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Soil Hydro-Physical Properties, Computed Tomography Measured Pore Parameters, and Soil Health Indicators as Influenced by Tillage and Crop Rotation Systems

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SOIL HYDRO-PHYSICAL PROPERTIES, COMPUTED TOMOGRAPHY MEASURED PORE PARAMETERS, AND SOIL HEALTH INDICATORS AS INFLUENCED BY TILLAGE AND CROP ROTATION SYSTEMS

BY

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THESIS ACCEPTANCE PAGE

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This thesis is approved as a creditable and independent investigation by a candidate for the master's degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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TABLE OF CONTENTS

ABBREVIATIONS

AMF	Arbuscular mycorrhizal fungi
ANOVA	Analysis of variance
AP	Acid phosphatase
AS	Arylsulfatase
C	Carbon
CC	Continuous corn
CS	Corn-soybean
CT	Conventional till
CWC	Cold water extractable carbon
CWN	Cold water extractable nitrogen
D	Fractal dimension
HWC	Hot water extractable carbon
HWN	Hot water extractable nitrogen
K_{sat}	Saturated hydraulic conductivity
MBC	Microbial biomass carbon
MBN	Microbial biomass nitrogen
MP	Macroporosity
MWD	Mean weight diameter
MesP	Mesoporosity
N	Nitrogen
NT	No-till
NMP	Number of macropores
NmesP	Number of mesopores
PAW	Plant available water content
PLFA	Phospholipid fatty acid
*p*NP	Para-nitrophenol
RT	Reduced till
SOC	Soil organic carbon
SWR	Soil water retention
TN	Total nitrogen
TNB	Total number of branches
WSA	Water stable aggregates
XCT	X-ray computed tomography
Ψ_m	Matric potential
λ	Thermal conductivity
ρ_b	Soil bulk density
τ	Tortuosity

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

SOIL HYDRO-PHYSICAL PROPERTIES, CT-MEASURED PORE PARAMETERS, AND SOIL HEALTH INDICATORS AS INFLUENCED BY TILLAGE AND CROP ROTATION SYSTEMS

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Long-term tillage and crop rotation systems are important agricultural management practices as these can have direct impact on the soil's key properties. The objectives of this study were to (i) quantify the soil pore characteristics under long-term tillage and crop rotation using X-ray computed tomography (XCT) and to assess the relationships between XCT-measured pore parameters and soil hydro-physical properties; and (ii) evaluate the impacts of long-term tillage and crop rotation on select soil health indicators. The objective (i) was carried out at Haskell Agricultural Laboratory (HAL), Concord, NE; and objective (ii) was carried out at South Central Agricultural Laboratory (SCAL), Clay Center, NE in addition to HAL study site. The SCAL and HAL experimental sites were initiated in 1985 and 1986, respectively. The experimental design was a randomized complete block design in split-plots with three and four replications in SCAL and HAL sites, respectively. The main plots were tillage and sub-plots were rotation treatments. The study treatments included: three tillage [no-till (NT), reduced till (RT) – disk till, and conventional till (CT) – moldboard plow] and two cropping systems [continuous corn (*Zea mays* L.) and corn-soybean (*Glycine max* [Merr.] L.)].

Results from objective (i) showed that NT with corn-soybean (CS) rotation decreased the soil bulk density (ρ_b) at 0-10 cm depth and increased the number of

macropores and mesopores at 0-10 and 10-20 cm depth as compared to the CT with continuous corn (CC) systems. Similarly, NT with CS also enhanced the saturated hydraulic conductivity (K_{sat}) at 0-10 cm depth. Though the crop rotation did not affect the soil organic carbon (SOC) and total nitrogen (TN), the NT improved the SOC by 24 and 49% and TN concentrations by 26 and 67% at 0-10 cm depth as compared to the RT and CT, respectively. Also, the NT increased the plant available water (PAW) content by 25 and 67% at 10-20 cm depth as compared to the RT and CT, respectively. Results from objective (ii) showed that the activities of β-glucosidase and urease were higher under NT with CS rotation as compared to the other treatments at HAL study site. At SCAL study site, similar effect of NT with CS was observed with enhanced arylsulfatase activity. At the HAL study site, though there was no interaction effect, the CS rotation enhanced the microbial biomass carbon (MBC) by 9% as compared to the CC. Similarly, the NT increased the MBC by 27 and 80% as compared to the RT and CT treatments. The NT with CC system has increased the mean weight diameter and water stable aggregates as compared to the other treatments. Overall, this study showed that NT with CS rotation enhanced the soil physical and hydrological attributes along with the other soil health indicators.

CHAPTER 1

INTRODUCTION

Intensive agricultural practices degrade soil quality by upsetting the natural balance (Lal, 2015; Matiyas, 2019). Additionally, the use of agrochemicals to increase agricultural yields drives the production of most of the crops. These external inputs degrade soil quality and interfere with ecological functions such as nitrogen cycling and biological pest control (Clermont-Dauphin et al., 2014). Hence, there is a great need for an agroecological approach to agricultural systems that aims to attain sustainability and yield profitability (Shrestha et al., 2020). Conservation practices such as no-tillage (NT), reduced tillage, and crop rotations are of increasing prominence in the agriculture sector during recent times. As per 2017 census, 42 million hectares (37% of tillable acreage) of cultivated land in the United States was under NT farming. Producers in the US planted around 73 million hectares of corn (*Zea mays* L.) and soybean (*Glycine max* [Merr.] L.) in 2021. The arable land is ought to be managed in a way to increase the crop yield and reduce ecological detriment. Sustainable agricultural approaches can boost climate change resistance and biodiversity protection (Koohafkan et al., 2012). Therefore, the conservation agricultural practices are important in enhancing the soil hydro-physical properties and other soil health indicators (Busari et al., 2015).

Soil hydro-physical factors regulate the critical functions of plant growth and development such as infiltration, C storage, water retention and transport (Blanco-Canqui, 2017). Soil physical structure can be influenced by a variety of factors such as soil texture, mineralogy, available organic matter, climate, tillage practices, cropping patterns, (Gould et al., 2016). Excessive long-term plowing can reduce aggregate stability, size and porosity, increase subsurface compaction (i.e., plow pan development), cause surface crusting, reduce infiltration and increase the risk of soil erosion (Nunes et al., 2020a). Contrastingly, long-term NT provides positive effects on various soil hydro-physical properties such as saturated hydraulic conductivity (K_{sat}), saturated thermal conductivity, porosity, plant available water content (PAW), and water retention (Schlüter et al., 2018). An increase in stable aggregates due to conservation tillage systems can usually decrease the rate of soil erosion, favoring environmental protection (Pires et al., 2017). Crop rotation with different species can increase the microbial richness and diversity, improve soil structure and enhance the hydro-physical properties (Venter et al., 2016). This is due to the facilitation and niche differentiation associated with distinct species as compared to the monocropping pattern (Smith et al., 2008). Crop rotation also benefits in breakdown of the pest cycle, and rotation with leguminous crops contributes to N cycling. It also reduces the crop stress from plant available nutrient levels and weeds (Smith et al., 2008). Hence, a better understanding of how tillage and rotation systems affect soil's hydro-physical characteristics is crucial to overall soil performance.

Tillage and crop rotation systems affect soil porosity, pore-volume, and pore size distribution and ultimately influence the soil’s hydraulic properties (Blanco-Canqui et al., 2017). Soil porosity can be assessed using traditional techniques of water retention method, Boyle’s porosimetry method, and thin section analysis that were destructive, time-consuming, and also failed to express spatial variability (Udawatta et al., 2006). X-ray computed tomography (XCT) scanning technology has emerged as a significant technical improvement in the imaging and measurement of soil pore characteristics in recent decades (Taina et al., 2008). The XCT approach is a non-destructive, non-invasive technology that uses the principle of attenuation of an electromagnetic wave beam focused on the item to explore the qualities of the 'interior' of objects of interest. Due to its non-invasive nature, the XCT technique helps to analyze the pore parameters of the same soil sample multiple times (Kumar et al., 2010b). The high-resolution images of the XCT scanning technique allow quantifying the microstructure of soil in 3D view (Peng et al., 2014). The expansion of the use of X-ray microtomography in soil research would almost probably lead to new XCT applications and breakthroughs in soil structure, such as more realistic studies on tortuosity, connectivity, form, size, and pore distributions (Pires et al., 2010). When combined with image processing techniques, the XCT method can be used to study many additional elements of soil micromorphology (Singh et al., 2021).

Agricultural management systems such as tillage and crop rotations can impact various soil properties and hence, can bring changes in overall soil health (Kibblewhite et al., 2008). The response of soil health indicators to agricultural management can usually observed with changes in soil parameters such as soil structure, porosity, infiltration, PAW, soil acidity, electrical conductivity, organic matter, microbial biomass and microbial diversity (Allen et al., 2011). Conservation agricultural systems (NT and crop rotation) can enhance the overall soil quality by improving soil organic carbon (SOC), microbial biomass carbon (MBC) and nitrogen (MBN), soil enzyme activity, aggregate stability, etc. (Gura and Mnkeni, 2019). Hence, NT and crop rotation systems can help in sustainable intensification. Therefore, the study on the long-term effects of tillage and crop rotation on important soil hydro-physical, chemical, and microbial properties is essential.

1.1 STUDY OBJECTIVES

The purpose of this study was to evaluate soil hydro-physical and other soil health indicators as influenced by different tillage and crop rotation systems. The objectives of this study were evaluated in two sub-studies and specific objectives were developed for each study as listed below.

Study 1. This study was entitled "Soil hydro-physical and computed tomography -measured pore characteristics as influenced by long-term tillage and crop rotation" with the specific objectives were to: (i) visualize and quantify the soil pore characteristics under long-term tillage and crop rotation systems using X-ray computed tomography (XCT), and (ii) correlate the XCT-measured pore parameters with soil hydro-physical properties.

Study 2. This study was entitled "Soil health indicators influenced by long-term tillage and crop rotations in two locations of Nebraska, USA" with the specific objective was to assess the influence of long-term tillage and crop rotation systems on soil health indicators.

STUDY HYPOTHESIS

Conservation agricultural practices such as no-till and crop rotation systems can enhance soil hydro-physical properties and soil health indicators

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CHAPTER 2

LITERATURE REVIEW

The growing concern of food security to the increasing population involves the risk of degradation in soil quality and damage to the environment (Busari et al., 2015). For instance, though the Midwest corn belt region is a huge part of the global food production system, it is also greatly responsible for soil health degradation and water pollution due to high fertilizer usage (Winkler et al., 2012). These problems pose a challenging situation for farmers to bridge between higher crop yields and mitigating undesirable effects on the environment (Hill et al., 2006). To overcome the prevailing challenge, there is a need of agricultural practices those establish a balance between production and environmental deterioration (Alhameid et al., 2019a). From the past few decades, conservation agricultural systems such as no-till and crop rotation have been used for enhancing production and sustainability (Hobbs, 2007). These different management practices influence soil hydro-physical, chemical, and biological properties in different ways (Doran, 2002). Therefore, the present review is focused on long-term tillage and crop rotation systems and their impacts on soil hydro-physical properties and other soil health parameters.

2.1. Cropping systems

A cropping system is defined as the *"type and sequence of crops grown and practices used for growing them"* (Blanco and Lal, 2008). Practices refer to the components of management methods in crop production using available technologies that can help to improve the growth environment for crop production (Cook, 2006). However, there is a necessity to consider the site-specific conditions, available resources, and cropping history while designing a cropping system. The new cropping systems are built comprehensively, considering the ecological, economic, and environmental considerations. Some of the components of cropping systems include such as tillage, crop diversification, nutrients and water management, erosion control practices (Blanco and Lal, 2008).

2.1.1 Tillage

Tillage is defined as the mechanical manipulation of the soil for crop production significantly affecting the soil characteristics (Busari et al., 2015). Based on climatic situations, type of soil and crop, accessible resources, the types of tillage practices followed in widespread are conventional, reduced, and conservation tillage.

Conventional tillage: It refers to the maximum disturbance to soil surface and burying of crop residues to deeper depths (Briones and Schmidt, 2017). The system is also referred to as intensive tillage practice that inverts the soil and alters the natural soil structure (Blanco-Canqui and Lal, 2008). Conventional tillage (CT) leaves less than 15% of residue cover on surface soil (Conservation Technology Information Center – CTIC).

Reduced tillage: These are the systems in which the intensity and/or frequency of tillage has been reduced relative to conventional based soil tillage (Van Kessel et al., 2013). In a reduced tillage system, there is 15-30% residue cover after planting (CTIC)

Conservation tillage: is defined as a tillage system in which at least 30% of crop residues are left after planting in the field on the surface (Mathew et al., 2012). As the name '*conservation*' implies, this system is an essential conservation practice to lessen soil erosion and sustain various properties of soil. Proper management of crop residues can help in the protection of soil resources, enhance soil quality, reduce surface runoff thus increasing water conservation and availability. Sustainable Agriculture Research and Education (SARE) program identifies different conservation tillage practices: no-till, strip-till, ridge-till, and mulch-till. The present study is focused on the no-till system among conservation tillage practices.

No-till system: No-till (NT) is a conservation farming system in which the crop is planted directly into untilled soil with previous crop or cover crop residues (Derpsch et al., 2011). In this system, all the residues such as leaves, stalks, cobs, etc. on the surface soil are left as such after harvest. The main aim of the practice is to disturb the soil as minimum as possible even when seeding, thus special NT seeding types of equipment are used to access narrow slots just wide enough to put seeds into residue-covered soil. Weed management operations in a NT system essentially include the adaption of crop rotations with suitable cover crops or crop associations and application of herbicides (Derpsch et al., 2011).

2.1.2 Crop rotation

Crop rotation is explained as the practice of growing different crops sequentially in the same field in sequential seasons or years. It is one of the strategies of sustainable farm management, meant to lower soil erosion (Shah et al., 2021). Different species in cropping pattern may help to enhance soil health, fertility, reduce soil erosion, water pollution, replenish nutrients in which the former crop has been removed out of the soil, and prevent diseases by breaking the life cycle chain due to non-host crops (Blanco and

Lal, 2008). Several factors such as soil type, weather conditions, availability of market, and various resources are also considered in choosing a crop in rotation (Chongtham et al., 2017). Different cropping patterns followed generally are mentioned below as:

Monocropping or monoculture**:** planting of the same crop in the same field season after season or year after year. Though monocropping is easy for planting and harvesting, it makes soil susceptible to erosion and pest infestation.

Short rotation: growing of two different crops in the same field in successive seasons or years. (e.g., 2-year rotation of corn (*Zea mays* L.) – soybean (*Glycine max* [Merr.] L.)

Extended or diverse rotation: cropping pattern involving more than 2-year rotation and three different crops (e.g., corn-soybean-oat [*Avena sativa*] -wheat [*Triticum aestivum* L.]).

2.2 Soil hydro-physical properties

Physical and hydrological properties of the soil are essential to carry out the soil functions effectively. The changes in these properties influence various ecological services provided by the soil such as water retention, soil C dynamics, and sequestration (Blanco-Canqui and Ruis, 2018). Physical and hydrological characteristics of the soil mediate necessary soil processes. For example, bulk density (ρ_b) which is affected by soil compaction influence soil pore size distribution and aeration; texture affects runoff and erosion; and heat capacity affects soil warming. A variety of soil physical indicators affecting plant available water (PAW) and field capacity are directly controlled by soil porosity and water retention properties (Dexter and Richard, 2009). Moreover, soil hydro-physical parameters with different soil management practices influence the flow and availability of water, air, and nutrients for plant development. Hence, the study of soil hydro-physical properties is essential to understand the water movement and balance in the soil as they have a significant impact on ecological, agronomical, and pedological processes (Lal, 2011).

2.2.1 Impacts of tillage on soil hydro-physical properties

Important hydro-physical properties of soil include aggregate stability, porosity, texture, rate of infiltration, saturated hydraulic conductivity, soil water retention, and PAW. Literature showed that the retention of plant residues in NT system helped to enhance the soil total porosity (Malobane et al., 2021). Also, their studies concluded that tillage treatment influenced aggregate stability, binding properties, and microstructure of soil. Conventional tillage (CT) system on the other hand enhances the susceptibility of soil erosion by breaking down the aggregates and reducing the stability (Xiao et al., 2019). The possible reason for this is that CT accelerates the residue and soil organic matter decomposition by disturbing the soil and exposing plant debris and soil aggregates to the action of soil microorganisms (Zuber et al., 2015). Impacts of tillage on ρ_b were not consistent with tillage intensity indicating that other factors such as sampling time, soil conditions, and duration of the experiment can also have influence on ρ_b. An increase in ρ_b under NT than CT was reported by Halvorson et al. (2002), whereas, no effect of tillage intensity on ρ_b was reported by Huggins et al. (2007). Tillage usually loosens the soil and generates macropores and hence, lowering the soil ρ_b; however, the absence of substantial changes between NT and CT might be due to an increase in SOC and wet aggregate stability, resulting in a higher accumulation of less dense surface material and hence lower ρ_b under NT (Coulter et al., 2009). Soil aggregation is also facilitated by the presence of additional binding agents such as glomalin-related soil protein from fungal hyphae. As a result, the stable aggregates maintain a range of pore diameters, impact the density and stability of soil physical structure, and increase the soil's capacity to store and supply water for plant growth (Amézketa, 1999). The residue retention helps to build SOC concentration and improves the soil physical and hydrological properties. The findings of Park and Smucker (2005) indicated that saturated hydraulic conductivity was higher under NT as compared to the CT. The higher porosity and increased aggregate stability in NT contributed to increase in saturated hydraulic conductivity. Similarly, higher water retention and increased porosity under long-term NT were reported by Sekaran et al. (2021). Hence, the tillage systems are important as they influence the soil hydro-physical properties.

2.2.2 Impacts of crop rotation on soil hydro-physical properties

Crop rotation majorly affects the quality and quantity of crop residue. For example, higher residues were deposited following corn than following the soybean crop (Zuber et al., 2015). Greater the retention of residues, higher is the amount of organic matter available to the soil. Stubble retention is one of the important management practices to enhance the soil organic carbon (SOC) and hence, the soil's hydro-physical properties (Chan, 2008). Literature is available regarding the changes that occurred in soil hydro-physical properties influenced by crop rotations (Alhameid et al., 2019b; Karlen et al., 2006). de Moura et al. (2021) reported increased soil ρ_b, decreased water-stable macro aggregation with continuous corn system (CC) as compared to a corn-soybean

(CS) rotation. Bulk density has a direct effect on soil's porosity and infiltration capacity. The inverse relationship between ρ_b and porosity was explained by Osunbitan et al. (2005) in their study. Also, the findings of Bansal et al. (2021) reported the higher aggregate weight with CS rotation as compared to the CC. This suggests that rotation with different crop species helps to build a diverse microbial community, hence improving the soil aggregation by binding substances (Tiemann et al., 2015). Hence, crop rotation with different species helps to enhance the important soil hydro-physical properties.

2.2.3 Impacts of tillage with crop rotation on soil hydro-physical properties

Combining the effects of tillage and crop rotation, the interaction effects have significant importance to changes in soil hydro-physical properties. Conservation tillage with crop rotation saves time and energy by reducing tillage operations (Triplett Jr and Dick, 2008). Studies of Hati et al. (2015) on different tillage systems with crop rotation reported that NT improved SOC that resulted in better hydro-physical properties such as aggregates, saturated hydraulic conductivity due to crop residue retention, and minimal disturbance to the soil. Similar findings were reported by Parihar et al. (2016) who reported that conservation tillage with Maize-Chickpea-*Sesbania* rotations reduced bulk density, penetration resistance, increased SOC, water-stable aggregates, and saturated hydraulic conductivity than compared to other maize based rotations. Hammerbeck et al. (2012) reported that removal of residues has a detrimental influence on the physical and hydraulic factors of the soil, emphasizing the importance of crop residue in maintaining the soil quality. This was supported by findings of Duru et al. (2015) in their study of 10-year NT with corn-soybean rotation, which concluded that retention of crop residues decreased water and wind erosion, increased earthworm population and accessible nutrients, and enhanced soil water retention. The NT with corn-soybean rotation reduced the soil ρ_b, penetration resistance and increased the saturated hydraulic conductivity and macroporosity as compared to the Maize-fallow-Maize system (Nebo et al., 2020).

2.2.4 X-ray CT scanning approach for measuring soil pore characteristics

Hounsfield (1975) developed X-ray Computed Tomography (XCT) technique for medical imaging. The XCT applies the principle of attenuation of an electromagnetic beam focused on the item to explore the internal qualities of the objects of interest. It is a non-destructive imaging technology that allows 3-dimensional (3D) view of object structural features (Carducci et al., 2017). Several researches applied XCT technique in their study for the 3D visualization of soil structural properties, (Luo et al., 2008; Naveed et al., 2013; Singh et al., 2020). Kumar et al. (2012) applied the XCT method to measure the soil macroporosity and coarse mesoporosity as influenced by agroforestry and grass buffers managed with grazed pastures. Müller et al. (2018) used the same approach to analyze the hydrological properties of macropores and concluded that water movement through macropores was influenced by connectivity, tortuosity, and pore size distribution. Garbout et al. (2013) assessed the tillage effects on soil structural quality using the XCT and concluded that direct drilled treatment has good structural quality as compared to the plow till. Several researchers utilized the XCT technique to measure various soil structural properties and pore characteristics, for example, aggregate structural analysis (Gao et al., 2017), macropore space organization (Rab et al., 2014), fractal properties of soil (Martín-Sotoca et al., 2018), pore size distribution (Jarvis et al., 2017). Hence, the

XCT technique can be used as an effective tool to describe various soil hydraulic and structural properties in spatial and temporal differentiation.

2.3 Soil health

Soil health plays an essential role in developing resilient agricultural systems (Lal, 2016). It is defined as the continued capacity of soil to function as a vital living ecosystem that sustains plants, animals, and humans (USDA – NRCS). The term also emphasizes the importance of sustainable soil management, as the soil contains living organisms that are involved in vital functions such as nutrient cycling (Sahu et al., 2017). Indicators of soil health are measurable properties of soil or plants that influence the functional capacity of the soil (Karlen et al., 2003). They are responsive to changes in land management systems such as tillage, crop rotation and integrate physical, chemical, and biological aspects of the soil (Doran et al., 2002). A few indicators related to soil health include soil structure, water holding capacity, aggregate stability – physical; total organic carbon and nitrogen (TOC, TN), water extractable carbon and nitrogen, pH, electrical conductivity – chemical; microbial biomass carbon and nitrogen (MBC and MBN), soil enzymatic activity, microbial community structure – biological properties. The literature review in this chapter is focused on the tillage and crop rotation practices impacts on selected soil health indicators.

2.3.1 Impacts of tillage on soil health indicators

Tillage has significant influence on soil health indicators, hence, reflecting the changes in overall soil health (Williams et al., 2020). The influence of tillage on soil physical, chemical, and biological properties is prominent and can impact the crop productivity and sustainability (Busari et al., 2015). Literature shows that the intensity of tillage can affect soil health indicators in several ways (Nunes et al., 2020). Conventional tillage negatively impacts the productivity of soil due to loss of soil fertility, soil organic matter (SOM), and increased soil erosion (Mathew et al., 2012). Intensive tillage can also increase the surface crusting and decrease the soil aggregate stability (Baumhardt et al., 2015). Contrastingly, in the NT system, the surface residues are left undisturbed in the soil that reduces the surface runoff and increase moisture retention capacity (Jemai et al., 2013). The NT soils can be moist with low temperatures due to residual plant biomass on the surface and provide a favorable environment for microbial activity including harmful diseases. Sekaran et al. (2021) reported an increased aggregate stability with enhanced SOC under NT system as compared to the CT (tilled once in fall with a disk ripper and in spring with a field cultivator). They also concluded that NT increased β-glucosidase and acid phosphatase activity compared to the CT. The β-glucosidase is a key enzyme in the carbon cycle that is mainly produced by saprotrophic microbes such as bacteria and fungi, and phosphatase enzymes are important in the release of accessible inorganic P from the organic form of P in soil. Several other studies reported that NT has a positive impact on aggregate stability (Sithole et al., 2019), soil organic carbon (Busari et al., 2015), and water retention capacity (Martínez et al., 2008). Abundance of soil microbial community is an important soil health indicator and is influenced by tillage practices. Dorr de Quadros et al. (2012) conducted a study to observe the effects of tillage on soil microbial diversity and found that NT system has higher microbial diversity. Similarly, Feng et al. (2003) measured soil microbial communities through phospholipid fatty acid analysis under CT and NT systems and observed a significant abundance of microbial community under NT than the CT. Nunes et al. (2018) in their study reported that long-term soil management under NT has the favorable soil biological, physical, and chemical conditions for plant growth and development with increased levels of soil organic matter, wet aggregate stability, total N, and infiltration rate. Thus, the conservation tillage system can enhance the microbial community, biomass, enzyme activity, aggregate stability, SOC storage and hence, improve the overall soil health (Bossuyt et al., 2002).

2.3.2 Impacts of crop rotation on soil health indicators

Crop rotation can influence the amount of plant-available nutrients, availability of SOC and hence, affect the soil functional activities (Neugschwandtner et al., 2014). Alteration of crops in every other growing season or year avoids the same host crop for pathogen, breaks its cycle and helps in control of pest and disease transmission. Plant diversity reductions are anticipated to lower soil microbial biomass, change microbial functions, and risk the soil ecosystem services (McDaniel and Grandy, 2016). Aziz et al. (2011) concluded that diverse crop rotation enhanced microbial biomass, basal respiration, aggregate stability, and organic matter values when compared to mono-cropping system. A meta-analysis conducted by Venter et al. (2016) revealed an increased microbial richness and diversity with crop rotation. Alhameid et al. (2019b) concluded that more diverse crop rotation systems have reduced soil ρ_b and soil penetration resistance when compared to less diverse rotation systems. Also, the chemical properties of soil such as carbon and nitrogen plays a major role in global C and N cycling and hence are the important indicators of soil health. Agomoh et al. (2021) found that crop rotation with different species enhanced the total C, total N, water extractable C and N as compared to the monocropping. Similar results of increased C and N fractions with crop rotation of different species were also demonstrated by Triberti et al. (2016); Van Eerd et al. (2014). The crop rotation practices has positive effects on microbial biomass C, community and hence are responsible for the increase of soil aggregate stability (Six et al., 2002). This was supported by the findings of Singh et al. (2018) who concluded that crop rotation has increased soil glomalin related protein and ultimately increased the aggregate stability than compared to monocropping practice. Therefore, the literature review of this chapter concludes that soil health indicators were influenced by crop rotation systems and their study is essential.

2.3.3 Impacts of tillage with crop rotation on soil health indicators

The interaction impacts of tillage by rotation on soil health are important because the conservation practices such as NT and crop rotation are being carried out simultaneously in the field. Hence, the soil functions and ecological interactions are the responses of tillage and rotation systems together. From the literature review of this chapter, it is clear that conservational cropping practices such as NT and crop rotation can have positive results on various soil physical properties – soil structure, aggregate stability, porosity, water holding capacity, ρ_b (Idoko Haruna and Vakanda Nkongolo, 2015); chemical properties – pH, cation exchange capacity, carbon and nitrogen content, soil organic carbon - (M. Tahat et al., 2020); biological properties –microbial biomass community, enzymatic activity, microbial respiration (Alhameid et al., 2019a) compared to conventional and monocropping systems. The overall beneficial effects on all these properties eventually results in improvement of soil health. Tillage and cropping systems directly affect soil health and crop yield. Nunes et al. (2018) conducted a study to demonstrate the effects of tillage and crop rotation on soil health and observed that NT with diverse crop rotation has increased soil health over intensive tillage and monocropping. Hence, the interaction impacts of tillage are rotation on soil health indicators are worth studying.

2.4 Research gaps

The literature reviewed revealed that previous studies have evaluated the impacts of tillage and crop rotation on soil hydro-physical properties and other soil health indicators separately. However, there are some research gaps among the studies those are mentioned below as:

i. Very few studies have explored the soil pore characteristics in the soils under long-term tillage and rotation using XCT technique that provides 3D spatial and geometrical visualization of soil pores.

ii. Previous studies investigated the impacts of tillage and crop rotation systems separately on various soil health indicators, and the studies that assessed the soil physical, chemical, and biological properties as a whole are limited.

Therefore, the present study takes an opportunity to address the above-mentioned research gaps with the main aim of the study as to assess the long-term tillage and crop rotation practices on (i) soil hydro-physical properties, and pore characteristics estimated using XCT technique, and (ii) various soil health indicators.

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CHAPTER 3

SOIL HYDRO-PHYSICAL AND COMPUTED TOMOGRAPHY MEASURED SOIL PORE CHARACTERISTICS AS INFLUENCED BY LONG-TERM TILLAGE AND CROP ROTATION

ABSTRACT

Soil hydro-physical and pore characteristics are crucial in crop production as they transfer water, air, and nutrients through the soil. This study assessed the impacts of long-term tillage and crop rotation on soil hydro-physical and X-ray computed tomography (XCT)-measured soil pore characteristics. Conventional methods of measuring soil porosity fail to provide information on spatial distribution and geometrical features of pore network at a micrometer scale. Thus, the present study utilized XCT technique (0.26 × 0.26 × 0.28 mm resolution) to identify the tillage with rotation treatments impacts on soil pore properties. The treatments included long-term tillage [no-till (NT); reduced till (RT) - disk till; conventional till (CT) - moldboard plow till] and crop rotation [continuous corn (CC) – *Zea mays* L. and corn-soybean (CS) – *Glycine max* [Merr.] L.] with four replications in split plots arranged as randomized complete block design. Intact soil cores of 7.62 by 7.62 cm were collected from all the treatments up to 40 cm soil depth in 10-cm increment. Data from the present study showed that NT with CS lowered the bulk density (ρ_b) than the other systems. Amount of soil water retained at matric potential (ψ_m) of saturation (0) to -30 kPa was higher under NT for 0-10 and 10-20 cm depths than the other tillage treatments and depths. The NT also increased the saturated hydraulic conductivity (K_{sat}) and plant available water (PAW) by 59.6 and 53.8%, respectively, than the CT treatment for 10-20 cm depth. The CC with NT system enhanced the SOC (33.3 g kg^{-1}), and TN (2.74 g kg^{-1}) as compared to the other treatments. However, the XCT-measured number of macropores and mesopores (5042 and 278, respectively) were higher with the CS with NT treatment. The XCT-measured soil pore properties were well correlated with ρ_b, SOC, PAW, and K_{sat}. The present study emphasizes that NT with CS can potentially improve soil pore characteristics and associated hydro-physical properties.

***Key words*:** X-ray Computed Tomography, no-till, conventional till, continuous corn, corn-soybean, plant available water, saturated hydraulic conductivity, number of pores

3.1. Introduction

Conservation agricultural practices such as crop rotations and minimum tillage systems have been gaining substantial recognition with respect to economic and environmental benefits. Conservation tillage with diverse crop rotations considered to be beneficial in various ways such as enhanced crop yield, controlled insect-pest diseases, lower erosion, and various others. Some of the advantages of reduced tillage operations over intensive plowing include: fewer expenses, high carbon storage, lower energy input/output ratio, decreased erosion, improved stability against compaction (Palm et al., 2014). No-till (NT) systems can have varied effects on soil structural quality parameters such as saturated hydraulic conductivity (K_{sat}), penetration resistance, water retention due to the less disturbance to soil and highest availability of residues (Blanco-Canqui and Ruis, 2018). Increase in stable aggregates in NT system can usually decrease the rate of soil erosion, thus favoring environmental protection (Pires et al., 2017). However, literature shows contrasting conclusions on effects of tillage and rotations on soil properties. Halvorson et al. (2002) reported an increase in soil bulk density (ρ_b) under NT compared to the conventional tillage (CT) system, whereas, findings of Huggins et al. (2007) showed that ρ_b was not affected by tillage. Studies of Kumar et al. (2012a) reported decrease in ρ_b under NT compared to the reduced and intensive (plow) till. The CT causes the disintegration of aggregates, and removal of surface residues in this system can result in the susceptibility to erosion.

Crop rotation has additional benefits to crops and soil such as enhanced crop yield, increased soil fertility, reduced soil erosion and improved soil structure (Dias et al., 2015). It also reduces the crop stress from plant available nutrient levels and weeds (Smith et al., 2008). Crop rotation with leguminous species (e.g., soybean *Glycine max* [Merr.] L.]) has an extra benefit of N fixing that is not originally present in soil. It can help in breakdown of pest infestation cycle from previous crop if a host crop is not present. Crop rotation when included with NT were shown to have beneficial results on many soils physical, chemical, and biological properties. However, it is not always possible to make a precise statement on the cause and impact of crop rotation on soil organic carbon (SOC) and other related soil properties such as water retention, K_{sat} and porosity. Russell et al. (2005) reported higher SOC in top 15 cm of CC as compared to 4-year rotation of corn (*Zea mays* L.)–corn–oat (*Avena sativa* L.)–alfalfa, whereas, Omonode et al. (2006) reported no effect of crop rotation on SOC at any depth in corn-corn and corn-soybean with chisel and disking tillage systems.

A better understanding of how tillage and rotation systems affect soil physical and hydrologic characteristics is crucial to overall soil performance. The present study focuses on tillage practices along with crop rotation impacting soil hydro-physical properties and soil pore characteristics. The traditional ways of assessing porosity such as water retention method (Anderson et al., 1990), Boyle's porosimetry method, and thin section analysis (van Golf-Racht, 1982) are time consuming and few are destructive. These methods also did not mention about other pore characteristics and distribution of pores in spatial variability (Udawatta et al., 2006). As a solution, X-ray Computed Tomography (XCT) provides information on various pore characteristics, pore distribution in both spatial and temporal variability without any destruction (Kumar et al., 2010a). The XCT scanning technology has emerged as a significant technical improvement in the imaging and measurement of soil structure in recent decades (Taina et al., 2008). Due to its non-invasive nature, the XCT technique helps to analyze the pore parameters of same soil sample in temporal distribution as well (Kumar et al., 2010b). The high-resolution images of XCT scanning technique allows to quantify the micro structure of soil in 3D view for pore continuity, fractal dimension, tortuosity etc. (Peng et al., 2014).The application of XCT scanning technique to assess the soil hydro physical properties were also supported by previous studies. Beckers et al. (2014) employed the technique in measuring XCT derived retention, unsaturated hydraulic conductivity curves with macroscopic measurements. In the present study we used XCT technique to visualize the soil pore characteristics influenced by tillage practices and crop rotation systems.

Majority of studies were limited to surface 10 or 15 cm depth, and very few have reported how soil properties varied with the deeper depths. Water retention, carbon storage, hydraulic conductivity play important role for the crop growth (Chalise et al., 2018). Assessing the impacts of management practices on root growing depth of soil (e.g., 12-18 inches or up to 45 cm for corn) can considerably benefits in various aspects of mangement. For example, the ρ_b can increase with increase in depth; however, NT has lower ρ_b as compared to the CT (Mishra et al., 2010). Contrastingly, the number of macropores, mesopores decreased with increase in depth from 0-40 cm (Udawatta et al., 2008).

The present study was conducted to study the impacts of tillage and rotation, and their interactions on soil hydrological and physical characteristics up to 40 cm depth. Specific objectives of the study are to: (i) assess the impacts of tillage and crop rotation impacts on soil hydro-physical properties up to 40 cm depth such as ρ_b, XCT-measured pore parameters, saturated hydraulic conductivity (K_{sat}), thermal conductivity (λ), water retention, plant available water (PAW) and organic carbon (SOC), and (ii) to estimate XCT-measured pore characteristics impacted by tillage and crop rotation interaction, and correlate measured soil hydro-physical properties with XCT-measured pore characteristics.

3.2. Materials and methods

3.2.1 Experimental site

The study site was located at Haskell Agricultural Laboratory of University of Nebraska Lincoln, near Concord, NE USA (42.38°N, -96.98°W) (Blanco-Canqui et al., 2014). The long-term trial was established in 1985 and was managed under rainfed conditions. Average annual precipitation for the last 10 years for the study site was 672 mm. The dominant soil series was Coleridge silty clay loam (fine-silty, mixed, mesic, Cumulic Haplustolls) with small amounts of Baltic silty clay (fine, smectitic, calcareous, mesic Cumulic Vertic Endoaquolls) and Maskell loam (fine-loamy, mixed, superactive, mesic Cumulic Haplustolls) (Blanco-Canqui et al., 2014). The experimental study was randomized complete block design with four replications arranged as split plots. The main plots were tillage treatments with each plot size of 30.5 m × 61 m. The sub-plots were crop rotation treatments with individual plot size of 10.7 m × 30.5 m. Different tillage treatments included with the study were reduced till (RT) – disk plow, no-till (NT), and conventional till (CT) – moldboard plow). Cropping patterns followed in the experiment site were continuous corn (CC); and corn-soybean rotation (CS).

3.2.2 Soil Sampling and analysis

Intact soil core samples from four replicates of all the treatments were collected in July 2020 from four different depths i.e., 0-10, 10-20, 20-30, and 30-40 cm. Soil core sampler was used to collect the samples by driving it vertically into the soil. Plexiglass cores of dimensions 76.2 mm long and 76.2 mm in diameter, with a 3.2-mm-thick wall were used. Samples were collected to measure the effects of different tillage treatments in interaction with crop rotation on soil physical properties and XCT-measured soil pore characteristics. The collected samples were wrapped, labelled, and transferred to the laboratory. The extra soil was trimmed, and the soil cores were stored at 4°C.

3.2.3 Bulk density, soil organic carbon, and total nitrogen

The ρ_b was measured using the core method (Blake and Hartge, 1986). Soil organic C and total N content for all the collected samples at different depths were measured by dry combustion method using TruSpec CN628 analyzer (LECO

Corporation, St. Joseph, MI). Around 0.25 g of soil sample weighed in tin foils was fed to the CN628 analyzer to measure C and N concentration and was expressed in g kg^{-1}.

3.2.4 Soil water retention, plant available water content, saturated hydraulic conductivity, and saturated thermal conductivity

The SWR in saturated cores were determined by capillarity and gradual draining at five matric potentials (0, −0.5, −5.0, −30.0 and −1500 kPa) using the combination of tension table, pressure plate extractors (Soil moisture Equipment Corp., Santa Barbara, CA, USA) (Dane and Topp, 2020; Klute and Dirksen, 1986) and WP4C Water Potential Meter (METER group Inc., Pullman, WA) . The PAW for the soil cores was determined by subtracting the moisture retained at field capacity (-30 kPa) and permanent wilting point (-1500 kPa). After measuring for SWR, K_{sat} was determined for all the cores using constant head method (Klute and Dirksen, 1986) by employing Darcy's equation:

$$K_{sat} = \left(\frac{Q}{At}\right)\left(\frac{L}{L+H}\right) \qquad [1]$$

where, Q is the outflow volume (cm^3), A is the cross-sectional area of soil column (cm^2), t is the time (hr), L is the length of soil column (cm), H is the height of pounded water at the top of soil column (cm). Also, thermal conductivity (λ) of all the sampled cores was determined using Tempos thermal analyzer (METER group Inc., Pullman, WA) and was expressed in W m^{-1} K^{-1}.

3.2.5 XCT Scanning and Image analysis

The cores were sealed with plastic caps on both the ends of plexiglass and masking tape and were stored in cold conditions before scanning. They were later transported in a cooler for XCT scanning to the Veterinary health center of University of Missouri, Columbia, MO. Toshiba Aquilion 64, Amber Diagnostics XCT scanner was used for the scanning of samples. The soil cores were placed horizontally on the scanner plank to acquire a 360-degree rotation scanning with a peak voltage current of 135 kV and an X-ray tube current of 250 mA. The X-ray beam width thickness was 0.28 mm, resulting in a voxel size resolution of 0.26 × 0.26 × 0.28 mm^3 (voxel is a unit representing a data point in a 3D grid). A field of view of 512 × 512 mm pixels was used to image the entire sample. The obtained scanned data was analyzed by a public domain software program *ImageJ 1.8.0* (Schindelin et al., 2012). Initially, the 3D images were cropped to acquire a region of interest which excludes the core walls and uneven surfaces of soil. To decrease noise, stacks were pre-processed with a median 3D filter with radius of 2.0 voxels (Luo et al., 2010), and contrast enhancement was applied with saturated pixels of 0.4 percent to increase the contrast between the soil matrix and pores in the picture. The stacks were then converted to 8-bit images to which the further processing was allowed. The adaptive local thresholding approach of Phansalkar method (Phansalkar et al., 2011) was used to segment the pores. The mean and standard deviation of the grey values of the nearby pixels were used to compute the threshold value of each pixel in this approach (Singh et al., 2021). Pores were recognized as pixels with gray values less than the threshold value. This technique produced a binary picture with white and black pixels representing the pores and soil matrix, respectively. A closure operation was used to eliminate the dispersed features with a one-pixel width. To assess the statistics of individual pores, porosity, pore size distribution etc., the Particle Analyzer plugin inside the BoneJ plugin in ImageJ (Doube et al., 2010) was employed. Image based soil porosity was calculated as follows:

$$\text{Porosity} = \frac{\text{Total volume of pores}}{\text{Volume of the ROI}} \qquad [2]$$

where, *ROI* is the region of interest.

Using *Skeletonize 3D tool in BoneJ plugin*, other pore structural parameters such as degree of anisotropy – an indicator of 3D pore symmetry; 3D fractal dimension – indicator of self-similarity and surface detail, estimated through a box-counting algorithm; and tortuosity – ratio of total actual lengths of all macropores to the sum of the shortest distance between two ends of macropores (Katuwal et al., 2015) were determined from the skeletons. The workflow showing the procedures involved in image processing of data scanned by XCT was given as Figure 3.1.

3.2.6 Statistical Analysis

The normal distribution of the data was tested using Shapiro-Wilk test and homogeneity of variance were tested using Levene's test. Analysis of variance (ANOVA) was performed at 5% level of significance (p<0.05) using R Studio software version 1.3.1093 (Team, 2013) to determine the effects of treatments on measured soil hydro-physical and XCT-measured soil pore properties . The treatment means were compared using Tukey's honest significant difference test. The Pearson correlation coefficients were used to create a correlation matrix and simple linear regression was used to determine the relationship between XCT-measured soil pore characteristics and other hydro-physical properties

3.3. Results

3.3.1 SOC, ρ_b, and TN

Data for SOC concentrations under different rotation and tillage systems for 0-10, 10-20, 20-30 and 30-40 cm depths were presented in Figure 3.3. The $p>F$ values at 5%

significant level for treatments was presented in Appendix 1.A. Crop rotation did not impact the SOC when averaged across tillage and depths. The tillage treatments influenced the SOC only at 0-10 and 30-40 cm depths. The NT system improved the SOC concentration by 24 and 49% at the 0-10 cm depth as compared to the RT and CT, respectively ($p<0.001$). At 30-40 cm, the NT and RT treatments had 58 and 73% higher SOC than the CT ($p<0.001$) (Appendix 1.C). The SOC concentration averaged across rotation and tillage, was greater for 0-10 cm depth as compared to the 10-20, 20-30, and 30-40 cm depth.

Soil ρ_b impacted by rotation, tillage, and depth was shown in Figure 3.3. The NT system under CS rotation decreased the ρ_b as compared to the other treatments (Appendix 1.A). Averaged across the rotation and tillage systems, the ρ_b for 0-10 cm depth was observed to be 11, 13, and 16% lower than 10-20, 20-30, and 30-40 cm depths, respectively (Appendix 1.C).

Data for TN concentrations influenced by rotation and tillage systems for 0-10, 10-20, 20-30 and 30-40 cm depths was presented in Figure 3.3. Crop rotation system did not affect the TN when averaged across tillage and depths (Appendix 1.A). The tillage treatments influenced the TN only at 0-10 depth. The NT system improved the TN concentration by 26 and 67% at the 0-10 cm depth as compared to the RT and CT ($p<0.001$) (Appendix 1.C). The TN concentration averaged across rotation and tillage, was greater for 0-10 cm depth by 47, 50, and 77% as compared to the 10-20, 20-30, and 30-40 cm depth.

3.3.2 K_{sat}, λ, and PAW

Data for K_{sat}, λ, and PAW affected by crop rotation, tillage, and depth was presented in Table 3.1. The interaction effect of rotation, tillage, and depth on K_{sat} was observed to be significant (p=0.001) (Appendix 1.B). The NT system under CS rotation increased the K_{sat} as compared to other treatments at 0-10 cm depth but is not different from NT-CC. However, at 30-40 cm depth, the NT system under CC was observed to have higher K_{sat} than the other treatments except CS-NT and CC-RT.

The rotation and tillage systems did not affect the λ at any soil depths except for 10-20 cm depth (Appendix 1.B). The NT system with CC has significantly higher λ mean value as compared to the NT-CS but not with the other interaction at 10-20 cm depth.

The interaction effects of rotation, tillage, and depth on PAW was not significant. However, the individual factors, tillage and depth affected the PAW content (Appendix 1.B). Averaged across the rotation within 10-20 cm depth, the NT increased the PAW content by 25 and 67% as compared to RT and CT respectively (p=0.027). The PAW content at 0-10 cm depth was observed to be higher by 1.1 and 1.8 times than 20-30 and 30-40 cm depths, respectively (p<0.001).

3.3.3 XCT-measured Pore Properties

The data for XCT-measured pore properties revealed the impacts of rotation, tillage, and depth on number of mesopores, macropores, mesoporosity, macroporosity, tortuosity (τ), fractal dimension (D), total number of branches, and mean of average branch length. The crop rotation and tillage treatments impacted the number of mesopores for every depth except for 30-40 cm depth (Figure 3.4). The NT under CS rotation increased the number of mesopores at 0-10 (5042) and 10-20 cm (3290) depth than compared to the other treatments (Appendix 1.D). However, at 20-30 cm depth, higher mean values for number of mesopores was observed under RT with CS rotation (3034) but is not significantly different from CC-RT (2443), CC-NT (2555), and CS-NT (2591) (Appendix 1.D). The interaction effect of rotation, tillage, and depth was significant on number of macropores (p<0.001) (Appendix 1.B). The NT system under CS rotation at 0-10 cm depth increased the number of macropores as compared to the other tillage and rotation treatments at all the depths (Appendix 1.D).

The tillage and rotation treatment effect on mesoporosity and macroporosity at different depths was presented in Figure 3.5. The crop rotation did not influence the mesoporosity when averaged across tillage and depth (Appendix 1.B). The NT at 0-10 cm depth was observed to have 14 and 99% higher mesoporosity than RT and CT respectively (Appendix 1.E). Similarly, the NT at 0-10 cm depth has higher macroporosity at all the depths when averaged across rotation (Appendices 1.B and 1.E). The CS rotation increased the macroporosity by 18 and 10% at 10-20 and 20-30 cm depth, respectively, as compared to the CC system (Appendices 1.B and 1.E).

Tillage and rotation have significant influence on fractal dimension (D) at 0-10 and 30-40 cm depths only (Table 3.2). The NT under CC was observed to have higher D mean values at 0-10 and 30-40 cm (2.57 and 2.56 respectively) than compared to the other treatments (p<0.001). No effect of tillage, rotation and depth was observed on τ except that CC-CT at 0-10 cm depth had less τ than compared to the other treatments (Table 3.2). The interaction of tillage, rotation, and depth significantly influenced the total number of branches (p<0.001) (Appendix 1.B). The RT system under CC increased the total number of branches at 0-10 cm depth than compared to the other tillage and rotation treatments (Table 3.3). Similarly, at 20-30 cm depth, CS-NT and CC-RT increased total number of branches than CS-CT. However, at 30-40 cm depth, CC with NT had increased number of branches than other interactions. The data for mean of average branch length was not consistent with the rotation, tillage treatments and depth. The higher mean values at 0-10 cm depth were observed under NT with CS rotation (Table 3.3). But the RT treatment with CC system was observed to have higher mean of average branch length than CC-NT at 20-30 cm depth.

3.3.4 Soil Water Retention (*SWR*)

The SWR differed among the 0, −0.5, −30.0 and −1500 kPa for 0-10 cm depth and 0, −0.5, −5.0, and −30.0 kPa for 10-20 cm depth (Figure 3.6). Crop rotation did not impact the SWR for any depth. However, tillage had significant influence on water retained at different ψ_m for 0-10 and 10-20 cm depths. At 0-10 cm depth, the NT treatment significantly enhanced the water retained by 28.2, 29.7, 48, and 15.4% for 0, −0.5, −30.0 and −1500 kPa, respectively, as compared to the CT. Similar trend was observed for 10-20 cm depth, where, NT had significantly increased the amount of water retained by 22.2, 22.9, 25.8, and 30.1%, for 0, −0.5, −5.0, and −30.0 kPa, respectively, as compared to CT system. However, no interaction effects of tillage by rotation on SWR was observed for all the ψ_m and depths. The amount of water retained decreased with increasing depth. The 0-10 cm depth retained significantly higher water for all the rotation (CC and CS) and tillage (NT, RT, and CT) treatments than the deeper depths. Though there was gradual reduction with depth in the water content at -1500 kPa, significant difference was not observed.

3.3.5 Correlation of soil pore parameters with other soil properties

The linear relationship of XCT-measured pore parameters and soil hydro-physical properties was assessed using Pearson's correlation coefficient (Table 3.4). Soil ρ_b had a strong negative correlation with number of pores (macropores and mesopores), macroporosity, SOC, and other soil properties such as PAW and K_{sat}. Soil water properties (PAW and K_{sat}) were positively correlated with XCT-measured parameters such as number of macro and mesopores, macroporosity. Similarly, the SOC was positively correlated with most of the measured hydro-physical properties and pore parameters. The measured soil properties such as K_{sat} and PAW were regressed with XCT-measured number of macropores and mesopores. The coefficient of determination (R^2) values ranged from 0.41 to 0.47 (Figure 3.7) for the fitted regression lines.

3.4 Discussion

3.4.1 SOC, TN, and ρ_b

Large body of literature is available on the effects of tillage and crop rotation system on the SOC and TN. The study of Van Eerd et al. (2014) on soil quality, organic carbon, total nitrogen impacted by long-term tillage and crop rotation concluded that adopting NT practices with crop rotation can improve the storage of SOC and TN in the soil. Also, the findings of Havlin and Kissel (2019) suggested that crop management strategies that combine high-residue-producing crop rotations and decreased or NT surface residue cover result in higher SOC and nitrogen, which may boost soil productivity. Similar results of zero tillage with crop rotation impacts on SOC was reported by Jat et al. (2019) in their study on cereal systems of semi-arid Northwest India. Supporting the above literature, Karlen et al. (2013) in their study reported that long-term moldboard plowing had negative effects on soil quality in central Iowa, USA. Also, Alhameid et al. (2017) in their study of SOC changes impacted by crop rotational diversity under NT in SD reported that SOC concentrations and other soil properties were increased under long-term diverse crop rotations with NT system. Literature shows that the NT system increased the TN concentration over the CT; for e.g., Zuber et al. (2015) in their study of crop rotation and tillage effects on soil physical and chemical properties reported 8.87 Mg ha^{-1} and 8.40 Mg ha^{-1} of TN concentration for NT and CT, respectively. Similar results of higher SOC concentrations under NT system was reported by Martínez et al. (2016) in their study of crop yield, SOC and nutrient distribution in the soil profile near Berne, Switzerland. Availability of crop residues on the soil surface for longer period can help in continuous supply of organic matter for decomposition and thus improves the SOC concentration which is in accordance to the findings of (Raphael et al., 2016). As a result, less soil disturbance is preferable to intense tillage in decreasing carbon losses in agricultural soils (Zibilske and Bradford, 2007). However, the rate of SOC buildup in NT soils, also varies depending on climatic circumstances, the quantity of residue and nitrogen (N) inputs, and the soils mineralogy (Kumar et al., 2014).

Soil ρ_b can provide a quantitative measurement of the impacts of tillage and rotation systems on soil physical property. Generally, soil ρ_b can indicate soil compaction to varying degrees depending on the type of tillage used. In the present study, CC crop rotation increased the soil ρ_b than compared to the CS which is in accordance to the findings of previous studies; for e.g. (Karlen et al., 2006). The CT resulted in higher ρ_b as compared to NT and RT systems. Similar findings were reported by (Huang et al., 2012) in their study of different tillage systems on soil properties in which NT resulted in lower ρ_b as compared to CT. Higher ρ_b under CT could be due to the settling of soil under the influence of rainfall after the tillage resulting in breaking up of aggregates. Other likely reason for lower ρ_b in long-term NT can be due to the undisturbed soil has continuous fissures and old root channels (Martino and Shaykewich, 1994). The results of ρ_b from this study are consistent with the findings of Topa et al. (2021) in which they observed an increased ρ_b under the CT. The results are also in accordance of (Gao et al., 2019) that reported increased ρ_b for CT at surface depths. Irrespective of the treatments, the ρ_b increased with increase in soil depth, can be due to particle resettlement and, wetting and drying cycles (Blanco-Canqui and Ruis, 2018).

3.4.2 K_{sat}, λ, and PAW

Various factors such as climate, topography, and parent material impact saturated hydraulic conductivity. Still, the tillage treatments can alter the K_{sat} by influencing the ρ_b and porosity (Indoria et al., 2017). The K_{sat} largely represents saturated water flow via the macropores. Earlier research has found that the quantity of macropores can explain up to 64% of the variability in K_{sat} measurements (Udawatta et al., 2006). It is also greatly impacted by the processes involving in the formation of soil structure and macropores. The K_{sat} for this study is higher under NT followed by CT and RT for shallow depths. However, the conductivity at deeper depths (30-40 cm) is least for CT treatment. The NT soils that are left undisturbed for longer periods provides favorable niche for the growth and development of earthworms. With time, the trend for NT is that an increase of macropore connectivity and hence K_{sat} (Strudley et al., 2008). Previous literature also supported the findings that NT increased K_{sat} as compared to CT; for e.g., (Schlüter et al., 2020). Similar results were reported by Anwar et al. (2017) in their study on the effect of five cropping systems on K_{sat}. Increased K_{sat} under crop rotation systems were in accordance to the findings of Dexter et al. (2001)

The proportion of water-and -air -filled soil pores affect the soil temperature by their contrasting effects on soil thermal conductivity and heat capacity. Water increases both soil thermal conductivity and soil heat capacity, whereas air has the reverse effect (Obia et al., 2020). The λ under NT is lower as compared to CT in the present study which is in accordance to the findings of Cook et al. (2006) who reported that conservation farming such as NT and crop rotation with residue cover reduced the near surface soil temperatures due to increased soil moisture. The NT farming can enhance soil moisture content by increasing the proportion of water-filled pores (Obia et al., 2018) and hence the soil thermal conductivity and heat capacity.

The PAW content in the present study observed to be higher under NT treatment which is in accordance to the findings of de Moraes et al. (2016) who reported that long-term NT for 24 years had improved PAW than that of other treatments of their study. Similarly, Celik et al. (2012) in their study of crop rotation and tillage effects on soil physical properties stated that the amount of PAW at surface depths was substantially reduced with CT system. Also, the literature supports the above findings that soils under NT retained higher quantity of available water to plants; for e.g., (Hernández et al., 2019). Reports of Kumar et al. (2012a) in their research of long-term NT impacts on organic carbon and properties of Ohio soils supports the present study findings of higher PAW under NT treatment as compared to the CT.

3.4.3 XCT-Measured Pore Characteristics

Tillage systems has been linked to an increase in soil porosity, particularly in macroporosity (Pöhlitz et al., 2018).The findings of Schlüter et al. (2018) in their study of 25 years of different tillage management at Wester Feld trial in Bernburg, Germany reported that long term NT had positive effects on porosity and pore sizes. Also, the works of Budhathoki et al. (2022) concluded that, under NT macropores were bigger and had more established pore networks. NT soils are thought to contain more stable particles on the top surface than tilled soils, resulting in higher overall porosity in NT plots. The review works of Busari et al. (2015) on conservation tillage in different agroecological regions reported that conservation tillage, which includes zero tillage and minimal tillage, has the capacity to break up the surface compact zone in soil while causing less soil disturbance. However, lower porosity in conventional tillage is most likely due to the collapse of larger unstable aggregates and the resulting rise in smaller aggregate sizes (So et al., 2009). Supporting the studies, the works of Abu and Abubakar (2013) reported that in the NT plot, the maximum quantity of water was retained throughout all soil matric potential ranges and soil depths, also, the CT reduced soil physical quality by 0.1–18.3% as compared to NT.

Fractal dimension determines the space-filling character of a pore that varies with the number of pores and the size distribution (Rachman et al., 2005). Hence, the higher D values under NT supports the increased number of pores than RT and CT. High tortuosity values are typical with soils having a large number of unconnected pores and in comparison to soils with higher pore connectivity, they are more likely to have lower hydraulic conductivity and gaseous diffusion. (Ferreira et al., 2018). Though there was no significant difference observed, the mean tortuosity values in the present study for the NT were lower than the CT except for 0-10 cm depth indicating better connectivity of the pores, which is in accordance to the study of (Galdos et al., 2019). The uneven distribution of number of branches at different depths was also reported by Wang et al. (2019). The higher number of branches under disturbed tillage system (RT) in the present study followed the findings of Dal Ferro et al. (2014). The study also concluded that NT has higher mean of branch length than the CT. Similar results of increased number of branches under disturbed soils was also reported by (Munkholm et al., 2013)The rotation impacts on soil pore properties were explained by (Singh et al., 2020) in their study of soil pore parameters influenced by crop rotation and cover crops; the study concluded that crop diversification had positive significant influence on XCT-measured pore properties. Similarly, Alhameid et al. (2019) in their study reported that, NT with diversified crop rotation improved soil physical and hydrological parameters as compared to CT with less varied systems.

3.4.4 Soil water retention (SWR)

The amount of water retained in the soils is linked to the volume of pores with storage size distribution. In the present study, the water retention for 0-10 and 10-20 cm depths is higher for NT treatment followed by RT and CT. The crop rotation systems did not affect the quantity of water retained at any depths, also, the tillage treatments from 20 cm depth and above had no influence of SWR. The findings are in accordance to the studies of (Hernández et al., 2019) who reported no significant effect of crop rotation patterns and no tillage effect at 20-30 cm on SWR. In comparison to the RT and CT treatments, the soils under NT had large volume of pores that hold available water, hence, the water retention at field capacity is greater in NT. The studies of long-term tillage and crop rotation effects on hydrological properties of Ohio soils by Kumar et al. (2012b) also reported that soils under NT had higher SWR than those under minimum tilled and plow tilled soils. Similar findings were reported by Bescansa et al. (2006) in which conservation tillage systems such as NT had considerably increased SWR capacity than the moldboard plow till. Also, the reports of (Arshad et al., 1999) were in accordance to the findings of the present study with higher water retention under NT system.

3.4.5 Correlation of soil pore parameters with soil properties

The strong negative correlation of soil ρ_b with pore properties observed in this study was supported by previous reports of Yang et al. (2018) and Udawatta et al. (2006). Also, Singh et al. (2020) in their study of XCT-measured soil pore parameters influenced by crop rotations and cover crops reported strong inverse relation of ρ_b with number of macropores, mesopores, and macroporosity. Similarly, the positive correlation of soil water properties such as K_{sat} with XCT-measured pore parameters were also supported by the same study of Singh et al. (2020). The significant positive correlation of K_{sat} and macroporosity with other soil properties was in accordance to the findings of Kumar et al. (2010a). This suggests the enhancement of XCT-measured parameters with increase in SOC and decrease in $\rho_{b.}$

The positive relation of K_{sat} and PAW with number of macropores and mesopores estimated with linear regression indicated that the soils with higher pore count can improve the water flow as well as the quantity of water available to the plants. Soli pore structure has a significant impact on water flow in the soil, that is connected to surface runoff and soil permeability. The minimal disturbance improves the soil aggregation and thus enhances the water conductivity and retention (TerAvest et al., 2015). Previous studies have also reported the positive correlation of K_{sat} with XCT measured pore characteristics. For example, Schlüter et al. (2020) reported that regression analyses of soil pore features assessed using XCT method and directly calculated saturated hydraulic conductivity exhibited a high level of congruence.

3.5 Conclusions

The present study applies the technology of XCT-scanning to investigate the soil pore characteristics for 0-40 cm depth (with 10-cm increment each depth) along with other measured soil hydro-physical properties as affected by long-term tillage and crop rotation. The data showed that CC rotation with NT improved the SOC and TN only at 0-10 and 10-20 cm depths, respectively. The corn crop residues with high C:N ratio provides organic matter for long term decomposition and constantly delivers the C and N. However, the other soil hydro-physical properties and pore parameters were higher under CS rotation than the CC. The CS-NT system decreased the soil ρ_b and increased K_{sat} compared to the other interactions. The XCT-measured soil pore parameters strongly correlated with different soil properties (e.g., ρ_b, K_{sat}, and PAW). Most of the measured properties such as number of pores, porosity, SOC, PAW, and K_{sat} etc., decreased with increasing depth. Among all treatments in this study, the combined application of long-term NT with CS rotation resulted in the best overall increase in soil hydro-physical and pore parameters. The findings also demonstrate the great potential for assessing soil structure and functions by combining XCT-derived soil pore parameters with traditional soil hydro-physical measures. Furthermore, the application of advanced tools, e.g., XCT scanning enables the visualization, characterization, and analysis of soil pore structure.

This helps in understanding the spatial distribution of pores and soil porosity in relation to soil water movement.

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Table 3.1. Saturated hydraulic conductivity (K_{sat}), thermal conductivity (λ), plant available water (PAW) as affected by crop rotation (corn-corn, CC; and corn-soybean, CS) and tillage (conventional tillage, CT; reduced tillage, RT; and no-tillage, NT) for 0-10, 10-20, 20-30, and 30-40 cm depths.

	K_{sat} -------------mm hr^{-1}-------------				λ -------------W m^{-1} K^{-1}-------------				PAW ------------cm^3 cm^{-3}------------			
	NT	**RT**	**CT**	$\overline{x}$	**NT**	**RT**	**CT**	$\overline{x}$	**NT**	**RT**	**CT**	$\overline{x}$
					0-10 cm depth							
CC	$86.2^{ab\dagger}$	65.3^{b}	68.4^{b}	$73.3^{A\ddagger}$	1.11	1.08	1.19	1.13	0.23	0.15	0.16	0.18^{A}
CS	134.2^{a}	76.4^{b}	58.8^{b}	89.8^{A}	1.15	1.01	1.18	1.12	0.24	0.19	0.20	0.21^{A}
$\overline{x}$	$110.2^{A\S}$	70.8^{A}	63.8^{A}		1.13^{A}	1.04	1.18		0.23^{A}	0.17^{A}	0.17^{A}	
					10-20 cm depth							
CC	81.1	40.3	41.4	54.2^{AB}	1.16^{a}	1.11^{a}	1.15^{a}	1.14^{A}	0.21	0.13	0.14	0.16^{AB}
CS	51.1	42.7	41.4	45.1^{B}	0.37^{b}	1.13^{a}	1.14^{a}	0.88^{A}	0.19	0.19	0.10	0.17^{A}
$\overline{x}$	66.1^{B}	41.5^{AB}	41.4^{B}		0.76^{B}	1.12	1.14		0.20^{A}	0.16^{AB}	0.12^{AB}	
					20-30 cm depth							
CC	46.7	33.9	17.4	32.6^{B}	1.04	1.10	1.14	1.09^{A}	0.12	0.11	0.10	0.11^{BC}
CS	61.6	57.7	15.7	45.1^{B}	1.02	1.04	1.07	1.04^{A}	0.08	0.08	0.07	0.08^{B}
$\overline{x}$	54.2^{B}	45.8^{AB}	16.5^{C}		1.03^{AB}	1.07	1.11		0.10^{B}	0.09^{B}	0.09^{B}	
					30-40 cm depth							
CC	43.9^{a}	40.5^{ab}	30.1^{b}	38.2^{B}	1.11	1.02	1.07	1.07^{A}	0.08	0.07	0.07	0.07^{C}
CS	31.7^{ab}	29.8^{b}	22.2^{b}	27.9^{B}	1.12	1.12	1.17	1.14^{A}	0.04	0.06	0.07	0.06^{B}
$\overline{x}$	37.8^{B}	32.6^{B}	26.2^{BC}		1.11^{A}	1.07	1.12		0.06^{B}	0.06^{B}	0.07^{B}	

[†]Mean values followed by different lowercase letters between each treatment within each depth and parameter represent significant differences due to a rotation by tillage interaction at $p<0.05$. No letters are shown if the rotation by tillage interaction was not significant.

[‡]Mean values within a column (averaged across NT, RT, and CT), rotation, and parameter across different depths followed by the different uppercase letters represent significant difference ($p<0.05$).

[§] Mean values within a row (averaged across the CC and CS rotations), tillage, and parameter across different depths followed by the different uppercase letters represent significant difference ($p<0.05$)

Table 3.2. X-ray Computed Tomography (XCT) -measured tortuosity (τ) and fractal dimension (D) as affected by crop rotation (corn-corn, CC; and corn-soybean, CS) and tillage (conventional tillage, CT; reduced tillage, RT; and no-tillage, NT) for 0-10, 10-20, 20-30, and 30-40 cm depths.

	Tortuosity (τ)				Fractal dimension (D)			
	NT	**RT**	**CT**	$\bar{x}$	**NT**	**RT**	**CT**	$\bar{x}$
				0-10 cm depth				
CC	1.29^{a†}	1.3^{a}	1.2^{b}	1.26^{A‡}	2.57^{a}	2.52^{a}	2.46^{a}	2.52^{B}
CS	1.25^{ab}	1.27^{ab}	1.24^{ab}	1.25^{A}	2.5^{ab}	2.54^{a}	2.4^{b}	2.48^{AB}
$\bar{x}$	1.27^{A§}	1.29^{A}	1.22^{A}		2.54^{A}	2.53^{AB}	2.43^{B}	
				10-20 cm depth				
CC	1.22	1.23	1.25	1.23^{A}	2.52	2.58	2.55	2.55^{A}
CS	1.25	1.24	1.24	1.24^{A}	2.56	2.56	2.56	2.56^{A}
$\bar{x}$	1.24^{B}	1.24^{A}	1.25A		2.54^{A}	2.57^{A}	2.56^{A}	
				20-30 cm depth				
CC	1.26	1.32	1.26	1.28^{A}	2.52	2.52	2.53	2.52^{A}
CS	1.25	1.25	1.27	1.26^{A}	2.6	2.56	2.49	2.55^{AB}
$\bar{x}$	1.26^{AB}	1.29^{A}	1.27^{A}		2.56^{A}	2.54^{AB}	2.51^{A}	
				30-40 cm depth				
CC	1.26	1.23	1.22	1.24^{A}	2.56^{a}	2.47^{a}	2.36^{b}	2.46^{B}
CS	1.27	1.29	1.44	1.33^{A}	2.44^{ab}	2.45^{ab}	2.46^{ab}	2.45^{B}
$\bar{x}$	1.27^{AB}	1.26^{A}	1.33^{A}		2.50^{A}	2.46^{B}	2.41^{B}	

[†]Mean values followed by different lowercase letters between each treatment within each depth and parameter represent significant differences due to a rotation by tillage interaction at P<0.05. No letters are shown if the rotation by tillage interaction was not significant.

[‡]Mean values within a column (averaged across NT, RT, and CT the tillage treatments), rotation, and parameter across different depths followed by the different uppercase letters represent significant difference (P<0.05).

[§] Mean values within a row (averaged across the CC and CS rotations), tillage treatments, and parameter across different depths followed by the different uppercase letters represent significant difference (P<0.05).

Table 3.3. Total number of branches, Mean of average branch length as affected by crop rotation (corn-corn, CC; and corn-soybean, CS) and tillage (conventional tillage, CT; reduced tillage, RT; and no-tillage, NT) for 0-10, 10-20, 20-30, and 30-40 cm depths.

	Total number of branches				**Mean of average branch length**			
	NT	**RT**	**CT**	$\bar{x}$	**NT**	**RT**	**CT**	$\bar{x}$
				0-10 cm depth				
CC	19300^{b†}	29122^{a}	18841^{b}	22421^{A‡}	0.46^{ab}	0.45^{ab}	0.44^{b}	0.45^{C}
CS	14053^{bc}	15302^{b}	6388^{c}	11914^{AB}	0.55^{a}	0.53^{ab}	0.44^{b}	0.51^{C}
$\bar{x}$	16677^{A§}	22212^{A}	12615^{A}		0.51^{B}	0.49^{B}	0.44^{B}	
				10-20 cm depth				
CC	15685	19114	13291	16030^{B}	0.63	0.63	0.66	0.64^{A}
CS	13936	18666	14029	15544^{A}	0.56	0.54	0.60	0.57^{AB}
$\bar{x}$	14811^{A}	18890^{A}	13660^{A}		0.59^{A}	0.58^{A}	0.63^{A}	
				20-30 cm depth				
CC	14668^{ab}	17142^{a}	15468^{ab}	15759^{BC}	0.49^{b}	0.61^{a}	0.57^{ab}	0.56^{BC}
CS	21962^{a}	13897^{ab}	8114^{b}	14658^{A}	0.58^{ab}	0.55^{ab}	0.56^{ab}	0.56B^{C}
$\bar{x}$	18315^{A}	15520^{AB}	11791^{A}		0.53^{AB}	0.58^{A}	0.56^{A}	
				30-40 cm depth				
CC	15710^{a}	9937^{b}	8068^{b}	11238^{C}	0.57	0.63	0.60	0.60^{AB}
CS	9791^{b}	10288^{b}	7923^{b}	9334^{B}	0.63	0.63	0.61	0.62^{A}
$\bar{x}$	12751^{A}	10113^{B}	7996^{A}		0.60^{A}	0.63^{A}	0.60^{A}	

†Mean values followed by different lowercase letters between each treatment within each depth and parameter represent significant differences due to a rotation by tillage interaction at P<0.05. No letters are shown if the rotation by tillage interaction was not significant

‡Mean values within a column (averaged across the NT, RT, and CT tillage treatments), rotation, and parameter across different depths followed by the different uppercase letters represent significant difference (P<0.05).

§ Mean values within a row (averaged across the CC and CS rotations), tillage treatments, and parameter across different depths followed by the different uppercase letters represent significant difference (P<0.05)

Table 3.4. Correlation matrix for soil hydro physical and CT-measured pore characteristics [ρ_b, bulk density; PAW, plant available water; SOC, soil organic carbon; TN, total nitrogen; K_{sat}, saturated hydraulic conductivity; λ, saturated thermal conductivity; NMP, number of macropores, MP, macroporosity; NmesP, number of mesopores; MesP, mesoporosity; τ, tortuosity; D, fractal dimension; TNB, total number of branches; MBL, mean of average branch length

	ρ_b	PAW	SOC	TN	K_{sat}	λ	NMP	MP	NmesP	MesP	τ	D	TNB	MBL
ρ_b	1													
PAW	0.506***	1												
SOC	-0.55***	0.35***	1											
TN	-0.17	0.16	0.23*	1										
K_{sat}	-0.44***	0.44***	0.50***	0.22*	1									
λ	0.24**	-0.11	-0.01	0.27**	0.03	1								
NMP	-0.52***	0.50***	0.56***	0.25*	0.63***	0.25**	1							
MP	-0.66***	0.40***	0.61***	0.14	0.59***	0.27**	0.75***	1						
NmesP	-0.65***	0.64***	0.55***	0.24*	0.68***	-0.09	0.64***	0.69***	1					
MesP	-0.15	0.29**	0.20*	0.27**	0.20*	0.09	0.12	-0.01	0.31**	1				
t	-0.03	-0.09	0.01	0.01	-0.07	-0.10	-0.06	-0.07	-0.13	0.03	1			
D	-0.14	0.12	0.26**	0.01	0.13	-0.22*	0.29**	0.32**	0.24*	-0.04	0.05	1		
TNB	-0.16	0.19*	0.38***	0.16	0.30**	-0.007	0.29**	0.41***	0.37***	0.10	-0.001	0.59***	1	
MBL	0.32**	-0.29**	-0.22*	-0.19*	0.37***	0.01	-0.28**	-0.32**	0.42***	-0.07	0.002	-0.02	0.31**	1

*Significant at level 0.1
**Significant at level 0.05
***Significant at level 0.001

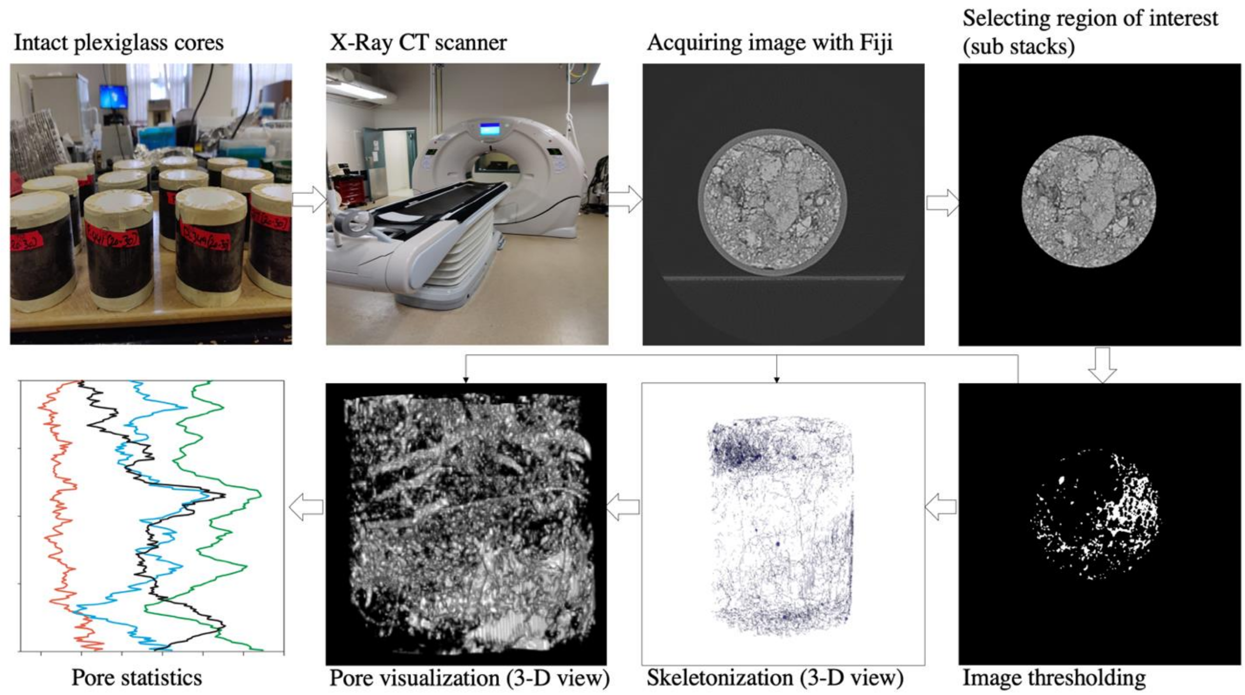

Figure 3.1. Workflow presenting the various steps involved in image processing of X-ray computed tomography–scanned data.

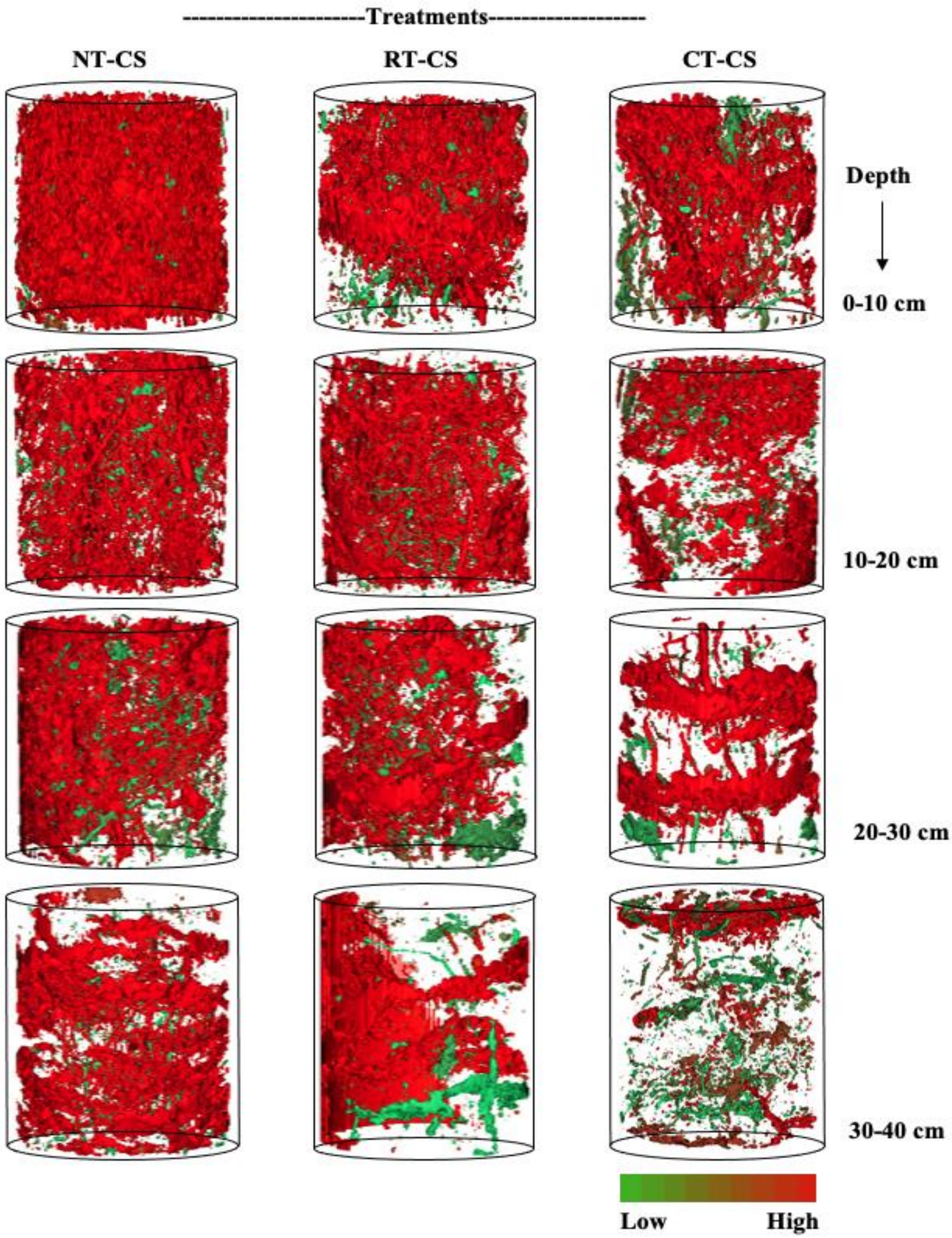

Figure 3.2. X-ray computed tomography derived images to visualize the 3-D macropore distribution in soil as influenced by no-till (NT), reduced till (RT), and conventional till (CT) in corn-soybean (CS) rotation treatments for the depths of 0-10, 10-20, 20-30, and 30-40 cm. Colored macropores are shown in non-porous white background.

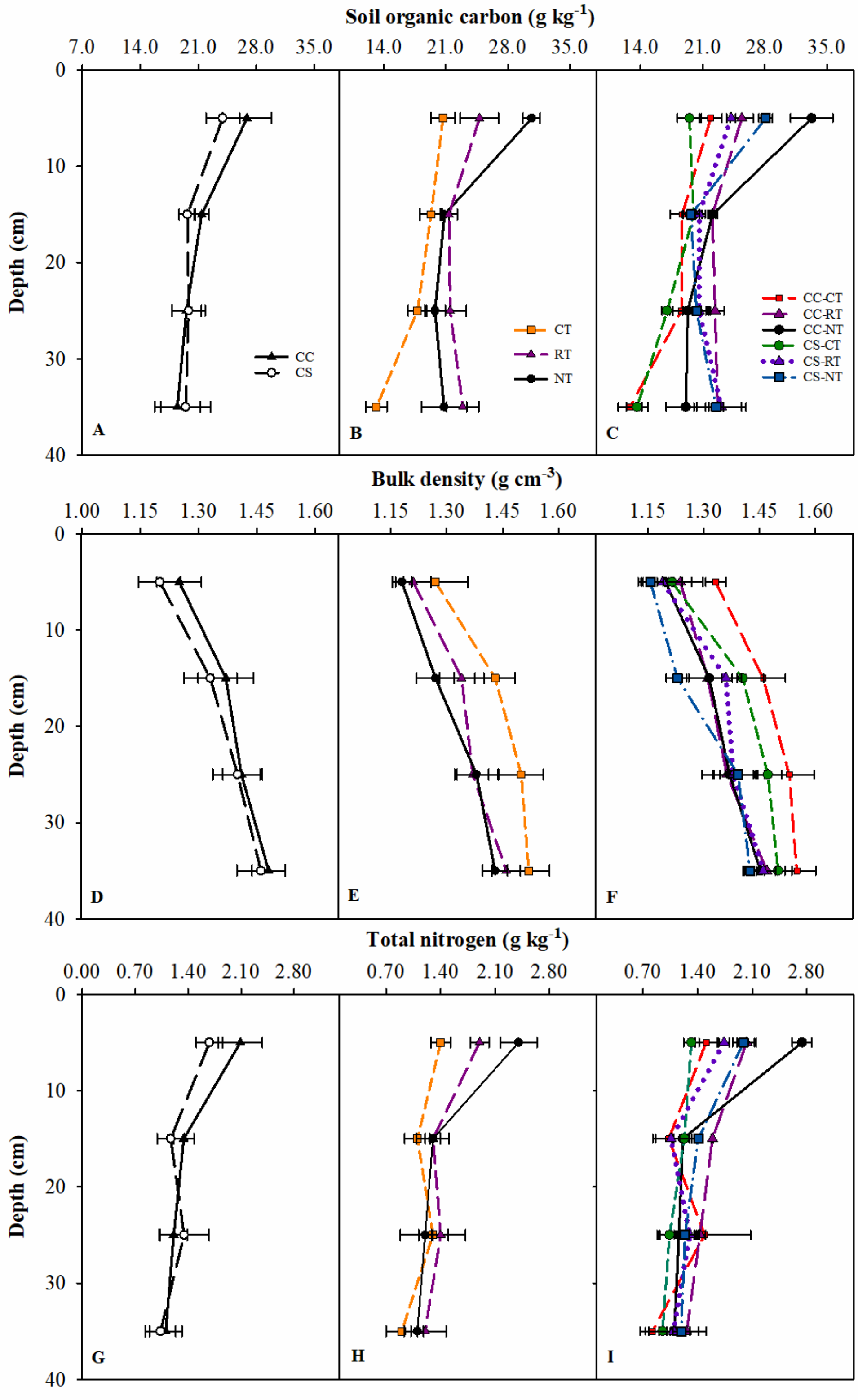

Figure 3.3. Soil organic carbon [A, B, and C], bulk density [D, E, and F], and total nitrogen [G, H, and I] as affected by crop rotation (corn-corn, CC; and corn-soybean, CS), tillage (conventional tillage, CT; reduced tillage, RT; and no-tillage, NT) and rotation-tillage interactions for 0-10, 10-20, 20-30, and 30-40 cm.

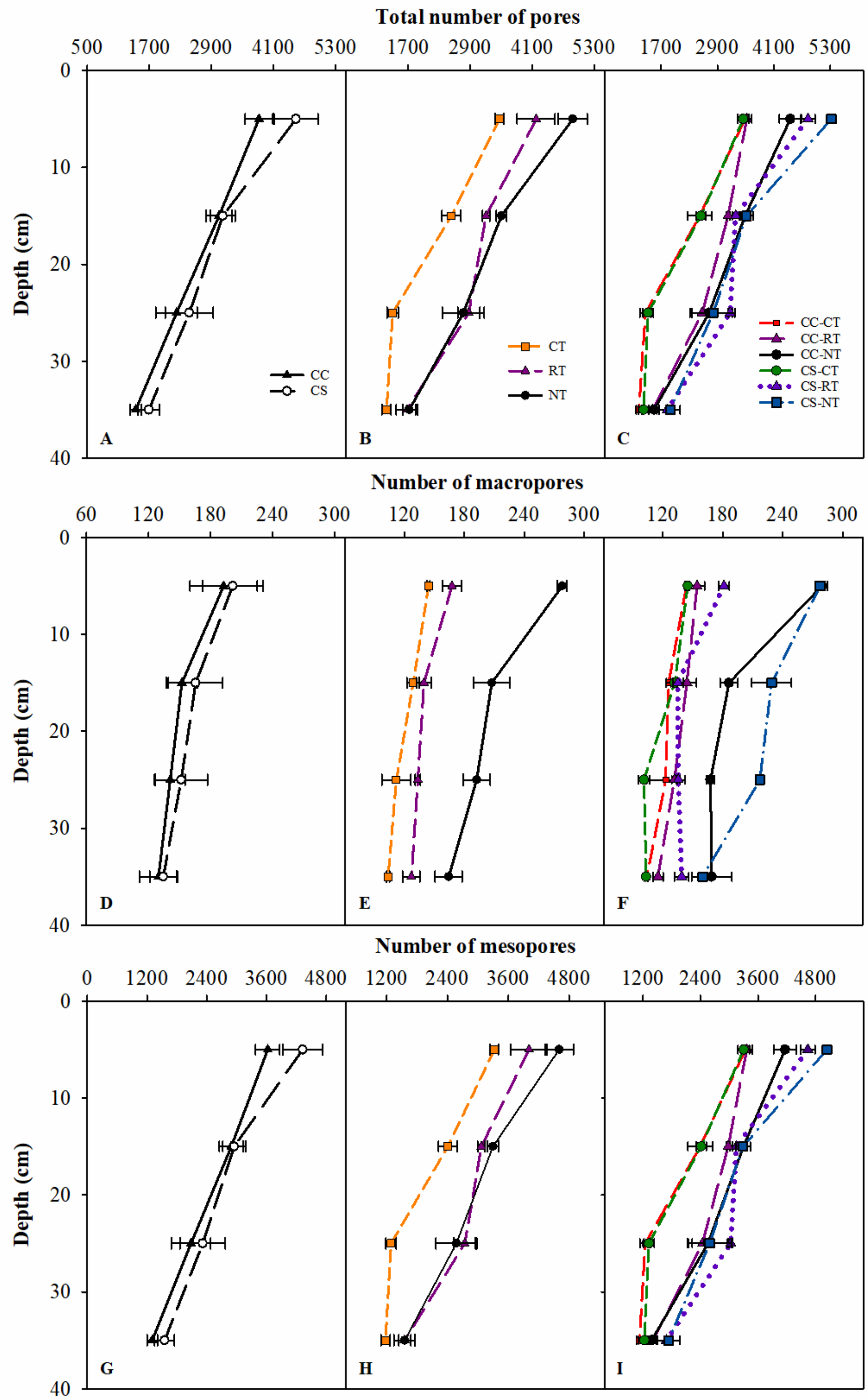

Figure 3.4. Total number of pores [A, B, and C], number of macropores [D, E, and F] and mesopores [G, H, and I] as affected by crop rotation (corn-corn, CC; and corn-soybean, CS), tillage (conventional tillage, CT; reduced tillage, RT; and no-tillage, NT) and rotation-tillage interactions for 0-10, 10-20, 20-30, and 30-40 cm.

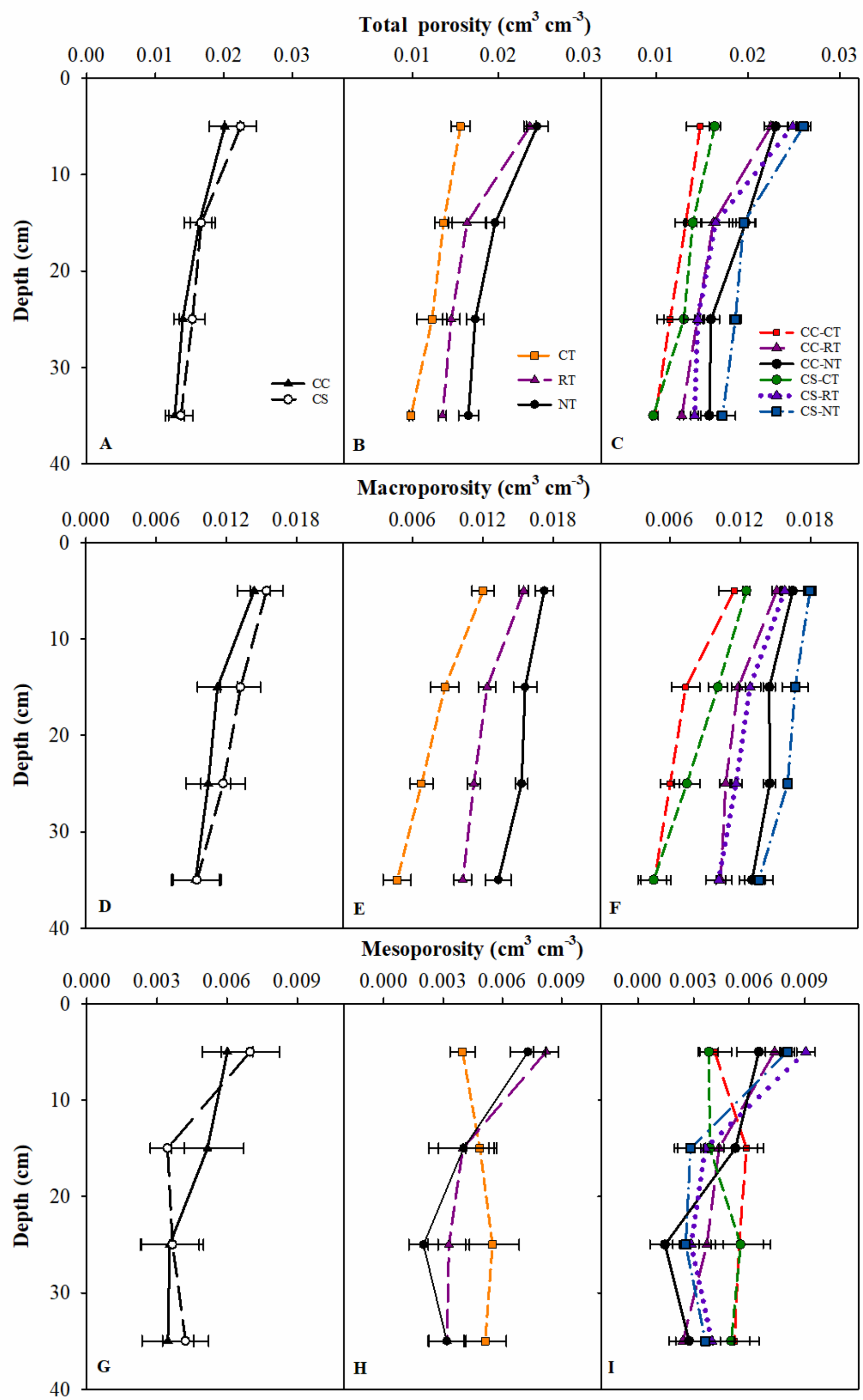

Figure 3.5. Total porosity [A, B, and C], macro-porosity [D, E, and F], and meso-porosity [G, H, and I] as affected by crop rotation (corn-corn, CC; and corn-soybean, CS), tillage (conventional tillage, CT; reduced tillage, RT; and no-tillage, NT) and rotation-tillage interactions for 0-10, 10-20, 20-30, and 30-40 cm

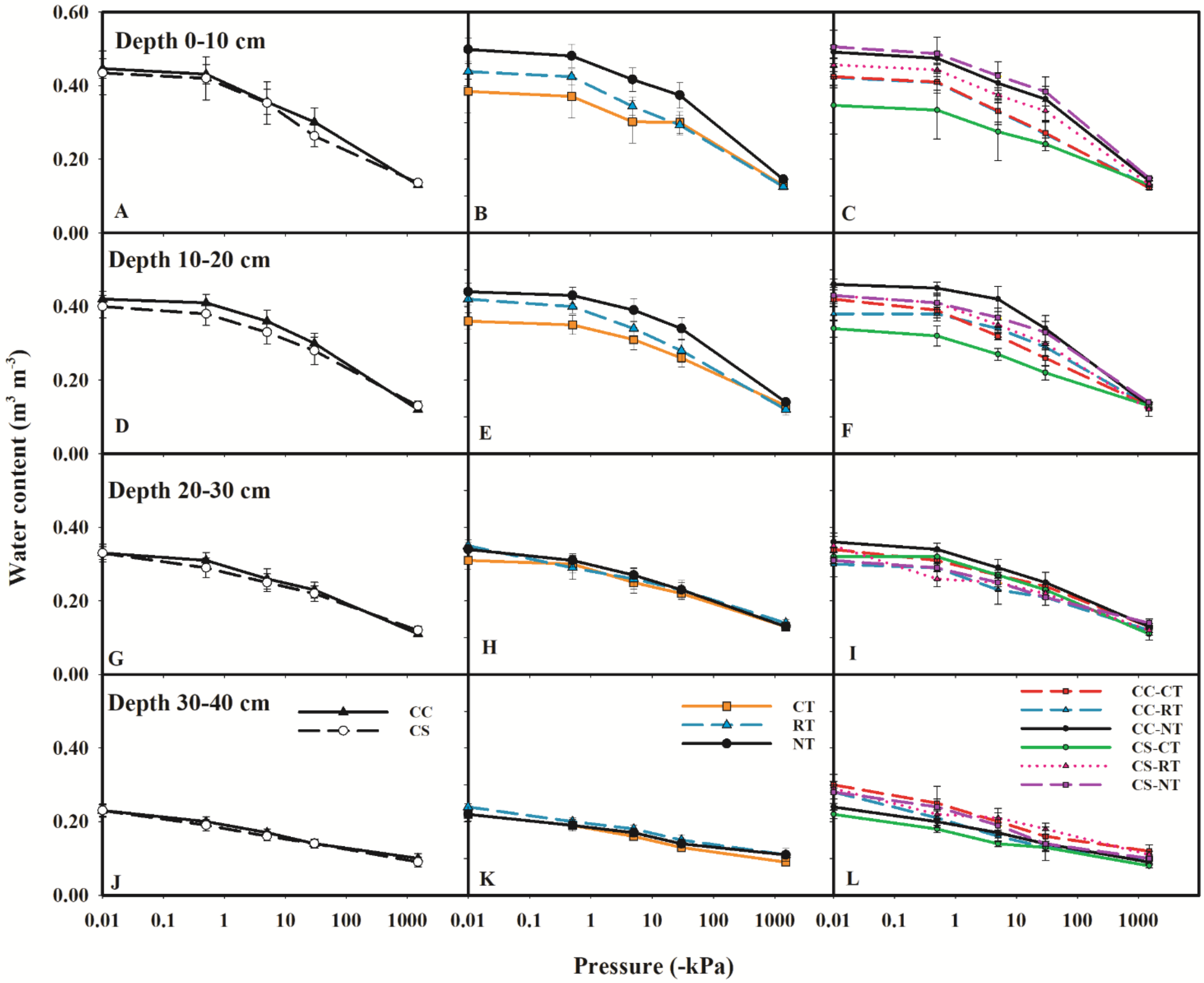

Figure 3.6. Soil water retention curves for crop rotation (corn-corn, CC; and corn-soybean, CS), tillage (conventional tillage, CT; reduced tillage, RT; and no-tillage, NT) and rotation-tillage interactions for 0-10 [A, B, and C], 10-20[D, E, and F], 20-30 [G, H, and I], and 30-40 cm [J, K, and L] depths

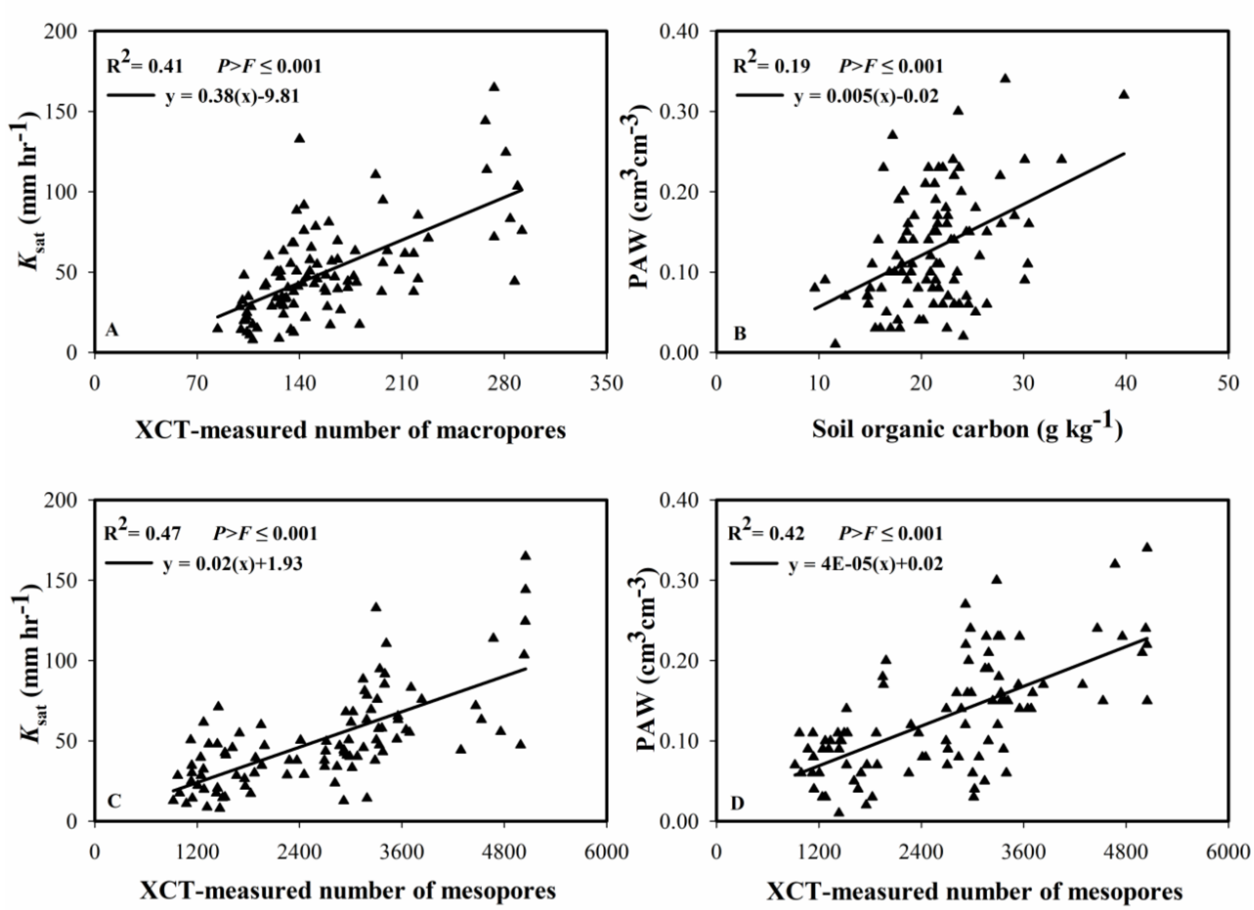

Figure 3.7. Relationships between A. XCT-measured number of macropores and saturated hydraulic conductivity (K_{sat}), B. soil organic carbon and plant available water content (PAW), C. XCT-measured number of mesopores and K_{sat}, D. XCT-measured number of mesopores and PAW

CHAPTER 4

SOIL HEALTH INDICATORS INFLUENCED BY LONG-TERM TILLAGE AND CROP ROTATIONS IN TWO LOCATIONS OF NEBRASKA, USA

ABSTRACT

Increased food demand for the growing population requires extensive agricultural practices for a higher production that can result in the degradation of soil quality and deterioration to the environment. Thus, sustainable cropping practices need to be identified to protect soil health and crop productivity without negatively impacting the environment. The purpose of this study was to evaluate the influence of long-term tillage and crop rotation practices on various selected soil health indicators such as enzymatic activities, protein content, carbon and nitrogen fractions, aggregate size distribution, and phospholipid fatty acid (PLFA). Two long-term experimental sites used for the study were: Haskell Agricultural Laboratory (HAL), Concord, and South-Central Agricultural Laboratory (SCAL), Clay Center in Nebraska (NE), USA. The treatments at both sites were tillage [no-till (NT), reduced till (RT) – disk till, and conventional-till (CT) – moldboard plow till] under continuous corn (*Zea mays* L.) (CC) and corn-soybean (*Glycine max* [Merr.] L.) rotation (CS). At the HAL site, the NT-CS enhanced the activity of β-glucosidase (9.88 μg p– nitrophenol g^{-1} soil h^{-1}) and urease (4.06 μg NH_4^+ g^{-1} soil h^{-1}) when compared to other treatments. At the HAL site, though there was no interaction effect, the CS rotation enhanced the microbial biomass carbon (MBC) by 9% as compared to the CC. Similarly, the NT and RT increased the MBC by and 80 and 42% as compared to the CT treatment. At the SCAL study site, the NT increased the mean weight diameter, water-stable aggregates, and microbial biomass carbon by 6, 13, and 15%, respectively, as compared to the CT. The CC rotation enhanced CWC, HWC, CWN

at SCAL study site and CWN at HAL site compared with CS. The NT and RT systems had greater SOC, TN, water-extractable C and N fractions compared to CT. This study showed that long-term conservation tillage system can enhance soil health.

Key words: no-till, conventional till, continuous corn, corn-soybean, soil enzymes, soil organic carbon

4.1 Introduction

The expanded efforts in agricultural sector for higher food production to meet the demand for growing population can led to degraded soil health and negative impacts to the environment (Busari et al., 2015). Agricultural practices such as diverse crop rotation and conservation tillage are shown to be effective in enhancing productivity and sustainability (Lal et al., 2020). However, these soil management practices influence soil health in different ways in short- or long-term (Doran, 2002). Adopting these conservation practices (e.g., crop rotations and conversation tillage) can likely have a positive effect on soil physical, biological, and chemical properties than the traditional systems such as continuous cropping and intensive tillage systems.

Conservation tillage such as no-till (NT) has gained an increasing attraction among researchers and producers not only from an economic perspective but also in terms of environment protection, as it cuts down the production cost and provides various benefits to soils (De Vita et al., 2007). Surface residues under NT system are left undisturbed on the soil that help to reduce surface runoff and can increase moisture retention. These tillage systems also have a significant positive impact on aggregate stability (Sithole et al., 2019), soil structure (Busari et al., 2015). One of the major soil health indicators, soil organic carbon (SOC) is also greatly influenced by tillage regimes. The NT system has shown to increase SOC accumulation and hence, improving soil health and sustainability (Govindasamy et al., 2021). Whereas, conventional tillage (CT) can negatively impact the productivity of soil due to loss of SOC and increased soil erosion (Sekaran et al., 2020). Also, the system has adverse impacts on long-term soil productivity and sustainability (Mathew et al., 2012).

Crop rotation with a leguminous crop is a cost-effective approach to enhance agroecosystem functions over time by increasing crop productivity while lowering fertilizer use (Chatterjee et al., 2016). It is an important land use system followed not only for the purpose of controlling pests and diseases (Neupane et al., 2021) but also in concern of ecological and crop environment benefits, and hence improving soil health (Dias et al., 2015). The practice can bring out changes in plant available phosphorus, potassium and SOC (Neugschwandtner et al., 2014). Carbon and N losses can be reduced by including a leguminous crop such as soybean into the cropping system, and produce residues with a low C/N ratio that increase C retention in soil (Bansal et al., 2021). Also, crop rotation with NT can substantially modify the microbial structure, C and N fractions in soil, and thus impacting the soil aggregate size (Mikha et al., 2015). Previous studies have shown that crop rotation in association with conservation tillage can potentially boost a system’s dynamic SOC and N pools; these changes, however, are dependent on climate-related interactions (McDaniel et al., 2014). Following an eight-year crop rotation treatment, Coulter et al. (2009) reported that continuous corn, corn–soybean rotation had no effect on SOC levels. However, According to Jagadamma et al. (2019), continuous corn showed greater SOC buildup than continuous soybean or corn–soybean rotation, owing to the higher corn residue, which results in slower residue breakdown. Where these studies The present study was conducted in two long-term sites of Nebraska region with two different soil types and water management (irrigation vs. rainfed) (Blanco-Canqui et al., 2014). Though extensive research was conducted in the study sites, the combined effects of tillage and rotation systems on various soil health parameters remained unclear. In Nebraska, as per 2006 survey, the cultivation under no till was about 45% of the total crop lands (Conservation Technology Information Center – CTIC); similarly, crop rotation practices were also being practiced in large arable areas with time. Thus, these provide a unique opportunity to examine the effects of tillage and rotation systems on soil health properties.

The effect of NT on increasing SOC as compared to the CT systems at the soil surface has been well demonstrated in long-term experiments. The intensive agriculture practices like CT can also potentially lead to soil erosion and degradation by affecting the stable soil aggregate sizes (Arriaga et al., 2017). Long-term studies of Hao et al. (2013) of tillage effects on SOC and dissolved organic C in Southwest China revealed that NT had much higher SOC concentrations than the reduced till and conventional tillage techniques. Identifying best management practices can help to maintain and enhance soil health, thus benefitting ecologically as well as economically with improved yields. In this study, we measured some of the physical, chemical, and biological indicators of soil influenced by diverse crop rotation and tillage practices. The main aim of this work is to evaluate the interaction effects of three tillage (no-till, NT; reduced till (disk till), RT, and conventional till (moldboard plow till), CT) and two crop rotation systems (continuous corn , CC; and corn-soybean, CS) on soil enzymatic activities, microbial biomass carbon (MBC), and microbial biomass nitrogen (MBN), SOC, total nitrogen (TN), C and N fractions within aggregates, water extractable C and N, mean weight diameter, water stable aggregates, and PLFA.

4.2. Materials and Methods

4.2.1 Experimental sites

The two study sites were located in Nebraska managed by University of Nebraska-Lincoln. The first site is located at Haskell Agricultural Laboratory (HAL) of the University of Nebraska, Concord, NE, USA (42.38^{o}N, -96.98^{o}W). This long-term dryland experiment was established in 1985 (Blanco-Canqui et al., 2014). The dominant soil series was Coleridge silty clay loam (fine-silty, mixed, mesic, Cumulic Haplustolls) with a sub-humid climate is predominant in the site. The long-term annual average rainfall in HAL is 672 mm (Irmak et al., 2019). The second site is furrow irrigated and was established in 1986. It is located at South Central Agricultural Laboratory (SCAL), Clay Center, NE, USA (42.49^{o}N, -99.90^{o}W). The dominant soil series at this site was Crete silt loam (*Pachic Arguistoll*) that are fine, smectic, and mesic (Blanco-Canqui et al., 2014). Climatic conditions are altered between sub-humid and semi-arid types influenced by cold continental dry air in winter and warm moist air during summer. The 10-year annual average rainfall in the SCAL site is around 680 mm (Irmak et al., 2019). The experiment is long-term research to understand how crop productivity and soil characteristics are affected by tillage management and crop rotation. The study treatments included in both sites were: three tillage [no-till (NT), reduced till (RT) – disk till, and conventional till (CT) – moldboard plow till] and two cropping systems [continuous corn and corn-soybean] . The experimental design was a randomized complete block design in split-plots with three and four replications in SCAL and HAL sites, respectively. The main plots were tillage and sub-plots were rotation treatments.

4.2.2 Soil sampling

The long-term plots were sampled in July 2020 to assess management impacts on soil health parameters. Soil samples were collected from the surface depth (0-10 cm) using a push probe with a diameter of 2.5 cm at both sites. Soil pH was determined using 1:1 soil to water (Cambardella and Karlen, 1999) with a pH meter. The pH was found to be moderately acidic to neutral for both SCAL and HAL study sites ranging from 5.42 to 6.65 and 4.63 to 7.02, respectively.

4.2.3 Soil enzymatic activity analysis

The β-glucosidase (EC 3.2.1.21) activity was assessed by following the methods outlined by Tabatabai (1994). One gram of moist soil (sieved through 2 mm) along with 0.25 ml of toluene was added to a 50 ml flask. Then to the same flask, 4 ml of 0.05 M modified universal buffer (MUB) of pH 6.0 and 1 mL of 50 mM *p*-nitrophenol-b-D-glucoside (PNG) were added. The flask was whirled and incubated for 1 hr at 37°C. To terminate the reaction, 1 ml of 0.5 M $CaCl_2$ and 4 ml of 0.2 M (pH 12.0) THAM – tris hydroxymethyl aminomethane buffer was added. The developed yellow color intensity was measured at 410 nm on a spectrophotometer (Dick, 2020). The β-glucosidase enzyme activity was expressed as μmol *pNP* g^{-1} soil h^{-1}.

Acid phosphatase enzyme activity was analyzed by following the procedure summarized by Tabatabai and Bremner (1969). One gram of 2 mm sieved moist soil was added along with 4 mL of modified universal buffer (pH 6.0) to a 50 ml flask. Then 1 mL of *p*-nitrophenyl phosphate solution was also added and incubated for 1 hr at 37°C. The reaction was stopped by adding 1 mL of 0.5 M $CaCl_2$ and 4 mL of 0.5 M NaOH. The soil suspension was allowed to develop yellow color. The color intensity of *p*-nitrophenol was measured at 400 nm with a spectrophotometer. The enzyme activity of acid phosphatase is expressed as μg *p*NP g^{-1} soil h^{-1}.

Arylsulfatase enzymatic activity was measured by following the methods outlined by Tabatabai and Bremner (1970). One gram of moist soil (sieved through 2 mm) along with 0.25 ml of toluene was added to a 50 ml flask. Then 4 ml of 0.5 M acetate buffer (pH 5.8) and 1 mL of 0.05 M *p*-nitrophenyl sulfate solution were added. The flask was swirled for few seconds to mix the contents and incubated for 1 hr at 37^{o}C. To terminate the reaction, 1 mL of 0.5 M $CaCl_2$ and 4 mL of 0.5 M NaOH were added. The yellow color intensity of soil suspension for *p*-nitrophenol was assessed at 420 nm by spectrophotometer. The developed *p*-nitrophenol yellow color was stable for several hours if stored in dark but fades away quickly if exposed to direct sunlight. Arylsulfatase enzyme activity was expressed as μg *p*NPg^{-1}soil h^{-1}.

Urease activity was determined by following the method given by Kandeler and Gerber (1988), where NH_4 release was determined for this measurement. Five grams of soil was placed in three 50 ml flasks, two of them were treated with 2.5 ml of substrate solution and 20 ml of borate buffer. Into the third flask, only 20 ml of borate buffer was added that acts as a control. All the flasks were incubated for 2 hr at 37^{o}C and then 2.5 ml of the substrate was added to the control sample. Thirty ml of 2 M KCl, 0.01 M HCl were added to all the flasks and placed in a rotary shaker for 30 minutes. After pipetting out 1 ml of filtrate, 9 ml of distilled water, 5 ml of sodium salicylate-sodium hydroxide solution, and 2 ml of sodium dichloro isocyanurate solution were added. Test tubes were swirled and allowed for color development for 30 min. Color intensity developed was measured at 660 nm using a spectrophotometer. Urease activity was expressed as μg NH_4-N g^{-1} h^{-1}.

4.2.4 Glomalin related soil protein (GRSP) and microbial biomass activity

Glomalin related soil protein content was measured according to the procedure given by Wright and Upadhyaya (1998). Three grams of air-dried soil sample was taken into pressure and heat-stable tubes along with 24 mL of sodium citrate extractant buffer (20 mM, pH 7.0) and mixed well for 5 min at 180 rpm. The tubes were subjected to autoclaving for 30 min at 121^{o}C, 103 kPa, and then cooled and centrifuged (10,000 x gravity). The soil protein concentration was measured using a pierce bovine serum albumin (BCA) protein assay kit (Thermo Scientific, IL, USA). The mixture was incubated for 1 hr at 60°C and was allowed for color reaction. The developed color was measured at 562 nm in a spectrophotometer for both the sample and blank. The protein concentration was calculated by comparing obtained values of samples with a standard curve of BCA (0-2000 μg mL^{-1}) and was expressed as mg g^{-1} of dry soil.

Microbial biomass C and N (MBC, MBN) were determined by following the method of chloroform fumigation direct extraction described by Anderson and Domsch (1978). The soil samples were stored in a cold room at 4^{o}C to preserve the moistness. For measuring the biomass activity of microbes, three subsamples of moist soil weighing 8 g each were taken. One of the subsamples was placed in a desiccator containing alcohol-free chloroform for 24 hours and was then evacuated. The other two samples were considered for non-fumigated and gravimetric soil moisture analysis. Both the fumigated and non-fumigated subsamples were subjected to extraction with 40 ml of 0.5 M K_2SO_4 and filtered through 0.453 µm filter papers. The filtered samples were then fed to a TOC analyzer (model TNM-L-ROHS, Shimadzu Corporation, Kyoto, Japan) for analyzing microbial biomass C and N contents. Microbial biomass C and N content was calculated by the difference between fumigated and non-fumigated with a correction factor of 0.45 (Beck et al., 1997) and was expressed as µg g^{-1} soil.

4.2.5 Soil organic carbon (SOC), total nitrogen (TN), and water extractable carbon and nitrogen fractions

Soil organic carbon and TN concentrations in bulk soil samples, and within the soil aggregates at both study sites were measured by dry combustion method using TruSpec CN628 analyzer (LECO Corporation, St. Joseph, MI). Approximately 0.25 g of sample was weighed in an aluminum cup and was fed to the CN628 analyzer to determine C and N content and was expressed as g kg^{-1}.

Estimation of water-extractable C and N fractions was performed following the methods described by Ghani et al. (2003). Three grams of soil samples were taken into 50 mL polypropylene centrifuge tubes along with 30 mL of distilled water. The tubes were subjected to shaking in an end-over-end shaker at 30 rpm for 30 minutes and centrifuged at 4^{o}C for 25 mins. The obtained supernatant was filtered through a 0.45 µm pore size syringe filter and the recovered filtrate was used for cold water extractable carbon and nitrogen (CWC, CWN). Thirty mL of distilled water was added to the remaining soil sample and was vigorously shaken for 10 seconds on a vortex shaker and was put in a hot water bath at 80^{o} C for 16 hrs. The extractant was again shaken for 10 seconds on a vortex shaker and then subjected to centrifugation at 3000 rpm and 25°C for 25 minutes. The supernatant was filtered through a 0.45 µm pore size syringe filter into glass vials and was considered as HWC, HWN. Obtained filtrates were finally fed in the TOC-L analyzer to measure water extractable C and N and was expressed as mg kg^{-1}.

4.2.6 Phospholipid Fatty Acid Analysis (PLFA)

The microbial community structure in soil samples was determined using a PLFA analysis (Clapperton et al., 2005). Briefly, 2 g of lyophilized soil was mixed with 9.5 mL dichloromethane (DMC): methanol (MeOH): citrate buffer (1:2:0.8 v/v) extraction solution to extract total soil lipids. The extracted solution was passed through a solid-phase silica column to separate phospholipids from other lipids. Fatty acid methyl esters (FAMEs) extracted were assessed using an Agilent 2030-GC equipped with a CP-7693 auto-sampler and a flame ionization detector (FID).

4.2.7 Soil Aggregate Size Distribution

Aggregate size distribution of soil was measured following the wet sieving method of Kemper and Rosenau (1986). The aggregate analysis was carried out using the Yoder equipment, that consisted of six successive sieves (4, 2, 1, 0.5, 0.25, and 0.053-mm diam.). The soil aggregates were progressively wetted and dispersed in the topmost sieve, then subjected to wet sieving by lowering and then rising the sieves with a stroke length of 13 mm and a frequency of 90 strokes min^{-1}. Six aggregate size fractions (>4, 2, 1, 0.5, 0.25, 0.053) were collected. After drying at 40°C for 2-3 days, the weight of each fraction was measured after the retained aggregates in the corresponding sieves were transferred to already weighed containers. With the assessed data, water stable aggregates

(WSA) were calculated, and the mean weight diameter (MWD) of stable aggregates was determined using WSA. The MWD was calculated as follows:

$$\mathrm{MWD} = \Sigma_{\mathrm{i=1}}^{\mathrm{n}} x_{\mathrm{i}} m_{\mathrm{i}}$$

where, n is the number of the aggregate size range (mm), x_i is the mass of the aggregates of that size range as a fraction of the total dry mass of the sample analyzed, and m_i is the mean diameter of any size range of aggregates separated by sieving.

4.2.8 Statistical Analysis

Tillage and rotation system effects on studied soil properties were analyzed using post-hoc test to compare least-squares means estimated by a model using GLIMMIX procedure in SAS 9.4 (2013). Analysis of Variance (ANOVA) was used to examine the fixed effects of tillage and rotation systems, as well as the random effect of their interaction on soil health indices by using this model. Normality of the dataset was observed using the Shapiro-Wilk test. Statistical significance was determined at α= 0.05 level. Also, principle component analysis (PCA) was created using the software JMP.pro to determine the impacts of tillage and crop rotation interactions on studied soil health properties.

4.3. Results and Discussion

4.3.1 SOC, TN, and Water Extractable C and N Fractions

Data for SOC, TN, CWC, HWC, CWN, and HWN fractions in bulk soil samples for both (SCAL and HAL) study sites were presented in Table 4.1. At the SCAL site, rotation and tillage did not affect SOC, however, they significantly impacted the CWC and HWC, except that tillage did not impact the HWC parameter. The CWC and HWC were 8.3 and 13.1% higher in the CC system as compared to the CT system. Also, the NT system increased the CWC content by 11% compared to CT and is not statistically different from RT. The TN and HWN parameters were also not impacted by rotation and tillage, however, the rotation influenced the CWN parameter. The CC rotation increased the CWN by 10.2% as compared to the CS rotation. Interactions of rotation by tillage on all these parameters (SOC, CWC, HWC, TN, CWN and TWN) were not statistically significant.

At the HAL study site, tillage significantly impacted only the SOC and HWC. The crop rotation main effect and the tillage with crop rotation interaction effect was not detected for SOC, CWC, and HWC. The RT increased the SOC by 39.9 and 11.7% as compared to the CT. The NT and RT increased HWC by 76.4 and 55.3% as compared to the CT system, respectively. Concentrations of TN, CWN, HWN were significantly impacted by tillage, however, rotation only impacted the CWN and HWN parameters. The NT and RT systems improved the TN by 47, 33% and HWN by 81, 80%, respectively, as compared to the CT system. The CC system enhanced the HWN by 1.2 times than the CS rotation. The CC-NT (19.8 mg kg^{-1}) and CC-RT (21.1 mg kg^{-1}) increased the HWN content compared to the other tillage and rotation interaction systems

Tillage practices can influence C cycling, storage and flow in an agroecosystem (Yoo et al., 2006), they also reported an increase in SOC through restoration by NT over the CT. Soils exposed to repeated tillage operations have lower amounts of SOC. These findings were similar to those reported by Singh et al. (2016). Due to intensive disturbance to the soil in CT systems, soils are prone to rapid mineralization and heavy loss of SOC (Tiessen and Stewart, 1983). Haddaway et al. (2017) also reported that NT has higher SOC concentration when compared to the CT. Due to the presence of residues on the surface of NT soil, it aids in slow decomposition rate and thus accumulation of high SOC concentrations. Hence, minimum disturbance to soil is beneficial over intensive tillage to agricultural soils in reducing C losses (Zibilske and Bradford, 2007). The loss of soil TN was reduced in many cropping systems with NT compared to CT practices (Malhi and Kutcher, 2007). Omara et al. (2019) reported higher accumulation of residues can result in increased TN concentration that could be possible with NT where there is no disturbance to soil. Hence, the results of present study were in accordance with the previous findings those reported that SOC and TN influenced by tillage systems.

High water temperatures (over 70°C) destroy microorganism vegetative cells and remove several components from microbial biomass, as well as many nonmicrobial organic compounds (Bu et al., 2011). Therefore, HWC has much higher biodegradability rates than the CWC (Hamkalo and Bedernichek, 2014). Additionally, the HWC is made up of easily accessible molecule (labile fraction) including carbohydrate, phenols, and lignin monomers according to Landgraf et al. (2006), and hence serves as a source of nutrients and energy for plants and microbes. Whereas, CWC is made up of more stable components that provide plants and microbes with a tight supply of nutrients and energy (Bu et al., 2011). Minimum tilled soils had a higher crop residue retention rate, which helps to reduce moisture losses and temperature changes, enhance soil aggregation (Hernanz et al., 2002), and hence accumulate more C and N than the intensively tilled soils (Kumar et al., 2012). The plant residues from conservation tillage soils can influence the C and N dynamics in the agroecosystem and was reflected in NT systems which is in accordance to study of Singh and Kumar (2021).

4.3.2 Soil Enzymatic Activity

Acid phosphatase, arylsulfatase, β-glucosidase, and urease enzymatic activity at the SCAL and HAL study sites revealed the tillage by rotation effect on soil enzymes (Table 4.2, Figure 4.1). At the SCAL site, the acid phosphatase enzyme activity under CC - NT was significantly higher as compared to the other tillage by rotation interactions. The arylsulfatase enzyme activity under CS-NT interaction was the highest among all rotation by tillage treatments. The interaction effect of tillage by rotation and rotation impact alone were not significant for β-glucosidase and urease enzyme activity. However, NT and RT system improved the β-glucosidase by 59, 58.5% and urease enzyme activity by 30, 33%, respectively, as compared to the CT.

At the HAL study site, tillage by rotation practices significantly influenced the activity of acid phosphatase and arylsulfatase enzymes. The CS-RT interaction significantly improved acid phosphatase activity (199.7 μg *p*– nitrophenol g^{-1} soil h^{-1}) as compared to the other tillage by rotation treatments. However, arylsulfatase activity was significantly improved under CC-NT interaction (236.3 μg *p*– nitrophenol g^{-1} soil h^{-1}) over other interaction treatments. Tillage by rotation interactions did not significantly impact the activity of β-glucosidase or urease enzymes. However, rotation and tillage treatments significantly affected the β-glucosidase and urease enzymes activity. The CS system increased urease activity by 34.5% compared to the CC. While β-glucosidase activity was similar between rotation systems. The NT and RT enhanced the activity of β-glucosidase by 1.5 times, 46.9% and urease by 75, 25%, respectively, as compared to CT.

The β–glucosidase, acid phosphatase, arylsulfatase, and urease enzymes play a major role in C cycling, mineralization of phosphorus, sulfur, and nitrogen containing compounds in soil (Chellappa et al., 2021). Findings from both study sites reveled that conservation tillage resulted in increased enzymatic activities than the CT system. This could be partially due to the fact that increased organic matter resulting in increased soil microbial activity (Heidari et al., 2016). The increased enzyme activity under conservation till system implies that adding crop residues is much more beneficial in the conservation till system than in the conventional till system. Results of reduced enzyme activity for CT soil were in accordance to the findings of Balota et al. (2004) who reported an increase of arylsulfatase by 2 times and acid phosphatase by 46% respectively, for NT system. The kind and amount of organic matter in the soil have a large impact on acid phosphatase activity, and its enhanced activity can alter the insoluble phosphate to available form for plant uptake (Wu et al., 2018). Crop residues were left on the surface in the NT system, where their slow disintegration can provide a long-term source of substrate for soil microorganisms and result in enhanced enzyme activity, whereas, crop residues were absorbed into the soil in the CT system (Tyler, 2019). It was supported from the findings that NT practices can improve overall biological activity of the soil than the intense tillage systems causing detrimental impact on enzyme activity. Previous studies had also reported similar effects of conservation tillage with high enzymatic activity than the conventional tillage (Roldán et al., 2005). Also, the inclusion of leguminous crop in a rotation can help to increase the microbial communities and enzymatic activities (Aschi et al., 2017). Singh et al. (2018) in their study of crop rotation and residue management effects on soil enzyme activities under zero tillage reported that legume-based cropping improved the soil enzymatic activity than cereal-cereal system. However, previous studies also reported an increase of soil enzymatic activity under continuous corn system. The findings of Eivazi et al. (2003) stated that the activities of studied enzymes were higher for CC under NT than CC under CT.

4.3.3 Soil Microbial Community Structure

Data on the effect of tillage and rotation on soil microbial community structure for SCAL and HAL study sites were presented in Table 4.3. The identified biomarker peaks in PLFA analysis included 10-methyl, straight-chain, 18:2 w6,9c, branched, monounsaturated fatty acids, 16:1 w5c, 18:1 w9c, polyunsaturated fatty acids, cyclopropane. At the SCAL site, the tillage and rotation treatments did not impact the soil microbial community structure. Whereas, for the HAL site, CC cropping system resulted in higher gram (-) bacteria (31.67 nmol g^{-1} soil) than the CS rotation (24.7 nmol g^{-1} soil). Moreover, the CC rotation had an 31% significantly higher AM fungi response than the CS. Though there were no significant differences observed in overall PLFA between tillage treatments, NT has the highest numeric mean values when compared to the RT and CT systems.

Soil microorganisms are essential to the long sustainability of agro-ecosystems, because of their critical involvement in essential soil processes such as organic matter decomposition, nutrient cycling, and the preservation of soil structure (Loranger-Merciris et al., 2006). Tillage and rotation can change the composition, variety, and function of the soil microbial population, resulting in major changes in soil processes and hence soil fertility (González-Chávez et al., 2010). Sun et al. (2018) reported that conservation tillage strategies such as NT can boost soil microbial diversity and abundance by directly altering the vertical distribution of soil microbial communities in the soil profile. However, tillage practice is not always necessarily the important element influencing the distribution of soil microbial communities. Findings of Lopes and Fernandes (2020) showed that crop variety and development, rather than tillage practice, are the important factors influencing microbial responses to land management in their study. The long-term availability of crop residues (in CC system) probably provided the required organic material for decomposition to enhance the microbial community structure. Similar findings were reported by Wang et al. (2021) in their study on impacts of long-term tillage and cropping system on soil fungal community where the soil fungal PLFA was higher in CC system than in CS rotation.

4.3.4 Soil Aggregate Size Distribution, Water Stable Aggregates, Mean Weight Diameter, and Aggregate Associated C and N

Data for soil aggregate size distribution, WSA, MWD and aggregate associated C and N revealed the significant interaction effect of tillage and rotation systems at the SCAL and HAL study sites (Table 4.4, Table 4.5). For the SCAL study site, the CC system increased the 0.053, 0.5 mm fraction by 14.9 and 22.7% than the CS rotation. The NT treatment significantly enhanced the aggregates of all sizes except for 2- and 4-mm fractions as compared to the RT and CT treatments. The CC-NT treatment significantly improved the 0.5 mm fraction (10.14 g), as compared to the other tillage by rotation combinations. Similarly, the CC-NT also enhanced the WSA and MWD by 30.1 and 23.5%, respectively, than the CS-CT. The significant differences for SOC and TN within the soil aggregates were observed only at 0.5 mm fractions (Table 4.5). The CC-RT significantly enhanced the SOC content (57 g kg^{-1}) for the 0.5 mm size fraction. However, the TN concentration was significantly higher for CS-CT (4.77 g kg^{-1}) as compared to CC-CT and CC-NT interactions.

At the HAL study site, the rotation impact on aggregate size distribution was observed for all sizes except for 4 mm faction. The lower size aggregate (0.053 and 0.25 mm) fractions were significantly higher under CS rotation than CC. However, the 1- and 2-mm fractions were significantly increased under CC than CS rotation. The NT treatment increased the 0.25, 0.5, 1, 2 mm fractions as compared to the other tillage treatments. Tillage by rotation interaction effects were significant only for 0.25- and 2-mm aggregate size fractions (Table 4.4). The CS-NT had significantly higher soil aggregates of 0.25 mm over the other tillage by rotation interactions. The 2 mm aggregate size fraction was improved under CC-NT interactions (8.92 g) as compared to other treatments. Tillage and crop rotation main effect was observed for WSA and MWD. The CS rotation increased the WSA by Further, the significant tillage by rotation interaction effect was observed for MWD but did not affect WSA. The MWD for the CC-NT system (1.00 mm) was significantly different as compared to the other interaction treatments.

The tillage by rotation interactions significantly influenced the SOC and TN concentrations within the soil aggregates (Table 4.4 and 4.5). However, the effect was not consistent with the treatments. The interaction effect of rotation (CC or CS) with NT and RT systems enhanced the SOC concentration within the aggregate sizes of 0.053, 0.25, 0.5, and 2 mm; except for 1 mm fraction that CC-NT had less SOC than other interactions. The CT treatment with CC or CS rotation was observed to have the decreased SOC concentration, except for 1 mm fraction. Similar trend was observed with the TN concentration in which the CT plots had significantly lower TN than the NT and RT treatments except for 1 mm fraction. The NT and RT treatments with CC or CS rotations increased the TN concentration for 0.053, 0.25, 0.5, and 2 mm (size fractions as compared to the CT with CC or CS.

The NT with no disturbance to soil promotes aggregate formation and improves stability due to increased soil organic matter and high crop residues (Briedis et al., 2012; Chellappa et al., 2021). By lowering soil disturbance frequency and maintaining a high residue cover, the NT system enhanced soil structure and increased the quantity of macroaggregates, preventing soil erosion and aggregate disintegration (Zheng et al., 2018). The presence of vegetation over the soil in NT systems promotes the formation of soil aggregates and improves aggregate stability (Maiga et al., 2019). Also, the residue cover in NT system can help in building up moisture and contributing to development of SOM, thus providing a habitat for soil microbes resulting in better aggregation.

However, intensive tillage treatments can greatly disrupt the soil and can reduce the stability and aggregate formation as well. (Hou et al., 2021). Macroaggregates, in particular are vulnerable to tillage degradation and serve as an essential mechanism for preserving and protecting soil organic matter (SOM), which can decrease in conventional till (Beare et al., 1994; Palm et al., 2014). The reduced proportion of macroaggregate fraction in the conventional tillage system might also be due to lower SOC concentrations (DU et al., 2013). Increased SOM in an undisturbed environment can help to improve aggregate formation and stability. Thus, it can be concluded that tillage regimes have significant impact on stability of soil aggregates, and conservation tillage system promotes the formation of soil aggregates while conventional tillage system can disrupt the aggregates.

Soil organic carbon is an effective predictor of aggregate stability. The rotations with corn, produce higher biomass, favor slower residue breakdown and can result in higher SOC levels (Bansal et al., 2021). Contrastingly, soybean rotations produce less residues that results in rapid decomposition and lower SOC accumulation (Jagadamma et al., 2019). Literature showed that retaining corn residues enhanced the soil aggregate stability as compared to soybean residues (Nouwakpo et al., 2018). The same study of Nouwakpo et al. (2018) on long-term tillage and crop rotations effects on soil structural stability also reported that NT improved the soil cohesion in surface layers as compared to other tillage treatments. Also, they concluded that soil sediment loss was significantly greater under conventional till than NT. SOC and MWD had a positive connection, which means that an increase in SOC can result in an increased aggregate MWD. Findings implied that soils with a higher SOC concentration have a better chance of forming stable aggregates (Nie et al., 2018). Land use system may have significant influence on particle size associated SOC and TN.

4.3.5. Glomalin Related Soil Protein, Microbial Biomass Carbon, and Microbial Biomass Nitrogen

At the SCAL site, tillage only impacted the MBC parameter while the crop rotation did not influence MBC, MBN, and GRSP parameters (Figure 4.2). The NT system significantly improved the MBC content by 27.8% than the RT.

At the HAL study site, crop rotation systems only influenced the MBC (Figure 4.2). The MBC content was 9% higher under CS rotation as compared to CC system. Tillage treatments had significant effect on the GRSP, MBC, and MBN contents. The NT and RT increased the MBC content by 80 and 27%, respectively, as compared to CT.

Also, the NT system increased the GRSP by 24% than CT and MBN content by 24 and 42% as compared to the RT and CT respectively.

Glomalin is a soil glycoprotein found abundantly on hyphae and spores produced by AM (arbuscular mycorrhizal) fungi in soils and roots that bind the soil particles together, and plays an important role in soil structural stability (Yang et al., 2017). The capacity of aggregates to withstand changes in the external environment and stay stable is referred to as soil aggregate stability (Zheng et al., 2018). Our findings indicated that using NT for a longer period increased soil aggregate stability and reduced unstable aggregates by minimizing tillage operations and maintaining a high residue cover on the surface, which prevents wind and water erosion. The aggregate stability can be increased with higher content of GRSP that acts a glue-like substance in adhering the soil aggregates or other aggregate forming components (Ji et al., 2019). Under NT treatments, the presence of GRSP enhances the number of macroaggregate fractions by combining more tiny aggregates into a macroaggregate (Liu et al., 2020). The conclusions from this study was supported by earlier works indicating that NT promoted macroaggregate formation by causing microaggregates to bind together (Tao et al., 2018).

The microbial biomass is a labile source of key plant nutrients that drives nutrient mineralization (C, N, P, and S) (Roldán et al., 2005). The increase in MBC content in the conservation tillage system supports that higher SOM in the soil providing niche to microorganisms and improved microbial activity (Balota et al., 1998). Because of the increased quantity of C trapped in microbial biomass, soil organic matter in NT systems offers more labile C than in conventional systems. In contrast to conventional till, where a transient level of microbial activity with each tillage event leads in substantial losses of

C as CO_2, the lack of a major disturbance event with conservation till likely offers a constant source of organic C to maintain the microbial population (Balota et al., 2003). Thus, the present results support the findings of Wright et al. (2005) study reporting that tillage activities and the consequent changes in the soil physicochemical environment may be more directly connected to changes in microbial biomass. Also, the non-significant impact of crop rotation systems on microbial activity is in accordance with Balota et al. (2003).

The PCA results showed that tillage and rotation system had a significant influence on C and N fractions, glomalin related soil protein (Figure 4.3). The first principle component (PC1) explained 33.7% of total variation whereas, PC2 explained 21.5% variation. The PCA results showed that NT with CC had significant influence on CWC, CWN, SOC, TN, MBN, and MWD as well. The interaction effect of RT with CC had impact on microbial community composition.

4.4. Conclusions

This study was conducted to determine the impacts of long-term tillage and crop rotation systems on soil health indictors such as β-glucosidase, acid phosphatase, arylsulfatase, urease, aggregate stability, C and N fractions, PLFA, and soil protein. Data showed that at the SCAL site, the RT with CC cropping system significantly increased soil enzymatic activity, aggregate stability, and mean weight diameter. However, at the HAL site, the CS rotation system with NT showed higher soil enzymatic activity and aggregate stability. However, the MWD was higher in CC rotation with NT system. The NT system at the HAL site positively affected the overall enzymatic activity, MBC, MBN, and glomalin. Whereas the effects of rotation were not consistent at either site. The overall study concluded that long-term conservation tillage (RT and NT) enhanced soil health indicators, however, rotation impact was not consistent at either site.

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Table 4.1. Crop rotation (continuous corn, CC; and corn-soybean, CS) and tillage systems (conventional tillage, CT; ridge tillage, RT; and no-tillage, NT) main effects on soil organic carbon (SOC), and carbon fractions (cold water extractable carbon, CWC and hot water extractable carbon, HWC), total nitrogen (TN) and nitrogen fractions (cold water extractable nitrogen, CWN and hot water extractable nitrogen, HWN) at South Central Agricultural Laboratory and Haskell Agricultural Laboratory, NE, USA.

Treatments	SOC	CWC	HWC	TN	CWN	HWN
	South Central Agricultural Laboratory Site					
Rotation	g kg^{-1}	-----mg kg^{-1}-----		g kg^{-1}	-----mg kg^{-1}-----	
CC	19.7	$70.4^{a\dagger}$	136.0^{a}	1.75	7.98^{a}	8.94
CS	19.4	65.0^{b}	120.2^{b}	1.73	7.24^{b}	8.20
Tillage						
CT	19.5	63.4^{b}	127.2	1.74	7.33	8.27
RT	18.7	69.4^{ab}	124.3	1.68	7.58	8.28
NT	20.2	70.4^{a}	132.9	1.82	7.91	9.15
	Analysis of Variance (*P*>*F*)					
Rotation (R)	0.60	0.02	0.03	0.79	0.02	0.14
Tillage (T)	0.37	0.03	0.57	0.15	0.25	0.25
R-T	0.79	0.75	0.13	0.48	0.24	0.14
	Haskell Agricultural Laboratory Site					
Rotation						
CC	24.8	76.1	133	1.90	10.91^{a}	17.0^{a}
CS	23.4	70.5	112	1.68	6.87^{b}	7.56^{b}
Tillage						
CT	19.8^{b}	63.5	85.5^{b}	1.41^{b}	6.21^{b}	7.77^{b}
RT	27.7^{a}	77.2	133^{a}	1.88^{a}	10.5^{a}	14.0^{a}
NT	24.8^{ab}	79.1	151^{a}	2.07^{a}	11.1^{a}	14.1^{a}
	Analysis of Variance (*P*>*F*)					
Rotation (R)	0.44	0.30	0.09	0.13	0.003	<0.0001
Tillage (T)	0.007	0.05	0.01	0.003	0.01	0.001
R-T	0.94	0.77	0.57	0.94	0.74	0.03

†Means followed by different letters within a column, treatment (rotation and tillage), and site are significantly different at $P<0.05$.

Table 4.2. Crop rotation (continuous corn, CC; and corn-soybean, CS) and tillage systems (conventional tillage, CT; ridge tillage, RT; and no-tillage, NT) interaction effect on soil enzymatic activity (acid phosphatase and arylsulfatase) at South Central Agricultural Laboratory and Haskell Agricultural Laboratory, USA.

Treatments	SCAL Site		HAL Site	
	Acid Phosphatase	Arylsulfatase	Acid Phosphatase	Arylsulfatase
	--------------------μg *p*– nitrophenol g^{-1} soil h^{-1}--------------------			
CC-CT	87.8[d†]	70.3[cd]	112[d]	183[b]
CC-RT	135[c]	93.3[b]	166[b]	194[b]
CC-NT	221[a]	102[ab]	137[cd]	236[a]
CS-CT	88.3[d]	57.1[d]	161[bc]	103[d]
CS-RT	137[c]	75.6[c]	200[a]	146[c]
CS-NT	166[b]	113[a]	126[d]	201[b]
	Analysis of Variance ($P>F$)			
Rotation (R)	<0.001	0.031	<0.001	<0.001
Tillage (T)	<0.001	<0.001	<0.001	<0.001
R-T	<0.001	0.001	<0.001	<0.001

[†]Means followed by different letters within a column, treatment is significantly different at P<0.05.

Table 4.3. Crop rotation (continuous corn, CC; and corn-soybean, CS) and tillage systems (conventional tillage, CT; ridge tillage, RT; and no-tillage, NT) main effect on Soil microbial community at South Central Agricultural Laboratory (SCAL) and Haskell Agricultural Laboratory (HAL) study sites, Nebraska, USA. [AM fungi – Arbuscular Mycorrhizal fungi; PLFA – phospholipid fatty acids)

Treatments	**South Central Agricultural Laboratory (SCAL) site**							
	AM Fungi	**Gram (-)**	**Gram (+)**	**Eukaryotes**	**Actinomycetes**	**Total Bacteria**	**Total Fungi**	**Total PLFA**
Rotation				**-------------------nmol g^{-1} soil-------------------**				
CC	2.69	23.3	22.10	0.53	10.7	45.4	3.53	59.7
CS	2.89	23.3	21.74	0.89	11.43	45.0	3.72	60.2
Tillage								
CT	2.66	23.97	21.63	1.12	10.1	45.6	3.41	59.2
RT	2.70	22.31	21.34	0.53	10.9	43.7	3.54	58.0
NT	3.01	23.60	22.77	0.48	12.2	46.4	3.92	62.5
				Analysis of Variance ($P>F$)				
Rotation (R)	0.69	0.99	0.89	0.54	0.60	0.94	0.79	0.94
Tillage (T)	0.82	0.89	0.90	0.59	0.46	0.92	0.83	0.88
R-T	0.13	0.08	0.08	0.33	0.01	0.08	0.83	0.08
				Haskell Agricultural Laboratory (HAL) site				
Rotation								
CC	2.26[a†]	31.6[a]	28.1	2.46	12.2	59.8	2.26	74.2
CS	1.72[b]	24.7[b]	23.2	2.09	10.4	48.0	2.66	61.1
Tillage								
CT	1.90	27.9	25.8	2.32	11.4	53.7	3.32	68.5
RT	1.72	25.5	24.5	2.05	10.3	50.1	1.72	62.2
NT	2.34	31.1	26.6	2.47	12.2	57.8	2.34	72.3
				Analysis of Variance ($P>F$)				
Rotation (R)	0.02	0.03	0.22	0.18	0.16	0.09	0.69	0.11
Tillage (T)	0.09	0.35	0.90	0.45	0.49	0.65	0.45	0.58
R-T	0.47	0.62	0.98	0.73	0.67	0.88	0.31	0.77

[†]Means with Means followed by different letters within a column, treatment (rotation and tillage), and site are significantly different at $P<0.05$.

Table 4.4. Crop rotation (continuous corn, CC; and corn-soybean, CS) and tillage systems (conventional tillage, CT; ridge tillage, RT; and no-tillage, NT) interaction effect on distribution of water-stable aggregates (WSA) and mean weight diameter (MWD) at South Central Agricultural Laboratory (SCAL) site and Haskell Agricultural Laboratory (HAL) study sites, Nebraska, USA.

Treatments	Aggregate size distribution (mm)							
	0.053	**0.25**	**0.5**	**1**	**2**	**4**	WSA	MWD
	----------------------------%--------------------------------						**%**	**mm**
	South Central Agricultural Laboratory (SCAL) Site							
CC-CT	46.3	23.1	5.81[b†]	3.61	4.29	2.20	85.3[b]	0.52[b]
CC-RT	36.5	24.7	5.75[b]	3.53	4.20	2.27	77.0[cd]	0.51[b]
CC-NT	42.6	28.4	10.1[a]	5.42	4.74	2.61	93.9[a]	0.63[a]
CS-CT	36.6	18.9	5.36[b]	3.69	4.72	2.49	71.8[d]	0.51[b]
CS-RT	31.3	25.8	5.77[b]	3.46	4.47	2.32	73.1[d]	0.51[b]
CS-NT	40.6	25.0	6.55[b]	4.14	4.48	2.02	82.8[bc]	0.52[b]
	Analysis of Variance (*P*>*F*)							
Rotation (R)	0.001	0.07	<0.001	0.19	0.65	0.57	<0.001	0.05
Tillage (T)	<0.001	0.003	<0.001	0.01	0.78	0.94	<0.001	0.01
R x T	0.11	0.15	<0.001	0.18	0.66	0.07	0.01	0.03
	Haskell Agricultural Laboratory (HAL) Site							
CC-CT	27.6	13.1[b]	11.5	6.04	10.4[ab]	3.37	72.1	0.78[ab]
CC-RT	25.7	14.6[b]	18.2	7.11	7.60[bc]	4.59	77.9	0.84[ab]
CC-NT	26.8	14.2[b]	18.1	8.92	11.3[a]	4.90	84.4	1.00[a]
CS-CT	32.1	15.6[b]	13.8	4.27	5.80[c]	3.10	74.7	0.63[b]
CS-RT	32.6	17.1[b]	16.6	6.18	7.67[bc]	4.12	84.3	0.81[ab]
CS-NT	32.5	25.9[a]	17.0	6.69	8.36[abc]	3.79	94.3	0.85[ab]
	Analysis of Variance (*P*>*F*)							
Rotation (R)	0.001	<0.001	0.90	0.007	0.001	0.08	0.019	0.01
Tillage (T)	0.92	0.009	<0.001	0.003	0.03	0.01	0.002	0.001
R-T	0.82	0.01	0.20	0.61	0.02	0.57	0.49	0.04

†Means followed by different letters within a column, treatment (rotation and tillage), and site are significantly different at $P<0.05$.

Table 4.5. Crop rotation (continuous corn, CC; and corn-soybean, CS) and tillage systems (conventional tillage, CT; ridge tillage, RT; and no-tillage, NT) interaction effect on soil organic carbon (SOC) and total nitrogen (TN) within aggregates at South Central Agricultural Laboratory (SCAL) site and Haskell Agricultural Laboratory (HAL) study sites, Nebraska, USA.

Treatments	**South Central Agricultural Laboratory (SCAL) Site**									
	SOC (g kg^{-1})					**TN (g kg^{-1})**				
	0.053 mm	0.25 mm	0.5 mm	1 mm	2 mm	0.053 mm	0.25 mm	0.5 mm	1 mm	2 mm
CC-CT	16.5	40.9	35.7^{b†}	45.2	42.1	1.23	2.82	2.50^{b}	3.15	5.01
CC-RT	14.3	27.1	57.0^{a}	51.5	66.4	0.98	2.09	3.65^{ab}	3.45	3.73
CC-NT	17.2	28.7	37.1^{b}	44.6	46.3	1.18	2.20	2.72^{b}	3.15	2.96
CS-CT	20.5	37.6	66.2^{a}	75.5	85.5	1.40	2.89	4.80^{a}	5.11	4.77
CS-RT	18.3	35.5	46.5^{ab}	51.6	72.6	1.26	2.63	3.38^{ab}	3.58	3.83
CS-NT	21.8	40.3	45.0^{ab}	48.0	50.0	1.36	2.57	3.67^{ab}	3.17	8.98
	Analysis of Variance (*P*>*F*)									
Rotation (R)	0.07	0.41	0.02	0.15	0.15	0.07	0.34	0.002	0.12	0.38
Tillage (T)	0.47	0.62	0.06	0.33	0.33	0.34	0.41	0.33	0.22	0.72
R-T	0.99	0.64	0.003	0.23	0.33	0.88	0.84	0.004	0.16	0.44
	Haskell Agricultural Laboratory (HAL) Site									
CC-CT	18.5^{b}	16.3^{b}	22.5^{b}	42.9^{a}	26.1^{b}	1.44^{b}	1.08^{b}	1.38^{b}	2.71	1.63^{b}
CC-RT	21.2^{ab}	23.9^{a}	27.5^{ab}	31.8^{a}	32.4^{a}	1.78^{a}	2.06^{a}	2.11^{ab}	2.42	2.56^{a}
CC-NT	23.7^{a}	27.6^{a}	33.6^{a}	29.1^{b}	35.5^{a}	1.96^{a}	2.42^{a}	2.76^{a}	2.24	2.79^{a}
CS-CT	19.6^{b}	20.9^{ab}	25.5^{b}	34.0^{a}	31.6^{a}	1.56^{b}	1.69^{b}	1.74^{b}	2.58	2.24^{ab}
CS-RT	22.6^{a}	25.2^{a}	34.2^{a}	35.8^{a}	37.2^{a}	1.85^{a}	2.02^{a}	2.65^{a}	2.63	2.74^{a}
CS-NT	23.2^{a}	27.6^{a}	33.3^{a}	36.9^{a}	33.4^{a}	1.92^{a}	2.33^{a}	2.77^{a}	2.88	2.53^{a}
	Analysis of Variance (*P*>*F*)									
Rotation (R)	0.48	0.13	0.12	0.72	0.38	0.33	0.30	0.03	0.31	0.37
Tillage (T)	0.004	<0.001	0.003	0.23	0.23	<0.001	<0.001	<0.001	0.92	0.02
R-T	0.004	0.02	0.02	0.004	0.02	<0.001	0.02	0.03	0.28	0.04

†Means followed by different letters within a column, treatment (rotation and tillage), and site are significantly different at $P<0.05$.

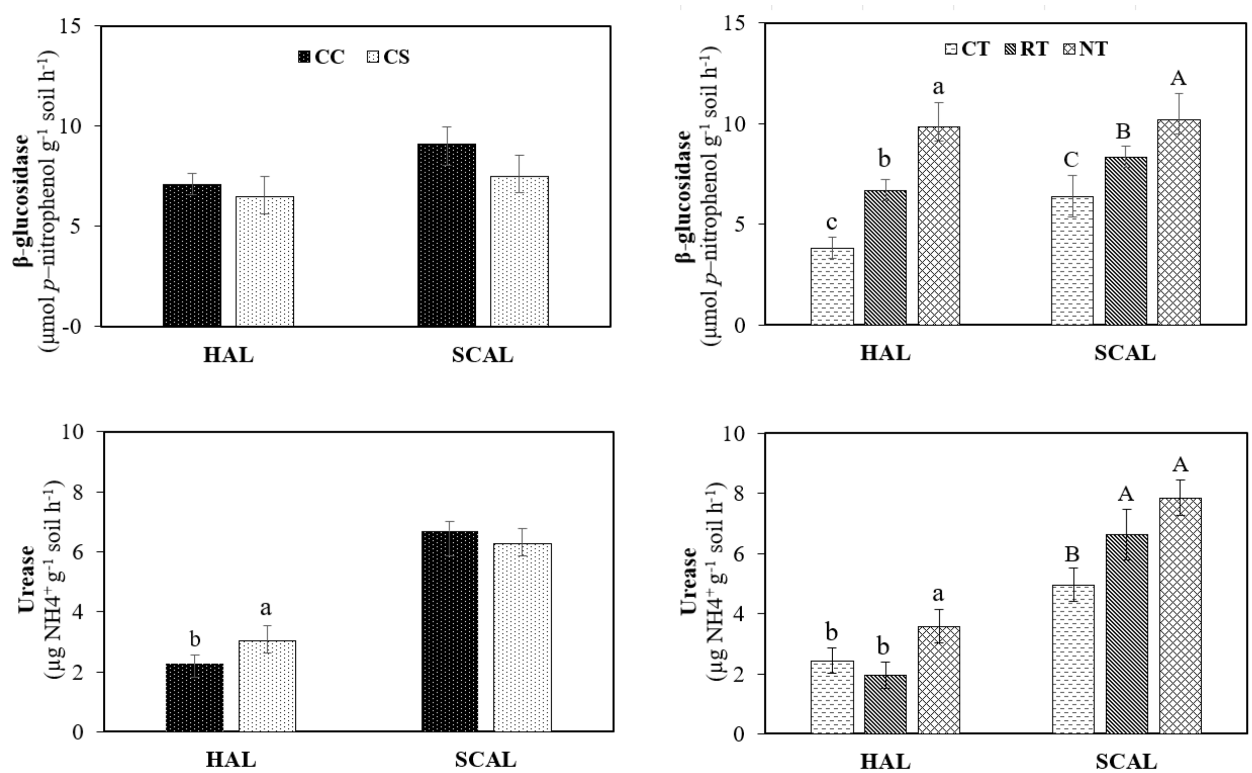


Figure 4.1. Soil enzymatic activity β-Glucosidase and urease as affected by crop rotation (continuous corn, CC; corn-soybean CS) and tillage (conventional tillage, CT; ridge tillage, RT; and no-tillage, NT) systems at South Central Agricultural Laboratory (SCAL) and Haskell Agricultural Laboratory (HAL) study sites, Nebraska, USA. Means with different lowercase (a) and uppercase (A) representing HAL and SCAL sites, respectively, are significantly different at *P* ≤ 0.05.

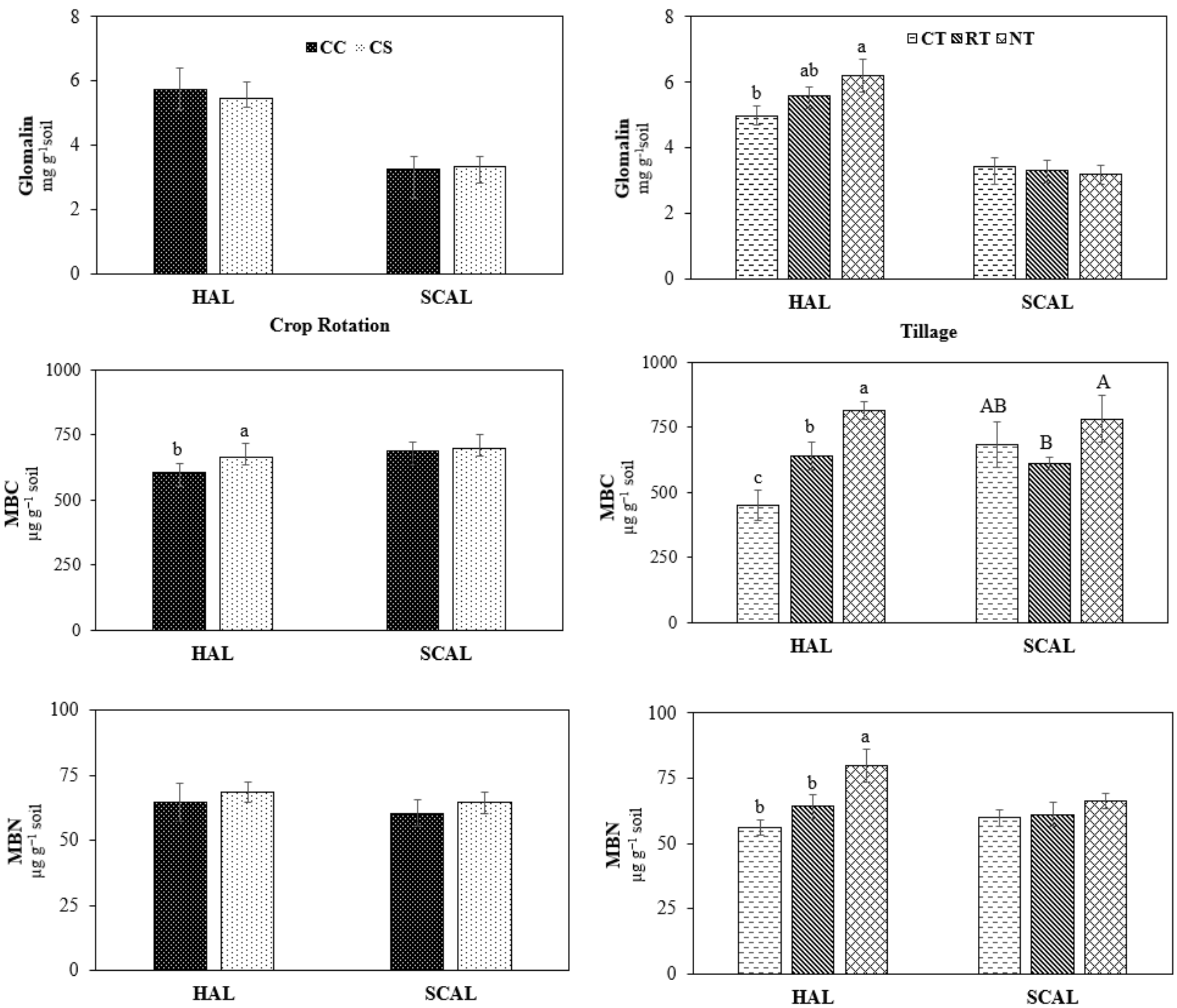


Figure 4.2. Glomalin, microbial biomass carbon (MBC) and nitrogen (MBN) as affected by crop rotation (continuous corn, CC; corn-soybean CS) and tillage (conventional tillage, CT; ridge tillage, RT; and no-tillage, NT) systems at South Central Agricultural Laboratory (SCAL) and Haskell Agricultural Laboratory (HAL) study sites, Nebraska, USA. Means with different lowercase (a) and uppercase (A) representing HAL and SCAL sites, respectively, are significantly different at $P \leq 0.05$.

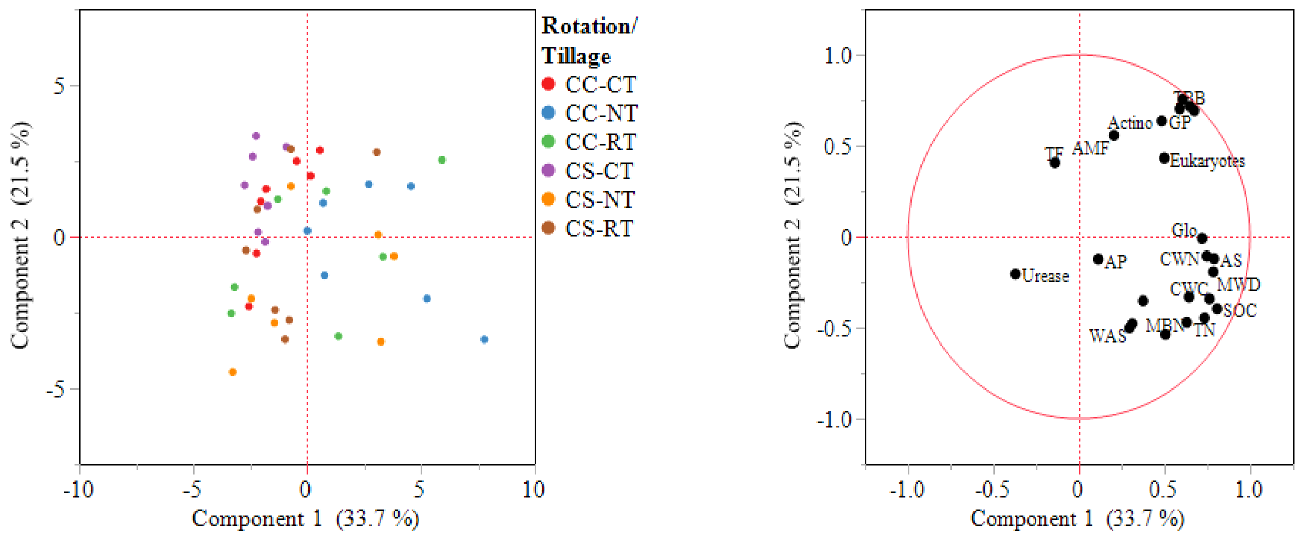


Figure 4.3. Principal component analysis (PCA) of the soil parameters with scores plotted in the plane of PC1 and PC2 (left) and eigenvectors (right). CC, continuous corn; CS, corn-soybean; CT, conventional tillage; RT, reduced tillage; NT, no-tillage.

CHAPTER 5

CONCLUSIONS AND SUMMARY

The thesis emphasizes the importance of conservation agricultural practices such as no-tillage farming and crop rotation systems. The study was conducted in two long-term experimental study sites - Haskell Agricultural laboratory (HAL) near Concord, NE (42°38'N, -96°98'W) with Coleridge silty clay loam (*fine-silty, mixed, mesic, Cumulic Haplustolls*) soils and South Central Agricultural Laboratory near Clay Center, NE (42°49'N, -99°90'W) with Crete silt loam (*fine, smectic, mesic, and Pachic Arguistoll*). Experimental design at both sites was a randomized complete block design in split plots with tillage as main-plot and rotation as sub-plot factors. The following conclusions were drawn from different objectives of this study, and are mentioned below as:

Objective 1. Soil hydro-physical properties

- No-till (NT) with CS rotation decreased the soil bulk density (ρ_b) and increased the saturated hydraulic conductivity at 0-10 cm depth.
- Plant available water (PAW) content at 10-20 cm depth was higher under NT system.
- The NT with CS rotation enhanced the number of mesopores and macropores as compared to the other treatments.
- The XCT measured pore parameters showed strong correlation with soil ρ_b, saturated hydraulic conductivity, and PAW content.

Objective 2. Soil health indicators

- No-till with CS rotation enhanced the activities of β-glucosidase and urease at the HAL study site.

- The NT with CS rotation enhanced the activity of arylsulfatase at SCAL study site.
- No-till with CC increased the water stable aggregates at HAL site and mean weight diameter at either (HAL or SCAL) site.
- At HAL study site, microbial biomass content was increased under NT and CS rotation

This study conclude that tillage and crop rotation systems impact different soil properties at different depths (objective 1). Long-term application of NT managed with CS rotation, in general, was beneficial in enhancing the soil physical and hydrological properties, and soil health indicators, however, differences were not significant always. This study emphasizes the significance of conservation management practices such as NT and crop rotations on soil hydro-physical properties and other soil health indicator attributes.

APPENDICES

Appendix 1

Appendix1.A. Obtained $P > F$ values at 5% significant level for rotation (R), tillage (T), and depth (D) factors as total depth (0-40 cm) included in the study. Note: SOC, soil organic carbon; ρ_b, bulk density; TN, total nitrogen; K_{sat}, saturated hydraulic conductivity; λ, saturated thermal conductivity; PAW, plant available water.

	SOC	ρ_b	TN	K_{sat}	λ	PAW
R	0.21	0.01	0.07	0.48	0.0012	0.89
T	<0.001	<0.001	0.003	<0.001	<0.001	0.02
D	<0.001	<0.001	< 0.001	<0.001	<0.001	<0.001
R-T	0.93	0.02	0.54	0.34	<0.001	0.31
R-D	0.09	0.34	0.26	0.02	<0.001	0.15
T-D	<0.001	0.64	0.03	0.003	<0.001	0.09
R-T-D	0.46	0.43	0.25	0.001	<0.001	0.21

Appendix 1.B. Obtained *P>F* values at 5% significant level for rotation (R), tillage (T), and depth (D) factors as total depth (0-40 cm) included in the study. Note: NmesP, number of mesopores; MesP, mesoporosity; NMP, number of macropores, MP, macroporosity; D, fractal dimension; τ, tortuosity; TNB, total number of branches; MBL, mean of average branch length

	NmesP	MesP	NMP	MP	D	τ	TNB	MBL
R	<0.001	0.93	0.009	0.002	0.73	0.28	<0.001	0.68
T	<0.001	0.36	<0.001	<0.001	<0.001	0.84	<0.001	0.35
D	<0.001	<0.001	<0.001	<0.001	<0.001	0.19	<0.001	<0.001
R-T	0.0148	0.59	0.02	0.57	0.57	0.11	0.0755	0.01
R-D	0.0197	0.12	0.84	0.32	0.24	0.03	<0.001	<0.001
T-D	<0.001	<0.001	<0.001	0.07	0.03	0.28	<0.001	0.008
R-T-D	0.21	0.89	<0.001	0.99	<0.001	0.47	<0.001	0.15

Appendix 1.C. Soil organic carbon (SOC), total nitrogen (TN), bulk density (ρ_b) as affected by crop rotation (corn-corn, CC; and corn-soybean, CS) and tillage (conventional tillage, CT; reduced tillage, RT; and no-tillage, NT) for 0-10, 10-20, 20-30, and 30-40 cm depths.

	SOC -------------g kg^{-1}-------------				TN -------------g kg^{-1}-------------				ρ_b ------------g cm^{-3}------------			
	NT	RT	CT	$\bar{x}$	NT	RT	CT	$\bar{x}$	NT	RT	CT	$\bar{x}$
0-10 cm depth												
CC	33.3^{a†}	25.4^{bc}	21.9^{bc}	26.8^{A‡}	2.74^{a}	2.03^{b}	1.51^{bcd}	2.09^{A}	1.20	1.23	1.33	1.25^{B}
CS	28.1^{ab}	24.2^{bc}	19.5^{b}	24.0^{A}	1.99^{bc}	1.74^{bc}	1.31^{d}	1.70^{A}	1.16	1.19	1.22	1.20^{B}
$\bar{x}$	30.7^{A§}	24.8	20.6^{A}		2.36^{A}	1.88^{A}	1.41		1.18^{C}	1.21^{B}	1.27^{B}	
10-20 cm depth												
CC	22.1	22.1	18.7	21.0^{AB}	1.21	1.58	1.03	1.27^{B}	1.32	1.31	1.47	1.37^{AB}
CS	19.6	20.6	19.9	20.0^{B}	1.41	1.26	1.23	1.30^{AB}	1.23	1.36	1.41	1.33^{B}
$\bar{x}$	20.1^{B}	21.3	19.3^{A}		1.31^{B}	1.32^{B}	1.13		1.27^{BC}	1.34^{A}	1.43^{A}	
20-30 cm depth												
CC	19.3	22.3	18.6	20.1^{B}	1.16	1.43	1.49	1.36^{B}	1.37	1.36	1.53	1.42^{AB}
CS	20.3	20.6	17.0	19.8^{B}	1.24	1.30	1.04	1.20^{B}	1.39	1.38	1.47	1.41^{AB}
$\bar{x}$	19.8^{B}	21.4	17.8^{A}		1.20^{B}	1.37^{B}	1.26		1.38^{B}	1.37^{A}	1.50^{A}	
30-40 cm depth												
CC	19.1	22.7	12.8	18.2^{B}	1.10	1.26	0.82	1.06^{B}	1.45	1.47	1.55	1.48^{A}
CS	22.5	23.1	13.6	19.7^{B}	1.19	1.09	0.95	1.07^{B}	1.43	1.46	1.50	1.46^{A}
$\bar{x}$	20.8^{B}	22.9	13.2^{B}		1.15^{B}	1.17^{B}	0.88		1.44^{A}	1.46^{A}	1.52^{A}	

†Mean values followed by different lowercase letters between each treatment within each depth and parameter represent significant differences due to a rotation by tillage interaction at $P<0.05$. No letters are shown if the rotation by tillage interaction was not significant.

‡Mean values within a column (averaged across NT, RT, and CT the tillage treatments), rotation, and parameter across different depths followed by the different uppercase letters represent significant difference ($P<0.05$).

§ Mean values within a row (averaged across the CC and CS rotations), tillage treatments, and parameter across different depths followed by the different uppercase letters represent significant difference ($P<0.05$)

Appendix 1.D. X-ray Computed Tomography (XCT) -measured number of mesopores and macropores as affected by crop rotation (corn-corn, CC; and corn-soybean, CS) and tillage (conventional tillage, CT; reduced tillage, RT; and no-tillage, NT) for 0-10, 10-20, 20-30, and 30-40 cm depths.

	Number of mesopores				Number of macropores			
	NT	RT	CT	$\bar{x}$	NT	RT	CT	$\bar{x}$
0-10 cm depth								
CC	4167^{b}	3370^{c}	3338^{c}	3625^{A}	279^{a}	154^{bc}	144^{c}	192^{A}
CS	5042^{a}	4642^{ab}	3303^{c}	4329^{A}	278^{a}	181^{b}	145^{c}	201^{A}
$\bar{x}$	4604^{A}	4006^{A}	3320^{A}		278^{A}	168^{A}	144^{A}	
10-20 cm depth								
CC	3300^{a}	2981^{a}	2397^{b}	2892^{B}	186^{ab}	144^{bc}	126^{c}	152^{B}
CS	3290^{a}	3149^{a}	2415^{b}	2951^{B}	229^{a}	135^{c}	132^{c}	165^{AB}
$\bar{x}$	3294^{B}	3064^{B}	2405^{B}		207^{b}	139^{B}	128^{AB}	
20-30 cm depth								
CC	2555^{ab}	2443^{ab}	1249^{b}	2080^{C}	168^{b}	132^{b}	123^{c}	140^{B}
CS	2591^{a}	3034^{a}	1325^{b}	2316^{B}	217^{a}	136^{bc}	101^{c}	151^{AB}
$\bar{x}$	2573^{C}	2738^{B}	1287^{C}		192^{BC}	133^{B}	112^{BC}	
30-40 cm depth								
CC	1381	1408	1128	1305^{D}	169^{a}	115^{b}	104^{b}	130^{B}
CS	1734	1693	1228	1551^{C}	160^{a}	139^{ab}	103^{b}	134^{B}
$\bar{x}$	1557^{D}	1550^{C}	1177^{C}		165^{C}	127^{B}	103^{C}	

†Mean values followed by different lowercase letters between each treatment within each depth and parameter represent significant differences due to a rotation by tillage interaction at $P<0.05$. No letters are shown if the rotation by tillage interaction was not significant.

‡Mean values within a column (averaged across NT, RT, and CT the tillage treatments), rotation, and parameter across different depths followed by the different uppercase letters represent significant difference ($P<0.05$).

§ Mean values within a row (averaged across the CC and CS rotations), tillage treatments, and parameter across different depths followed by the different uppercase letters represent significant difference ($P<0.05$).

Appendix 1.E. X-ray Computed Tomography (XCT) -measured mesoporosity (cm^3 cm^{-3}) and macroporosity (cm^3 cm^{-3}) as affected by crop rotation (corn-corn, CC; and corn-soybean, CS) and tillage (conventional tillage, CT; reduced tillage, RT; and no-tillage, NT) for 0-10, 10-20, 20-30, and 30-40 cm depths.

	Mesoporosity				Macroporosity			
	NT	RT	CT	$\bar{x}$	NT	RT	CT	$\bar{x}$
				0-10 cm depth				
CC	0.006^{ab}	0.007^{ab}	0.003^{b}	0.005^{A}	0.017^{a}	0.015^{ab}	0.012^{c}	0.014^{A}
CS	0.008^{a}	0.009^{a}	0.004^{b}	0.006^{A}	0.018^{a}	0.016^{ab}	0.013^{bc}	0.015^{A}
$\bar{x}$	0.007^{A}	0.008^{A}	0.003		0.017^{A}	0.015^{A}	0.012^{A}	
				10-20 cm depth				
CC	0.005	0.004	0.003	0.004^{B}	0.015^{ab}	0.012^{bc}	0.007^{d}	0.011^{AB}
CS	0.006	0.004	0.004	0.005^{A}	0.017^{a}	0.013^{abc}	0.010^{cd}	0.013^{AB}
$\bar{x}$	0.005^{B}	0.004^{B}	0.003		0.016^{AB}	0.012^{B}	0.009^{B}	
				20-30 cm depth				
CC	0.001	0.004	0.005	0.003^{B}	0.015^{ab}	0.011^{bc}	0.006^{d}	0.010^{B}
CS	0.002	0.003	0.005	0.003^{A}	0.017^{a}	0.013^{abc}	0.010^{cd}	0.011^{AB}
$\bar{x}$	0.002^{B}	0.003^{B}	0.005		0.016^{AB}	0.011^{BC}	0.008^{BC}	
				30-40 cm depth				
CC	0.003	0.002	0.005	0.003^{B}	0.013^{ab}	0.010^{ab}	0.005^{b}	0.009^{B}
CS	0.004	0.004	0.005	0.003^{A}	0.014^{a}	0.010^{ab}	0.005^{b}	0.010^{B}
$\bar{x}$	0.003^{B}	0.003^{B}	0.005		0.013^{B}	0.010^{C}	0.005^{C}	

† Mean values followed by different lowercase letters between each treatment within each depth and parameter represent significant differences due to a rotation by tillage interaction at P<0.05. No letters are shown if the rotation by tillage interaction was not significant.

‡ Mean values within a column (averaged across NT, RT, and CT the tillage treatments), rotation, and parameter across different depths followed by the different uppercase letters represent significant difference (P<0.05).

§ Mean values within a row (averaged across the CC and CS rotations), tillage treatments, and parameter across different depths followed by the different uppercase letters represent significant difference (P<0.05).

APPENDIX 2

Appendix 2.A. Glomalin, microbial biomass carbon (MBC) and nitrogen (MBN) as affected by crop rotation (continuous corn, CC; corn-soybean CS) and tillage (conventional tillage, CT; ridge tillage, RT; and no-tillage, NT) systems at South Central Agricultural Laboratory (SCAL) and Haskell Agricultural Laboratory (HAL) study sites, Nebraska, USA.

Treatments	South Central Agricultural Laboratory (SCAL) site		
	Glomalin	MBC	MBN
	mg g^{-1}soil	μg g^{-1} soil	
Rotation			
CC	3.27[a†]	686[a]	60.4[a]
CS	3.34[a]	697[a]	64.3[a]
Tillage			
CT	3.41	683[ab]	59.7[a]
RT	3.33	611[b]	61.1[a]
NT	3.18	782[a]	66.3[a]
	Analysis of Variance (*P*>*F*)		
Rotation (R)	0.74	0.83	0.07
Tillage (T)	0.69	0.05	0.05
R-T	0.47	0.86	0.85
	Haskell Agricultural Laboratory (HAL) site		
Rotation			
CC	5.73[a†]	607[b]	64.7[a]
CS	5.46[a]	662[a]	68.4[a]
Tillage			
CT	4.99[b]	450[c]	56.0[b]
RT	5.58[ab]	640[b]	64.1[b]
NT	6.21[a]	813[a]	79.6[a]
	Analysis of Variance (*P*>*F*)		
Rotation (R)	0.32	0.005	0.24
Tillage (T)	0.006	<0.0001	<0.0001
R-T	0.18	0.27	0.96

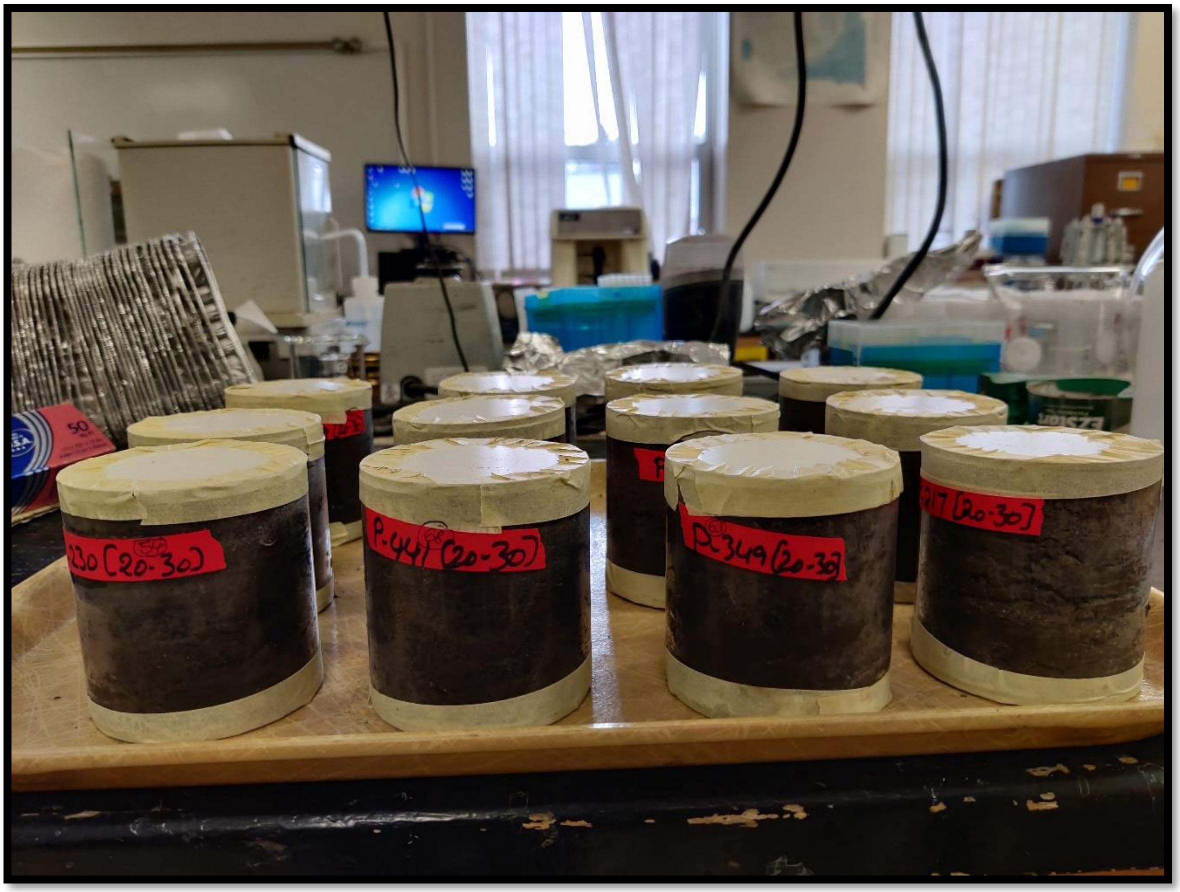
[†]Means with Means followed by different letters within a column, treatment (rotation and tillage), and site are significantly different at $P<0.05$.

Appendix 2.B. Soil enzymatic activity β-Glucosidase and urease as affected by crop rotation (continuous corn, CC; corn-soybean CS) and tillage (conventional tillage, CT; ridge tillage, RT; and no-tillage, NT) systems at South Central Agricultural Laboratory (SCAL) and Haskell Agricultural Laboratory (HAL) study sites, Nebraska, USA.

Treatments	SCAL Site		HAL Site	
	β-Glucosidase	urease	β-Glucosidase	urease
	μg p– nitrophenol g^{-1} soil h^{-1}	μg NH_4^+ g^{-1} soil h^{-1}	μg p– nitrophenol g^{-1} soil h^{-1}	μg NH_4^+ g^{-1} soil h^{-1}
CC-CT	87.8[d†]	70.3[cd]	112[d]	183[b]
CC-RT	135[c]	93.3[b]	166[b]	194[b]
CC-NT	221[a]	102[ab]	137[cd]	236[a]
CS-CT	88.3[d]	57.1[d]	161[bc]	103[d]
CS-RT	137[c]	75.6[c]	200[a]	146[c]
CS-NT	166[b]	113[a]	126[d]	201[b]
	Analysis of Variance ($P>F$)			
Rotation (R)	<0.001	0.03	<0.001	<0.001
Tillage (T)	<0.001	<0.001	<0.001	<0.001
R-T	<0.001	0.001	<0.001	<0.001

APPENDIX 3

Appendix 3.A. Collecting plexiglass core samples using soil core sampler.



Appendix 3.B Prepared plexiglass cores for computed tomography scanning

Appendix 3.C. X-ray Computed Tomography scanner used for the scanning of soil cores at University of Missouri, Columbia, USA.

VITA

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