 2024, enzyme activity was significantly higher under CT at pre-season, likely from tillage-induced aeration and under RCA at V6/V7 and RC at R6 growth stage, driven by the steady supply of labile carbon and nutrients from cover crops and poultry litter (Pokhrel et al., 2021; Lupwayi et al., 2012).

Overall, the influence of the treatments that received poultry litter and/or biochar increased over time, suggesting a cumulative impact of the amendments. It is interesting to note that the reduced tillage treatment by itself was not as impactful against the conventional tillage as when it was combined with poultry litter and/or biochar, indicating the positive impact of integrating soil health management practices to improve microbial activity. It is well documented that reduced tillage provides many benefits, but it takes several years for the benefits to be realized (Triplett & Dick, 2008). One of the most important benefits of integrating soil health practices could be to speed up the realization of the benefits associated with soil health practices.

Correlation Analysis

β -glucosidase correlates positively with Ca, Mn, and P, highlighting their roles facilitating microbial C cycling. β -glucosidase, a cellulolytic enzyme, often requires Ca^{2+} and Mg^{2+} . Also, available P alleviates P limitations on microbial biomass, allowing microbes to allocate resources to C-degrading enzymes (Muraleedharan et al., 2013; Trap et al., 2024).

Summary and Conclusions

The impact of soil health management practices on microbial activities and soil nutrient levels varied. The soil pH remained between 6.17 and 6.81, indicating that the management practices did not significantly affect pH. The soil pH buffering capacity showed no significant treatment effects either over the two-year period. Treatments RCA and RCAB both enhanced base cations and P compared to conventional tillage, resulting mainly from poultry litter addition. The increased soil respiration rates indicated improvement in soil biological health in 2024 due to the integrated soil health management practices. Urease activity was not affected by treatment in 2023, but there were treatment and sampling time effects driven by RC and RCAB at the R6 growth stage, indicating a cumulative effect of the integrated soil health practices. β -glucosidase activity peaked in RCA and RCAB while phosphatase activity did not reflect significant change overall. The correlation analyses indicated strong positive associations between base cations and β -glucosidase, as well as between macronutrients (K^+ , P) and micronutrients (Mn, Zn) and soil respiration. Overall, the integrated soil health management approach resulted in improved microbial activity as a result of nutrient and organic matter input from poultry litter, cover crop and biochar. The changes were gradual and more pronounced in treatments with poultry litter application because of the more labile nature of nutrients and organic materials than in freshly incorporated cover crop or a more stable organic input in biochar.

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Table 3.1: Inorganic fertilizer sources and application rates used for corn during 2023 and 2024 growing seasons.

Fertilizer/Nutrients Applied	Rate (kg ha ⁻¹)	Nutrient sources
CT, RT, and RC		
N	280	Urea, ammonium nitrate solution, diammonium phosphate, ammonium sulfate
P	135	Diammonium phosphate
K	168	Potassium chloride
S	11	Ammonium sulfate
RCA		
N	165 + 116	Urea/inorganic sources + poultry litter
P	150	Poultry litter
K	173	Poultry litter
S	51	Poultry litter
RCAB		
N	219 + 61	Urea/inorganic sources + organic sources (poultry litter and biochar)
P	53 + 82	Diammonium phosphate + organic (poultry litter and biochar)
K	89 + 84	Potassium chloride + organic (poultry litter and biochar)
S	26	Organic (poultry litter and biochar)

CT: Conventional tillage; RT: Reduced tillage; RC: Reduced tillage with cover crop; RCA: Reduced tillage with cover crop and poultry litter; RCAB: Reduced tillage with cover crop, poultry litter, and biochar.

Table 3.2: Yearly estimated nutrient amounts available to corn from poultry litter in RCA (4.48 Mg ha⁻¹) and from poultry litter (2.24 Mg ha⁻¹) and biochar (2.24 Mg ha⁻¹) in RCAB

Treatment	N	P	K	Mg	Ca	S	B	Zn	Mn	Fe	Cu
	(kg ha ⁻¹)										
	Poultry litter										
RCA	116	150	173	32.4	121	50.8	0.44	1.56	1.12	8.74	1.56
RCAB	57.9	74.8	86.7	16.2	60.6	25.4	0.22	0.78	0.56	4.37	0.78
	Biochar										
RCAB	3.2	7.3	7.5	5.27	41.0	0.44	0.22	0.22	0.45	4.1	0.22

RCA (Reduced Tillage with Cover Crop and with Poultry Litter) and RCAB (Reduced Tillage with Cover Crop and with Poultry Litter and Biochar). RCA received 4.48 Mg ha⁻¹ of poultry litter, and RCAB received 2.24 Mg ha⁻¹ poultry litter and 2.24 Mg ha⁻¹ of biochar.

Table 3.3. Sampling times correlating to vital corn growth stages

Sampling stage	Description
Pre-season	Samples were collected to assess plot variability and management of soil nutrients and microbial activity.
V6	Exhibit 6 fully developed collars and is affected by stress levels.
VT	The ultimate branch is now evident. Pollination takes place here.
R6 (Harvest)	The plant has attained its physiological stage, and the base has developed with a black coating. 15.5 % of it is moisture
Preseason (before planting), V6-V7 (6-7 leaf stage), VT-R1 (tasseling-silking), and R6 (physiological maturity).	

Table 3.4. Soil pH and selected nutrients monitored under RC, RCA, RCAB, RT and CT for corn during 2023 and 2024 growing seasons.

	Treatment	2023	2024	Nutrient	Treatment	2023	2024
pH	CT	6.64aA	6.55aA	P (kg ha ⁻¹)	CT	56.70aA	62.93bA
	RCA	6.49aB	6.81aA		RCA	52.98aB	117.8aA
	RC	6.47aA	6.35aA		RC	62.68aA	60.58bA
	RCAB	6.17aB	6.67aA		RCAB	53.75aB	81.3bA
	RT	6.17aA	6.38aA		RT	49.30aA	53.83bA
Ca (mg kg ⁻¹)	CT	440.5aA	476aA	Zn (kg ha ⁻¹)	CT	3.86aA	4.35bA
	RCA	427.8abA	474.5aA		RCA	4.18aB	8.15aA
	RC	392abB	678.75aA		RC	4.60aA	4.45bA
	RCAB	348bB	564aA		RCAB	3.97aB	5.57abA
	RT	344bA	443.75aA		RT	3.54aA	4.21bA
K (mg kg ⁻¹)	CT	25.25aA	35.07bA	Mn (mg kg ⁻¹)	CT	6.06aA	6.13bA
	RCA	33.75aB	80.58aA		RCA	5.60aB	8.78aA
	RC	23aA	32.78bA		RC	5.93aA	5.95bA
	RCAB	19aB	40.65bA		RCAB	5.76aA	6.67abA
	RT	22.50aA	33.8bA		RT	5.71aA	5.61bA
Mg (mg kg ⁻¹)	CT	78.50aA	71.63bA	LBCeq (mg CaCO ₃ kg ⁻¹ soil pH ⁻¹)	CT	254.00aA	252.33aA
	RCA	74.75aB	110.40aA		RCA	246.00aA	294.50aA
	RC	79.75aA	66.75bA		RC	224.00aA	228.50aA
	RCAB	46.75aB	85.90abA		RCAB	243.25aA	265.00aA
	RT	60.25aA	64.23bA		RT	207.50aB	285.50aA

Calcium; Ca, Potassium; K, Magnesium; Mg, Manganese; Mn, Phosphorus; P, Zinc; Zn, Lime Buffering Capacity Equivalent; LBCeq.

Different lowercase letters within a column indicate significant differences within the year for the treatments using Tukey's HSD test, $P \leq 0.05$.

Different uppercase letters within a row indicate significant differences across both years for the treatments, as determined by Tukey's HSD test, $P \leq 0.05$.

Table 3.5: Soil microbial activities under different soil health management practices (RC, RCA, RCAB, RT and CT) for corn during the 2023 growing season.

Microbial Activities	Treatments	V7/V8	VT/R1	R6
Soil Respiration (mg CO ₂ kg ⁻¹ soil hr ⁻¹)	CT	35.33abcA	16.08aB	17aB
	RT	38.21abA	15.72aB	14.95aB
	RC	29.26bcA	22.17aAB	16.6aB
	RCA	22.05cA	19.11aA	17.44aA
	RCAB	48.03aA	21.61aB	18.36aB
Urease (μmol NH ₃ g ⁻¹ soil hr ⁻¹)	CT	1.57aC	6.49aB	16.00aA
	RT	1.11aC	6.73aB	16.33aA
	RC	1.60aB	6.78aB	18.14aA
	RCA	1.13aB	6.80aB	16.59aA
	RCAB	1.13aC	8.46aB	17.21aA
Phosphatase (μmol pNP g ⁻¹ soil h ⁻¹)	CT	0.23aA	0.04aB	0.04bB
	RT	0.21aA	0.94aA	0.05bA
	RC	0.19aA	1.52aA	0.81bA
	RCA	0.19aA	0.14aA	0.08bA
	RCAB	0.25aB	1.11aB	4.38aA
β-glucosidase (μmol pNP kg ⁻¹ soil h ⁻¹)	CT	13.59aA	11.92aA	10.90aA
	RT	6.94bA	7.62aA	8.90aA
	RC	13.94aA	11.58aA	13.05aA
	RCA	7.47bA	9.01aA	9.04aA
	RCAB	3.92bB	8.24aA	10.80aA
FDA hydrolysis (μg flu g ⁻¹ soil)	CT	21.08abA	12.78aA	18.14bA
	RT	35.88aA	14.23aB	19.52bB
	RC	12.2bB	18.72aAB	21.80abA
	RCA	12.5bB	16.69aAB	21.86abA
	RCAB	29.00abA	14.54aA	26.86aA

FDA, Fluorescein Diacetate Hydrolysis; RC, Reduced tillage with Cover Crop; RCA, Reduced tillage with cover crop and with poultry litter; RCAB, Reduced tillage with Cover Crop and with poultry litter and biochar; RT, Reduced tillage; CT, Conventional tillage.

Different lowercase letters within a column indicate significant differences within growth stages for the treatment using Tukey's HSD test, $P \leq 0.05$.

Different upper-case letters within a row indicate significant differences across growth stages for the treatments using Tukey's HSD test, $P \leq 0.05$.

Table 3.6. Soil Microbial Activities Under Different Soil Health Management Practices (RC, RCA, RCAB, RT and CT) for Corn 2024 Growing Season.

Microbial Activities	Treatments	Preseason	V6V7	VTR1	R6
Soil Respiration (mg/kgCO ₂ -C/g soil/ hr)	CT	47.33aA	26.13bB	22.97aB	29.57abB
	RT	42.77aA	26.21bAB	34.21aAB	28.29bB
	RC	46.61aA	32.65bAB	22.21aB	33.07abAB
	RCA	61.28aA	57.93aAB	35.47aB	40.76aAB
	RCAB	52.76aA	39.81abAB	28.39aB	27.26bB
Urease (μmol NH ₃ g ⁻¹ soil hr ⁻¹)	CT	7.30bC	24.70aB	35.74aA	15.82cB
	RT	15.30bC	28.09aB	40.20aA	27.36abB
	RC	15.75abB	25.18aAB	31.87aA	26.16abA
	RCA	15.77abC	26.59aB	37.30aA	33.48aA
	RCAB	16.27aB	24.08aA	31.52aA	24.82bA
Phosphatase (μmol pNP g ⁻¹ soil h ⁻¹)	CT	0.31aA	0.37aA	0.30aA	0.42aA
	RT	0.32aAB	0.26aB	0.29aB	0.43aA
	RC	0.32aB	0.28aB	0.32aAB	0.43aA
	RCA	0.35aA	0.32aA	0.36aA	0.41aA
	RCAB	0.30aAB	0.25aB	0.31aAB	0.36aA
β-glucosidase (μmol pNP kg ⁻¹ soil h ⁻¹)	CT	27.08aB	28.09bAB	14.89cC	39.17cA
	RT	24.40aBC	22.05bC	31.08abB	62.65bA
	RC	26.80aB	38.01abB	21.47bcC	64.28bA
	RCA	26.76aC	26.57bC	42.59aB	92.36aA
	RCAB	24.31aC	54.17aB	38.10aC	99.28aA
FDA (μg flu g ⁻¹ soil h ⁻¹)	CT	63.96aA	53.85abAB	41.01aB	15.43bC
	RT	47.48bcA	34.20cA	28.40aA	17.71abA
	RC	37.79cA	34.63cA	34.67aA	25.63aA
	RCA	53.76abA	65.08aA	36.19aB	25.33aB
	RCAB	54.92abA	39.77bcB	27.43aC	15.66bD

FDA, Fluorescein Diacetate Hydrolysis; RC, Reduced tillage with Cover Crop; RCA, Reduced tillage with cover crop and with poultry litter; RCAB, Reduced tillage with Cover Crop and with poultry litter and biochar; RT, Reduced tillage; CT, Conventional tillage.

Different lowercase letters within a column indicate significant differences within growth stages for the treatment using Tukey's HSD test, $P \leq 0.05$.

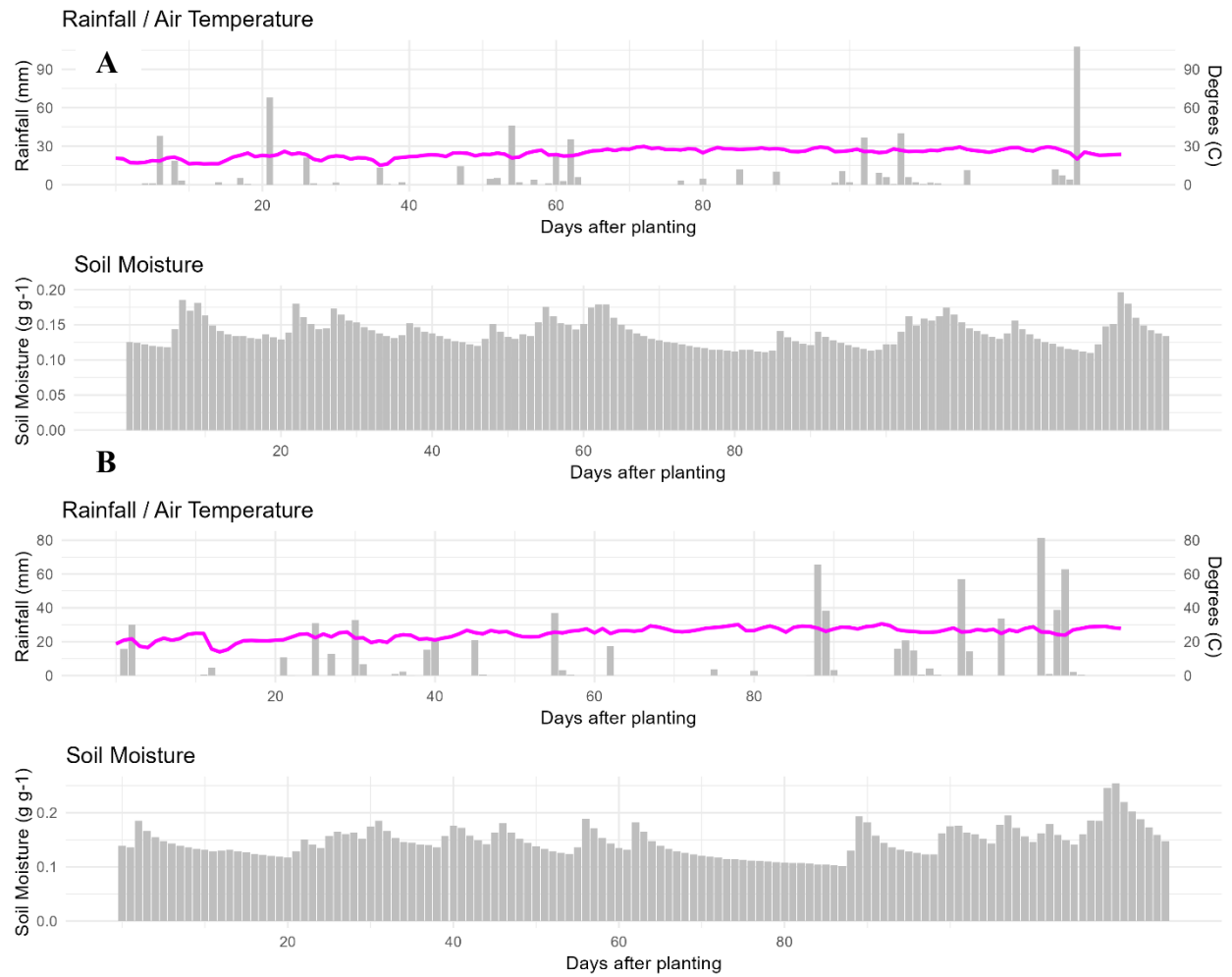


Figure 3.1. Daily patterns of rainfall(mm), temperature(°C), and soil moisture content (g g⁻¹) observed in 2023 (A), and 2024 (B) during the corn Production seasons.

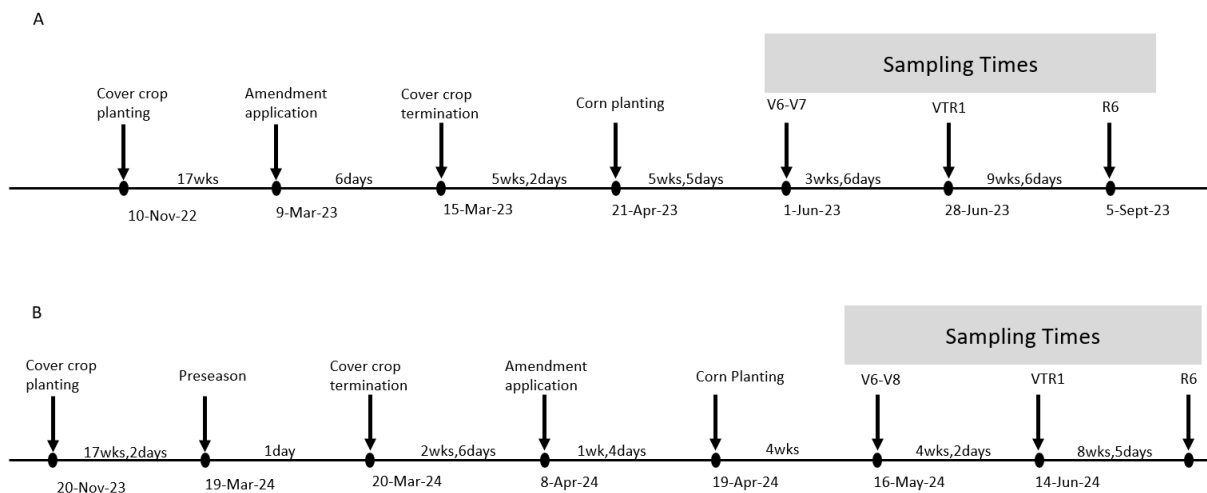


Figure 3.2. Chronological Field Operations and Sampling Times of the Study in 2023 (A), and 2024 (B): 2024 Growing season.

Correlation Plots

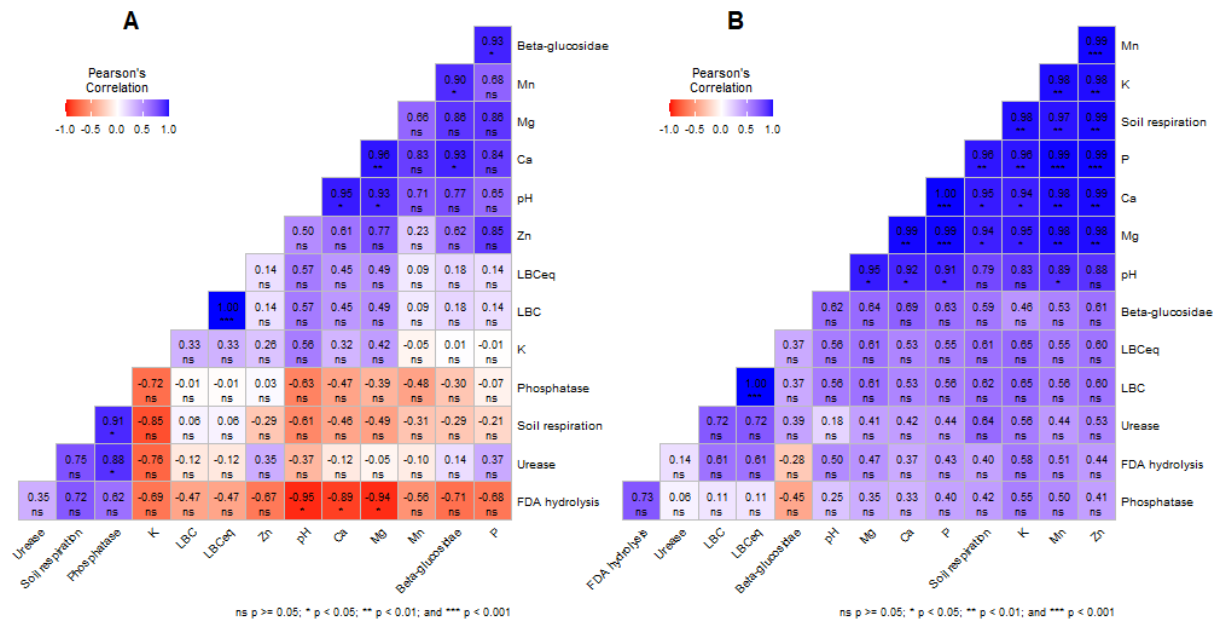


Figure 3.3: Correlation matrix of variables, including soil respiration, enzyme activities, pH, and nutrient concentration in 2023 and 2024 growing seasons.

CHAPTER 4

THE IMPACT OF INTEGRATED SOIL HEALTH MANAGEMENT PRACTICES ON
MICROBIAL ABUNDANCE AND COMPOSITION²

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Abstract

While individual soil health management practices are advocated for improving soil health, their impact on soil microorganisms when integrated is not well understood. This study evaluated the impacts of reduced tillage (RT) and combinations of RT with cover crop (RTC), RTC and poultry litter (RTCA) and RTCA and biochar (RTCAB) against a conventional tillage (CT) on soil microbial abundance and composition in a corn system. Field studies were conducted at the University of Georgia Southeast Georgia Research and Education Center in Midville, GA, over two growing seasons. Microbial composition was characterized by sequencing the 16S rRNA gene while absolute microbial abundance was determined for ammonia-oxidizing archaea (AOA) and bacteria (AOB) with digital and quantitative polymerase chain reactions. In 2023, no treatment had significant impact on the relative abundance at phyla level. During the growing season of 2024, Acidobacteriota was the only bacterial phylum whose abundance was significantly impacted by RTCA that had significantly higher relative abundance than RTC. Alpha diversity showed an increasing trend over time under the integrated soil health practices, while beta diversity revealed distinct microbial communities between RTCAB vs the rest, indicating the effect of integrating multiple practices. AOB abundance peaked under CT at the R6 growth stage in 2024, and AOB were dominant in all the treatments. Overall, the study indicated that there is a clear trend towards a shift in soil microorganisms in response to integrating the soil health management practices as opposed to RT or CT, but the changes were subtle.

Introduction

Microorganisms play a major role in nutrient cycling and ecosystem stability and are responsive to agricultural land use management (Mendes et al., 2015; Ryan & Adley, 2010). Thus, both quantitative and compositional alterations can act as essential and responsive markers for monitoring the short and long-term modifications in soil health (Hill et al., 2000). Agricultural land management practices profoundly alter the physical, chemical, and biological features of soil. These alterations affect microbial abundance and composition and their role in ecosystem functioning. (Jangid et al., 2008; Pampulha & Oliveira, 2006). Soils disturbed by intensive agricultural practices such as tillage may exhibit increased vulnerability to declines in ecosystem functions mediated by soil microorganisms because of mechanical disturbance, soil compaction, and obstruction of access to nutritional supplies (Giller, 1996).

Adoption of soil health management practices can help alleviate some of the problems associated with intensive agricultural practices. Soil health management practices such as reduced tillage, cover cropping, and the use of poultry litter and biochar as organic amendments have the potential to enhance microbial activity and diversity, therefore improving soil health (Enebe & Babalola, 2020). Research has shown that reduced tillage increases the soil's fungal to bacterial ratio, which is an indicator of the capacity of microbial community to degrade, assimilate and store carbon (van Groenigen et al., 2010; Bailey et al., 2002). The use of cover crops improved microbial abundance and diversity and nutrient availability in the soil (Schmidt et al., 2018). Similarly, the use of biochar and poultry litter was also reported to have a positive impact on the soil microbial community (Ajayi et al., 2025). Furthermore, adding organic amendments mitigates nutrient losses by strengthening soil structure and improving water retention capacity (Zhang et al., 2023). However, the individual use of these soil health

management practices has its own drawbacks. For instance, continuous use of poultry litter can lead to accumulation of some nutrients and metals (Dalólio et al., 2017). Cover crops can compete for available soil moisture, which may adversely affect production of the main crop (Tribouillois et al., 2018). Reduced tillage and frequent use of biochar can also cause soil compaction (Ravindra et al., 2023; Soane et al., 2012). In this study, we are testing the hypothesis that integrating these practices might reduce their drawbacks and amplify their positive impact on soil health.

Among soil microbial communities that are often targeted as indicators of biological soil health are ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA). They play a very important role in regulating the availability and mobility of nitrogen in soil as they mediate the first and rate limiting step of nitrification (Prosser & Nicol, 2008, 2012; Norton & Ouyang, 2019). The two groups respond differently to different nitrogen sources and management practices. As such, they can be excellent markers for detecting changes associated with various agricultural management practices (Habteselassie et al., 2013; Mundepi et al., 2019; Ouyang et al., 2016).

The objective of this study was to examine the impact of individual and integrated soil health management practices on microbial abundance and composition in a field study in a corn system at the University of Georgia Southeast Georgia Research and Education Centre in Midville, GA.

Materials and Methods

Study site and experimental set-up

The study evaluated the impact of five soil health management practices, two baseline treatment that included conventional Tillage (CT), reduced tillage (RT) and three integrated

practices combining reduced tillage with cover crop (RC), reduced tillage with cover crop and poultry litters (RCA), reduced tillage with cover crop, poultry litter and biochar (RCAB) in a field study at the University of Georgia Southeast Georgia Research and Education Centre in Midville, GA (32° 52'46.44N, 82° 12'33.86W) from 2023 -2024. The poultry litter used in the experiment was sourced from Powell Poultry Farms, LLC, in Omega, GA, while the biochar was obtained from Wakefield BioChar, located in Valdosta, GA. The biochar was produced through pyrolysis at a temperature of 600 °C and had an alkaline pH of 10.7. The amendments were broadcast uniformly across the designated plots on the surface prior to tillage operations.

Temperature, rainfall, and soil moisture data were obtained from the nearby Georgia Automated Weather Monitoring Network (Georgia AEMN, 2023, 2024) and are shown in Figures 3.1A and 3.1B. Total rainfall amounts for the 2023 and 2024 corn-growing seasons were 82 mm and 90 mm, respectively. The mean average air temperature were 18.77 °C in 2023 and 22 °C in 2024. Initial soil pH was 6.39_{1:2(soil/water)}, 6.54_{1:2(soil/water)}, and lime buffering capacity (LBC) 235 and 266 mg CaCO₃ kg⁻¹ soil pH⁻¹ in 2023 and 2024, respectively. The study site's soil is a Dothan sandy loam (Fine-loamy, kaolinitic, thermic Plinthic Kandiudults). Texture analysis of the 0-15 cm layer was 93.26% sand, 6.50% clay, and 0.24% silt (Soil Survey Staff, 2022). Organic matter content was determined by using the loss on ignition method and averaged 0.86 % in the 0-15 cm depth.

The five treatments were organized in a randomized complete block design with four replications, and the size of each treatment plot was 7.3 by 9.1 m. The experimental field was laid out in three equal sections, allowing for the rotation of corn, peanut, and cotton over three years (2021-2023) under each management system. The crops were sequenced in the first section as corn (2021), peanut (2022), and cotton (2023). In the second section, peanuts were planted in

2021, cotton in 2022, and corn (planted each April) in 2023. For the third section, cotton was planted in 2021, corn in 2022, and peanuts in 2023. All plots received fertilizers at rates that meet the University of Georgia Agricultural and Environmental Services Laboratories (UGA-AESL) recommendations to achieve the desired corn yield of 15.7 Mg ha⁻¹, as summarized in Table 3.1. A combination of ammonium sulfate, diammonium sulfate, urea and potassium chloride were used to supply required amounts of N, P, K and S. Treatments CT, RT, and RC received the full nutrient rates of 280 kg N ha⁻¹, 135 kg ha⁻¹ P, 168 kg ha⁻¹ K, and 11 kg ha⁻¹ S from the above-mentioned fertilizers. The nutrient levels expected to be available from the poultry litter and biochar were considered before applying synthetic fertilizers for RCA and RCAB. Table 3.2 shows estimated levels of available nutrients from poultry litter and biochar based on the 2.24 Mg ha⁻¹ application rate, assuming 60% of the N and 80% of the other nutrients in poultry litter were available (Bryant et al., 2020). Thus, treatment RCA received only 165 kg N ha⁻¹. The remaining N and all the other nutrients came from poultry litter. Treatment RCAB received 219 kg N ha⁻¹, 53 kg ha⁻¹ P, 89 kg ha⁻¹ K and 26 kg ha⁻¹ S from synthetic sulfur fertilizers. The remaining amounts came from a combination of poultry litter and biochar. Nitrogen was applied before the planting of the corn, at the V3-V4 and liquid side dress was applied at the V6 stage to all treatments at 112 kg ha⁻¹ N with urea ammonium nitrate (34-0-0) at 14 days after planting (DAP).

The conventional tillage involves tilling and preparing the soil using a harrow and field cultivator. The reduced tillage is a one-time strip tillage, and cereal rye was used as a cover crop. The cereal rye was planted in the fall, using the John Deere 750 no-till drill with a seed drill at a rate of 56 kg ha⁻¹. The cover crop was planted in winter preceding the year before planting and were terminated by spraying glyphosate [isopropylamine salt of N-(phosphonomethyl) glycine]

in spring. A concise overview of the key field operations, including the dates for amendment applications, corn planting, and soil sampling, is shown in Figure 3.2. The strip tillage implement had a roller-crimper attachment that lays down the rye cover as mulch. Poultry litter was applied at 4.48 Mg ha^{-1} in the RCA treatments and 2.24 Mg ha^{-1} in RCAB treatment, respectively. The corn variety planted was different in 2021 and 2022; however, in 2023, the Corn variety planted was Croplan® Genetics 5678, at a rate of $79,074 \text{ seeds ha}^{-1}$.

Soil sampling and processing

Soil sampling was conducted throughout the study at four corn growth stages: Pre-season and the following corn growth stages: V6-V8, VT/R1 and R6 as indicated in Table 3.3. A composite of up to five cores of samples were collected from each plot from the top 15 cm. The pre-season soil samples were taken before planting. The V6-V8 samples were taken 4-6 weeks after seed emergence, with leaf collars determining the stages. VT/R1 was taken around 6-10 weeks after emergence, and R6 was 60 days after silking, determined by the average silking of the whole field. These soil samples were stored in a chest cooler with ice packs and transported to the University of Georgia campus (Griffin, GA) for analysis. Upon arrival at the laboratory, the soil samples were sieved through a 2mm sieve mesh (USDA standard) to remove debris and stones. A portion of each soil sample was stored at $-20 \text{ }^{\circ}\text{C}$ for DNA extraction and molecular analysis.

Extraction and Quantification of Microbial Genomic DNA

Following soil sampling and processing, the soils were removed from the freezer and put into the refrigerator 24 hrs prior to using, then allowed to thaw for 2 hours before analysis. Genomic DNA was extracted by weighing 0.25g of soil using the power beads for cell lysis according to the manufacturer's protocol and instructions (DNeasy ® PowerSoil ® Pro Kit) with a total eluted volume of $100\mu\text{l}$. After this procedure, $10\mu\text{l}$ of the extracted genomic DNA was

thawed, centrifuged, and quantified using the Qubit flex Fluorometer (Invitrogen, Waltham, MA) to determine the concentration and using the reagent Qubit 1X dsDNA HS Assay Kit that included the Qubit™ 1X dsDNA HS Working Solution and Qubit™ 1X dsDNA HS Standard #1 and #2 with Qubit™ Flex Assay Tube Strips, following the procedure described in the protocol.

Abundance of Ammonia Oxidizers

The abundance of ammonia-oxidizing bacteria (AOB) was quantified by quantitative polymerase chain reaction (qPCR) through the amplification of bacterial amoA gene primer pair amoA-1F (5'-GGGGTTTCTACTGGTGGT-3') and amoA-2R (5'-CCCCTCGGGAAAGCCTTCTTC -3') in 20 µL reactions (Rotthauwe et al., 1997; Wyngaard et al., 2016). The 20 µL total volume reaction contained 10 µL of the PowerUp SYBR™ Green (Applied Biosystems), 1.5 µL of both forward and reverse primer of amoA-1F and amoA-2R (5µM), 2 µL of DNA template, and 5 µL of DNA nuclease-free water. The qPCR amplification program consisted of an initial denaturation step set at 95 °C for 10 min followed by 40 cycles of 95 °C for 1 min, 57 °C for 1 min (annealing), and 72 °C for 3 min (elongation). Each run had a negative template, which comprised the DNA nuclease-free water instead of the DNA template. Bacteria amoA standards were prepared as described in Wyngaard et al. (2016). Serial dilution was prepared from the stock solution to generate the standards with concentrations varying from 10¹ to 10⁵ copies per µL. Standards were run in analytical triplicate to generate the standard curves. The amplification efficiency ranged between 85 – 110 %, with $r > 0.97$ for the standard curve. The qPCR assays were performed on Quant Studio 3 from Applied Biosystems. The amplification reactions were carried out in a transparent MicroAmp® Optical 96-well Reaction Plate with a barcode (Applied Biosystems by Life Technologies) and sealed with MicroAmp™ Optical Adhesive Film (Applied Biosystems).

The abundance of AOA was determined using digital PCR (dPCR), using the Qiacuity digital PCR system from Qiagen (Germantown, MD). The amplification reactions were carried out in Qiacuity nanoplate 26k 8-well and sealed with Qiacuity™ nanoplate seals. The amplification of archaeal *amoA* was performed with *ArchamoAF* (5' TTATGGTCTGGCTTAGACG-3') and *ArchamoAR* (5'-GCGGCCATCCATCTGTATGT-3') that resulted in 635 bp amplicon in 40 µL reaction volume. The reaction contained 13.3 µL 3x EvaGreen PCR Master Mix (FAM channel), 3.2 µL of both forward and reverse primers (0.4 µM), 5 µL of DNA template and 15.3 µL of RNase-free water. The thermocycling condition was programmed based on Qiacuity instrument instructions as follows: PCR initial heat activation 95 °C for 2 min, 40 cycles of 95 °C for 15s (denaturation), 56 °C for 15s (annealing), an extension of 72 °C for 15s, and cooling at 40 °C for 5 min.

Amplicon Metagenomic Sequencing

Following genomic DNA extraction (gDNA) and quantification, 40 µL of gDNA was collected and sent to Novogene Corporation (Sacramento, CA) for amplicon metagenomic sequencing targeting the V3-V4 region of 16S rDNA on NovSeqX Plus (PE250) with 30k tag per sample data output. The paired-end reads were first demultiplexed based on the barcodes specific to each sample, and the barcodes and primers were trimmed before further analysis. The overlapped read pairs were then assembled into raw tags using FLASH (v1.2.7, <http://ccb.jhu.edu/software/>), leveraging the paired reads that are overlapped to reconstruct the original DNA fragment. After this, the raw tags went through quality filtering in QIIME (v1.7.0, <http://qiime.org/index.html>) to eliminate the low-quality sequences and to retain the high-quality clean tag. Next, chimera detection was carried out against the Gold database using the UCHIME algorithm. Operational Taxonomic unit (OTU) clustering was performed using UPARSE

(v7.0.1001,<http://drive5.com/uparse/>) at 97% sequences. The ACE (<http://www.mothur.org/wiki/Ace>) and Chao1 (<http://www.mothur.org/wiki/Chao>) richness estimator in the Mothur package was used to calculate alpha diversity. A heatmap (Weighted Unifrac) was used in QIIME (v1.7.0) to visualize beta diversity distances, facilitating comparisons of community composition across different samples.

Statistical Analysis

The microbial abundance data were changed into copies/g soil before analysis. Analysis of variance was performed ($\alpha = 0.05$) with the aov function in R studio to determine significance in treatments. Post-hoc analysis for mean comparison was performed using Tukey's honest significant difference (HSD) to identify significance among treatments. Logarithmic base 2-fold change was performed based on the phylum level on the relative abundance across the different years in 2023 and 2024 using the ggplot2 package in R.

Results

Relative Abundance of Bacteria at Phyla level

Actinobacteria, Proteobacteria, Firmicutes, and Acidobacteriota emerged as the most abundant bacterial phyla across all treatments (Table 4.1). Throughout the growing seasons of 2023 and 2024, these four groups consistently dominated the soil communities, although their proportions shifted subtly in response to different management regimes over time. In 2023, no treatment had significant impact on the relative abundance at phyla level. During the 2024 growing season, Acidobacteriota was the only bacterial phylum whose abundance was significantly impacted by Treatment (Table 4.2), RTCA had significantly higher relative abundance of Acidobacteria than RTC. Although it was not significant, the relative abundance of Acidobacteria in RTCA showed an increasing trend as compared to the other treatments by an

average of 3.75%. Over time (between 2023 and 2024), the relative abundances of Actinobacteriota and Chloroflexi were significantly increased by RTCA as well. Similarly, the relative abundances of Acidobacteriota, Firmicutes and Gemmatimonadota were significantly increased by RTCAB, RTC, and RT ($p < 0.001$) respectively.

Alpha and Beta Diversities of Bacterial Community

When testing for each year separately, no significant differences in the alpha diversity indices of ACE or Chao1 were observed among treatments; however, a significant treatment $\times$ time interaction was observed, with the RTC in 2023 exhibiting significantly higher ACE and Chao1 values than RTCAB in 2023 (Figure 4.1). Moreover, both indices under RTCAB in 2023 were significantly lower than all of the treatments in 2024, including the RTCAB treatment. There was a big variation, demonstrated by an outline in the boxplot shown in Figure 4.1, associated with the RTCAB indices in 2023. Otherwise, the trend for both indices suggests an increasing diversity with the reduced tillage and integrated soil health management practices as compared to the conventional treatment.

A weighted UniFrac heatmap was used to analyze the beta diversity (dissimilarities in microbial communities among samples in different treatments) among the different soil management treatments in 2023 and 2024. The color intensity indicates how similar or dissimilar the microorganisms are to one another (Figure 4.2). The highest beta diversity (0.232) was between RTCAB in 2023 and RT in 2024, respectively. This suggests that the microbial communities in these two treatments were markedly different. The next biggest dissimilarity (0.204) was between RTCAB in 2023 and RTCA in 2024, followed by treatments RTCAB in 2023 and 2024 (0.196) shows minimal. What is consistent in the data pattern is the difference is mainly between RTCAB vs other treatments separated by time.

Ammonia Oxidizers Abundance

Abundances of bacterial and archaeal *amoA* gene copies did not differ significantly among treatments in either 2023 or 2024 at the VTR1 growth stage (Figure 4.3). However, at the R6 growth stage in 2024, conventional tillage (CT) showed a significant treatment effect, with bacteria *amoA* abundance in CT plots exceeding that of all of other treatments (RT, RTC, RTCA, RTCAB) (Table 4.3). There was a treatment x year interaction effect on bacterial *amoA* abundance, with treatment RTCAB exhibiting lower abundance in 2024 than in 2023. The bacterial to archaeal *amoA* ratio in 2023 and 2024 ranged from 1.1 to 1.3 (Figure 4.3), with increasing trend in 2024 than 2023 especially in the VTR1 samples.

Discussion

Copiotrophic microorganisms represented by phyla Actinobacteria and Proteobacteria were expected to be more dominant in the CT treatment vs the other treatments that received less amount of readily available nutrients. Tillage disturbance common in conventional practices are also expected to favor copiotrophs vs oligotrophs (e.g., Acidobacteria) as they accelerate organic-matter turnover and disrupts microhabitats, favoring fast-growing taxa and diminishing slower-growing, resource-efficient groups (Fierer, 2017; Sun et al., 2025). By and large, this was not the case in this study (Figure 4.4), and it could be because of several reasons. Firstly, all the other treatments received substantial amount of synthetic and inorganic fertilizers (Table 3.1) to meet the N requirement of the corn and hence might have allowed the growth of copiotrophic organisms to a level that was comparable to the CT treatment. Secondly, those treatments that have poultry litter are also able to provide readily mineralizable nutrients that can support the growth of copiotrophs in the integrated soil health management practices.

The significant changes observed in relative abundance of bacteria in 2024 and, the nonsignificant but increasing trends we saw in relative abundance in 2024 were associated with treatments that received poultry litter (mainly RTCA) vs those that did not receive poultry litter (e.g., RT) under the soil health management practices. This indicated that poultry litter played a significant role over time in influencing the bacterial communities. The treatment RTCA, for example, significantly increase the abundance of copiotrophic decomposers such as Actinobacteria and Chloroflexi between 2023 and 2024. Interestingly, the RTCAB treatment that incorporated the application of combined biochar and poultry litter increased the abundance of Acidobacteriota between 2023 and 2024, indicating this group's response to a more recalcitrant C sources from the biochar. Some oligotrophic phyla (e.g., Acidobacteria) have subgroups that respond to C input (Chen et al., 2024; Navarrete et al., 2013).

The alpha diversity indices were highly skewed by big variabilities among replications in each treatment, particularly in the RTCA and CT in 2023. Similar but smaller level of variabilities were also observed in 2024. These variabilities in both years might have masked the significance of the clear trend that can be observed in the 2024 data, with the RT and all of the integrated soil health management practices showing higher alpha diversity than the CT treatment by about 5 to 10%. These findings are similar to Wang et al., (2025) that reported similar margin of increase in alpha diversity under reduced tillage systems due to enhanced soil habitat heterogeneity and reduced disturbance. These findings align with long-term studies that reported reduced soil disturbance preserves habitat heterogeneity and labile organic-matter pools, thereby sustaining higher bacterial diversity (Li et al., 2020). High input of readily available N was also reported to have reduced bacterial diversity in a corn system (Habteselassie et al., 2022).

As indicated by the beta diversity, the most dissimilar microbial communities were between RTCAB and RT, stressing the influence of layering the RT treatment with cover crop, poultry litter and biochar, all of which not only introduce nutrient of organic natures but also some external microorganisms. The impact of individually applying these amendments have been reported by several studies variability (DeBruyn et al., 2011; He et al., 2024; Lauber et al., 2013; Zhang et al., 2011). In this study, integrating the individual practices as in RTCAB seemed to have caused the biggest difference in microorganisms as opposed to the RT, potentially suggesting a synergistic effect of the integrating the practices.

For ammonia oxidizers, the significant treatment impact was observed at the R6 growth stage in 2024 in which CT samples had higher bacterial *amoA* abundance than all of the other treatments, likely reflecting the readily available inorganic N from the synthetic fertilizers applied in CT. Under high inorganic N input, AOB are known to be more competitive than AOA (Prosser & Nicol, 2012). The dominance of AOB over AOA in most of the treatments is shown by the bacterial *amoA* to Archaeal *amoA* ratios that were higher than 1 (Figure 4.3). This suggests that while ammonia was not in short supply in all the treatments, its availability was better under CT treatment than the others in the R6 stage, resulting in higher AOB abundance. AOB abundance seems to be a good indicator in detecting differences between CT vs the soil health management practices.

Summary and Conclusion

In this two-year field study at the University of Georgia Southeast Georgia Research and Education Center, we evaluated the effects of conventional tillage vs soil health management practices on soil microbial abundance and composition. The soil health management practices were tested individually (reduced tillage, RT) or integrated in which the reduced tillage practice

was integrated with one or more additional practices. These included combinations of reduced tillage with cover crop, reduced tillage with cover crop and poultry litter and reduced tillage with cover crop, poultry litter and biochar, with the objective of examining the idea that integrating the soil health management practices can lead to better outcomes than the adoption of the individual practices separately. In 2023, no treatment had significant impact on the relative abundance at phyla level. During the 2024 growing season, Acidobacteriota was the only bacterial phylum whose abundance was significantly impacted by Treatment in which RTCA had significantly higher relative abundance of Acidobacteria than RTC. Although it was not significant, the relative abundance of Acidobacteria in RTCA showed an increasing trend as compared to the other treatments by an average of 3.75%. Bacterial alpha diversity showed a trend of increasing diversity with the reduced tillage and integrated soil health management practices as compared to the conventional treatment. The highest beta diversity (0.232) was between RTCAB in 2023 and RT in 2024, respectively, indicating that the microbial communities in these two treatments were markedly different. What is consistent in the data pattern with the beta diversity was that the differences were mainly between RTCAB vs other treatments separated by time. Ammonia-oxidizing bacteria reflected a clear distinction between the conventional tillage vs the soil health management practices. Overall, the study indicated that there is a clear trending towards a shift in soil microorganisms in response to integrating the soil health management practices as opposed to the RT or CT, but the changes are subtle and might require several more years to be fully realized and its effect felt on soil health.

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Table 4.1: The proportion of the ten most abundant bacterial phyla in soil treated with RTCA (Reduced Tillage with Cover Crop and Poultry Litter), RTCAB (Reduced Tillage with Cover Crop, Poultry Litter, and Biochar), CT (Conventional Tillage), RT (Reduced Tillage), and RTC (Reduced Tillage with Cover Crop).

Phyla	CT		RT		RTC		RTCA		RTCAB	
	Y23	Y24	Y23	Y24	Y23	Y24	Y23	Y24	Y23	Y24
Actinobacteria	41.1	34.5	40.7	31.7	37.5	31.7	39.6	30.7	42.1	30.4
Proteobacteria	23.2	23.5	19.7	22.6	24.7	25.7	23.0	22.5	25.5	25.9
Firmicutes	8.4	9.6	11.0	9.9	7.5	12.4	8.9	11.3	10.9	11.3
Acidobacteriota	7.7	11.5	9.7	11.5	9.8	9.5	9.6	14.6	5.7	11.9
Chloroflexi	6.3	7.4	5.9	7.0	6.1	7.0	5.4	7.4	5.3	7.0
Gemmatimonadota	3.4	4.2	2.8	4.6	3.5	3.3	3.1	3.3	3.3	3.3
Crenarchaeota	2.2	2.1	2.8	4.7	3.2	2.5	3.6	2.7	2.1	2.9
Bacteroidota	1.3	1.1	0.8	0.7	1.0	1.5	1.3	1.2	1.0	0.9
Verrucomicrobiota	0.6	0.7	0.9	0.7	0.6	0.6	0.3	0.7	0.2	0.6
Cyanobacteria	0.4	0.3	0.7	1.4	0.9	1.1	0.4	0.3	0.2	0.4

RCA received 4.48 Mg ha⁻¹ of poultry litter, and RCAB received 2.24 Mg ha⁻¹ of poultry litter and 2.24 Mg ha⁻¹ of biochar.

Table 4.2: Comparison of the top ten soil bacterial phyla across conventional tillage (CT), reduced tillage (RT), reduced tillage with cover crop (RTC), reduced tillage with cover crop and poultry litter (RTCA), and reduced tillage with cover crop, poultry litter, and biochar (RTCAB) during two growing seasons.

Phyla	Treatment	Y23	Y24	Phyla	Treatment	Y23	Y24
Actinobacteriota	CT	0.41aA	0.35aA	Gemmatimonadota	CT	0.03aA	0.04aA
	RT	0.41aA	0.32aA		RT	0.03aB	0.05aA
	RTC	0.38aA	0.32aA		RTC	0.04aA	0.03aA
	RTCA	0.40aA	0.31aB		RTCA	0.03aA	0.3aA
	RTCAB	0.43aA	0.30aA		RTCAB	0.03aA	0.03aA
Proteobacteria	CT	0.23aA	0.24aA	Cyanobacteria	CT	0.004aA	0.003aA
	RT	0.20aA	0.23aA		RT	0.01aA	0.01aA
	RTC	0.25aA	0.26aA		RTC	0.01aA	0.01aA
	RTCA	0.23aA	0.23aA		RTCA	0.004aA	0.003aA
	RTCAB	0.26aA	0.26aA		RTCAB	0.002aA	0.004aA
Acidobacteriota	CT	0.08aA	0.11abA	Bacteroidota	CT	0.01aA	0.01aA
	RT	0.10aA	0.12abA		RT	0.01aA	0.01aA
	RTC	0.10aA	0.10bA		RTC	0.01aA	0.02aA
	RTCA	0.10aA	0.15aA		RTCA	0.01aA	0.01aA
	RTCAB	0.06aB	0.12abA		RTCAB	0.01aA	0.01aA
Firmicutes	CT	0.08aA	0.10aA	Verrucomicrobiota	CT	0.02aA	0.10aA
	RT	0.11aA	0.10aA		RT	0.001aA	0.01aA
	RTC	0.08aB	0.12aA		RTC	0.01aA	0.01aA
	RTCA	0.09aA	0.11aA		RTCA	0.003aA	0.01aA
	RTCAB	0.11aA	0.11aA		RTCAB	0.002aA	0.01aA
Chloroflexi	CT	0.06aA	0.07aA				
	RT	0.06aA	0.07aA				
	RTC	0.06aA	0.07aA				
	RTCA	0.05aB	0.07aA				
	RTCAB	0.05aA	0.07aA				

Table 4.3. ACE and Chao1 Kruskal-Wallis test response to soil health management strategies with statistically significant differences ($p < 0.05$)

Treatment	LCL	UCL	P-value	LCL	UCL	P value
	ACE			Chao1		
RTC.Y23 - RTCAB.Y23	3.08	35.42	0.02*	3.06	35.44	0.02*
RT.Y24 - RTCAB.Y23	1.08	33.42	0.04*	1.81	34.2	0.03*
RTC.Y24 - RTCAB.Y23	3.83	36.17	0.02*	5.06	37.44	0.01*
RTCA.Y24 - RTCAB.Y23	4.58	36.92	0.01*	3.31	35.7	0.02*
RTCAB.Y23 - RTCAB.Y24	-37.42	-5.08	0.01*	-37.94	-5.56	0.01*

RT, Reduced Tillage; RTC, Reduced tillage with cover crops; RTC, Reduced tillage with cover crops and poultry litter; RTCAB, Reduced tillage with cover crops, poultry litter and Biochar; LCL, Lower confidence level; UCL, Upper confidence level.

Table 4.4: Abundance of ammonia oxidizers (AOA & AOB) in two corn growth stages in 2024.

Abundance	Treatment	VTR1	R6
AOB (copies/g soil)	CT	4.2×10^6a	5.4×10^6a
	RT	2.2×10^6a	2.0×10^6b
	RC	2.4×10^6a	1.7×10^6b
	RCA	1.5×10^6a	1.0×10^6b
	RCAB	1.4×10^6a	1.3×10^6b
AOA (copies/g soil)	CT	8.2×10^4a	2.7×10^5a
	RT	7.4×10^4a	2.6×10^5a
	RC	9.0×10^4a	3.0×10^5a
	RCA	1.1×10^5a	4.8×10^5a
	RCAB	1.5×10^5a	3.5×10^5a

Different lowercase letters within a column indicate significant differences within a growth stage for the treatments using Tukey's HSD test, $P \leq 0.05$.

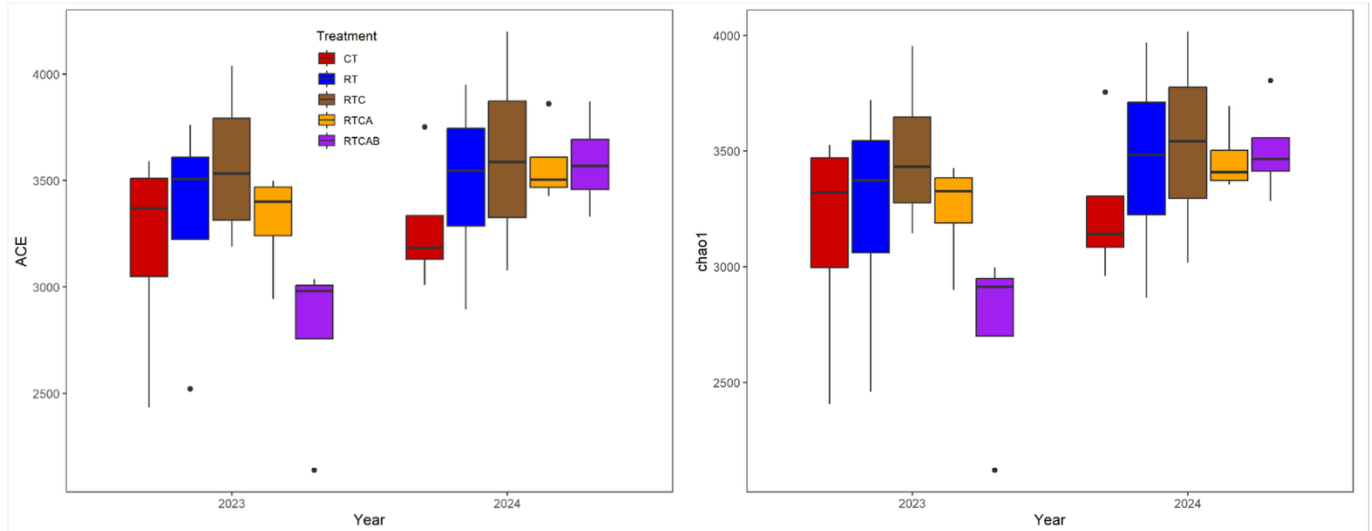


Figure 4.1: Alpha diversity on the bacterial community in response to soil health management practices implemented during the 2023 to 2024 corn growing season, measured by the ACE index and Chao1, with statistically significant differences (Kruskal-Wallis test, $p < 0.05$).

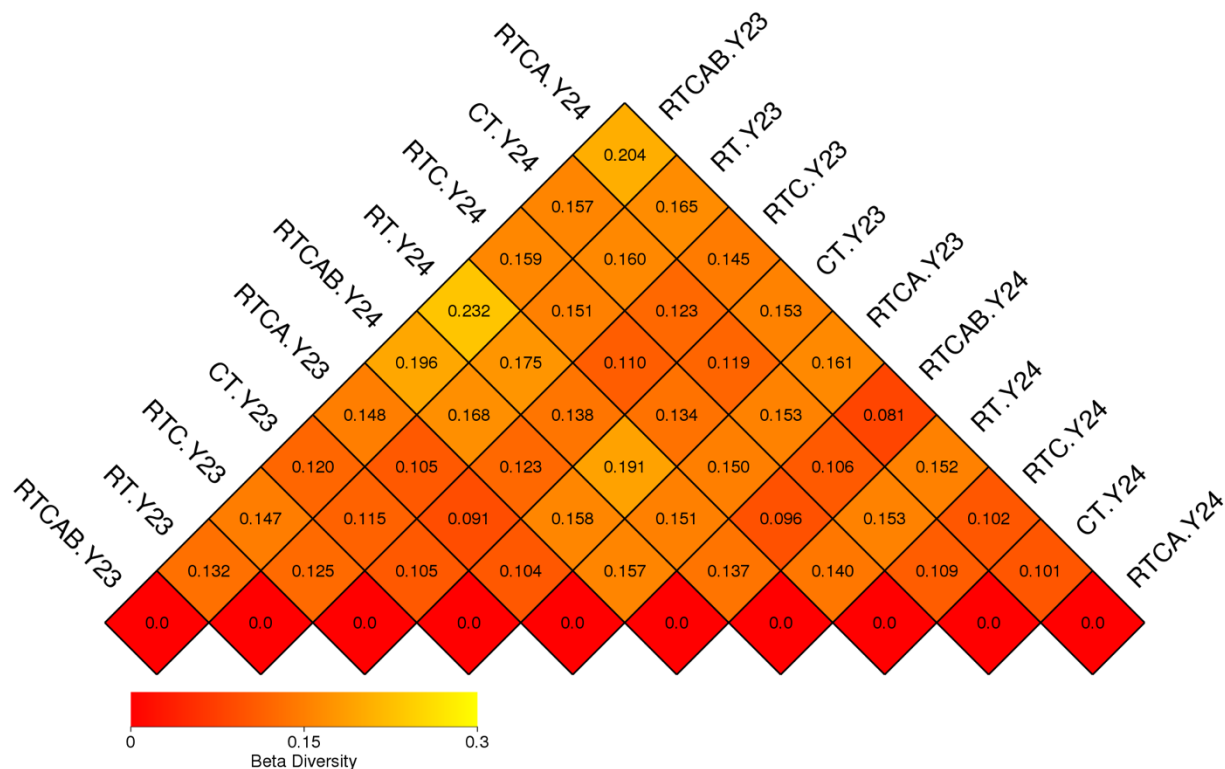


Figure 4.2. Weighted UniFrac heatmap showing soil microbial beta diversity across treatments in the 2023 – 2024 corn growing season. RT, Reduced Tillage; RTC, Reduced tillage with cover crops; RTC, Reduced tillage with cover crops and poultry litter; RTCAB, Reduced tillage with cover crops, poultry litter, and Biochar.

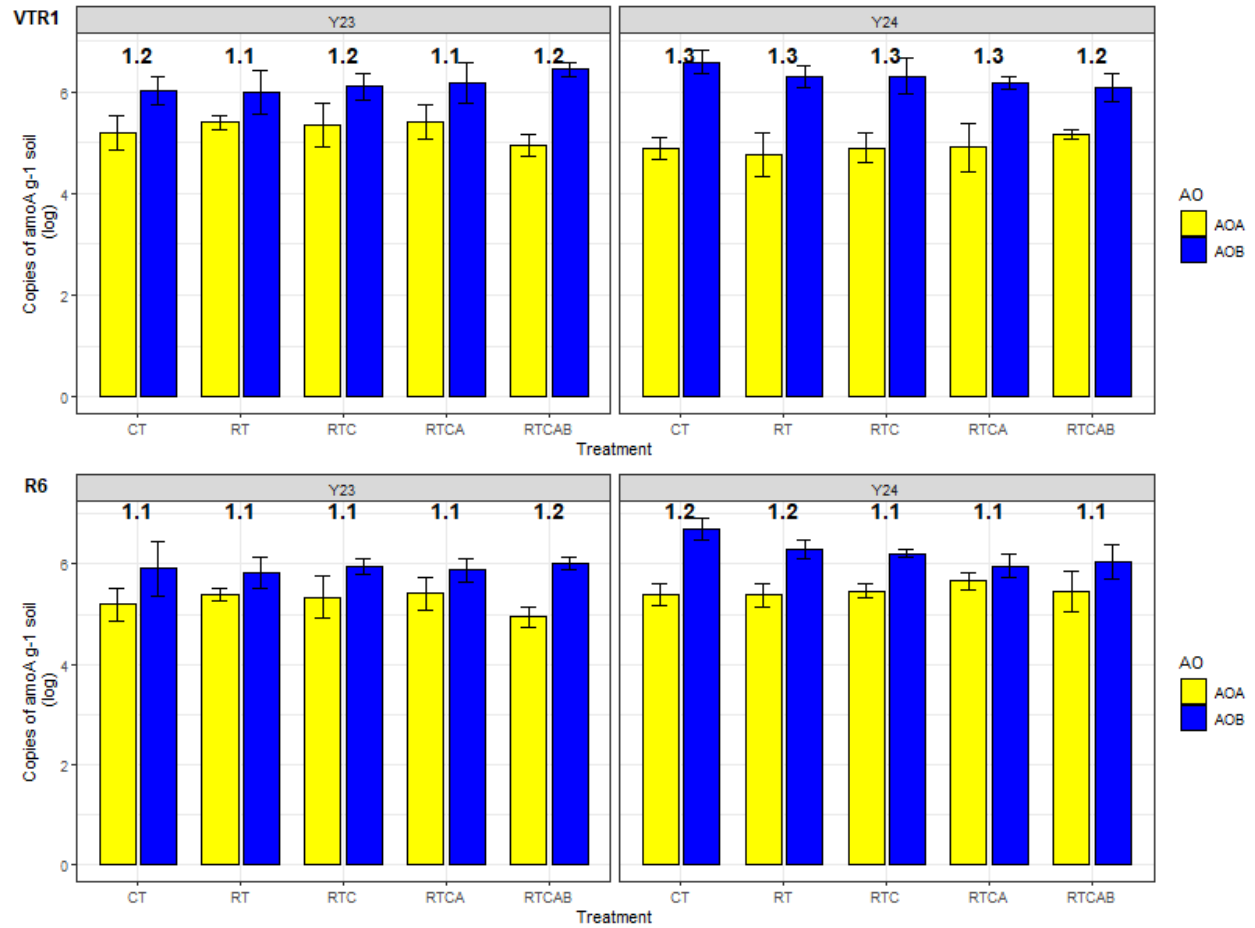


Figure 4.3: Ammonia oxidizers (AOA & AOB) abundance in soil samples from plots under CT (Conventional tillage), RT (Reduced tillage), RTC (Reduced tillage with cover crop), RTCA (Reduced tillage with cover crop and poultry litter), and RTCAB (Reduced tillage with cover crop, poultry litter, and biochar) for growth stages VT/R1 (A), and R6 (B) across two growing seasons.

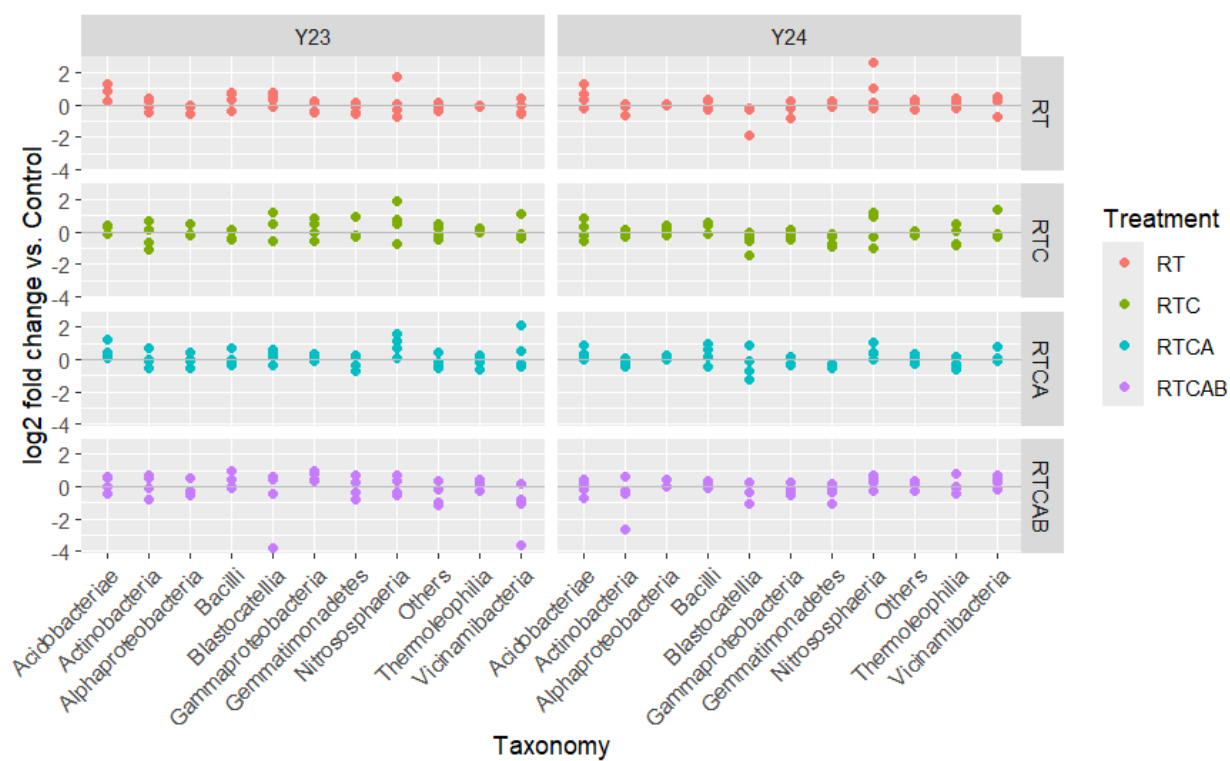


Figure 4.4: Log2 fold change in the abundance of selected microbial taxa under different soil health management practices relative to Conventional Tillage (CT) over two years.

CHAPTER 5

SUMMARY AND CONCLUSION

The overreliance on synthetic fertilizers and intensive tillage has long been implicated in the degradation of soil structure, declines in microbial diversity, and imbalances in nutrient cycling that undermine long-term productivity and environmental sustainability. This two-year field study at the University of Georgia's Southeast Georgia Research and Education Center evaluated the potential of individual and integrated soil health management practices in improving soil biological health in a corn system. We compared conventional tillage (CT), reduced tillage (RT), reduced tillage with cover crops (RTC), reduced tillage with cover crop and poultry litter (RTCA), and reduced tillage with cover crop, poultry litter and biochar (RTCAB) during the 2023 and 2024 growing seasons. Soil biological health was characterized by measuring microbial activity (soil respiration and enzyme assays) and microbial abundance and composition (16S rDNA sequencing). Across both years, soil pH remained stable (6.17–6.81), and buffering capacity did not differ among treatments. Treatments RCA and RCAB both enhanced base cations and P compared to conventional tillage, resulting mainly from poultry litter addition. The increased soil respiration rates indicated improvement in soil biological health in 2024 due to the integrated soil health management practices. Urease activity was not affected by treatment in 2023, but there were treatment and sampling time effects driven by RC and RCAB at the R6 growth stage, indicating a cumulative effect of the integrated soil health practices. β -glucosidase activity peaked in RCA and RCAB while phosphatase activity did not reflect significant change overall. The correlation analyses indicated strong positive associations

between base cations and β -glucosidase, as well as between macronutrients (K^+ , P) and micronutrients (Mn, Zn) and soil respiration. Overall, the integrated soil health management approach resulted in improved microbial activity as a result of nutrient and organic matter input from poultry litter, cover crop and biochar. The changes were gradual and more pronounced in treatments with poultry litter application because of the more labile nature of nutrients and organic materials than in freshly incorporated cover crop or a more stable organic input in biochar.

In terms of microbial community composition and diversity, no treatment had significant impact on the relative abundance at phyla level in 2023. During the 2024 growing season, Acidobacteriota was the only bacterial phylum whose abundance was significantly impacted by Treatment in which RTCA had significantly higher relative abundance of Acidobacteria than RTC. Although it was not significant, the relative abundance of Acidobacteria in RTCA showed an increasing trend as compared to the other treatments by an average of 3.75%. Bacterial alpha diversity showed a trend of increasing diversity with the reduced tillage and integrated soil health management practices as compared to the conventional treatment. The highest beta diversity (0.232) was between RTCAB in 2023 and RT in 2024, respectively, indicating that the microbial communities in these two treatments were markedly different. What is consistent in the data pattern with the beta diversity was that the differences were mainly between RTCAB vs other treatments separated by time. Ammonia-oxidizing bacteria reflected a clear distinction between the conventional tillage vs the soil health management practices. Overall, the study indicated that there is a clear trending towards a shift in soil microorganisms in response to integrating the soil health management practices as opposed to the RT or CT, but the changes are subtle and might require several more years to be fully realized and its effect felt on soil health.