SOIL PROFILE PROPERTIES AND GREENHOUSE GAS EMISSIONS AS INFLUENCED BY LONG-TERM CATTLE MANURE AND INORGANIC FERTILIZER APPLICATIONS UNDER CORN-SOYBEAN-SPRING WHEAT ROTATION IN EASTERN SOUTH DAKOTA

BY

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A dissertation submitted in partial fulfillment of the requirements for the

Doctor of Philosophy

Major in Plant Science

South Dakota State University

2023

DISSERTATION ACCEPTANCE PAGE
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This dissertation is approved as a creditable and independent investigation by a candidate for the Doctor of Philosophy degree and is acceptable for meeting the dissertation 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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ACKNOWLEDGMENTS

I would first like to express my appreciation to my advisors, Dr. Sandeep Kumar, and Dr. Peter Kovacs, for their support, patience, motivation, and excellent guidance for my doctoral research. Without them, this work would not have been possible. I would also like to thank my committee members, Dr. Sutie Xu, Dr. Peter Sexton, and Dr. Gary Hatfield, for all useful comments and being a part of my advisory committee.

I would like to thank the Research Farms of South Dakota State University for providing the study site for my research. My sincere thanks also go to the faculty and staff of the Department of Agronomy, Horticulture and Plant Science at South Dakota State University for their support and for sharing their knowledge. Next, I would like to thank Dr, Gandura Abagandura, Dr. Virginia Jin, Dr. Udayakumar Sekaran, Dr Navdeep Singh, Dr. Jashanjeet Kaur Dhaliwal, and Dr. Omolayo Olorunwa for sharing their knowledge and assisting me whenever needed.

I would like to say thanks to the current and past members of Soil Physics and Hydrology Lab at SDSU; Dr. Poulamee Chakraborty, Dr. Sangeeta Bansal, Dr. Jemila Chellappa, and my colleagues Goutham Thotakuri, Vaishnavi Varikuti, Namrata Ghmire, Sainfort Vitali, Varshith Kommineni. Finally, I would like to thank my family, Kayode Sangotayo Ejura Ibrahim, Ifeoluwa S., Ebunoluwa S., and Okikioluwa S., Hope Dudusola, and Chidinma Olewunne whose unconditional love, support, and sacrifice can never be forgotten.

Brookings, SD, USA (Anuoluwa Ojonoka Sangotayo)

CONTENTS

ABBREVIATIONS

ANOVA	Analysis of Variance
BL	Average branch length,
C	Carbon
C: N	Carbon: Nitrogen ratio
CWC	Cold water extractable carbon
CWN	Cold water extractable nitrogen
CH_4	Methane
CO_2	Carbon dioxide
CT	Computed tomography
DA	Degree of anisotropy
FD	Fractal dimension,
GC	Gas chromatograph
GHG	Greenhouse gas
HM	High manure,
HF	High fertilizer,
HWC	Hot water extractable carbon
HWN	Hot water extractable nitrogen
LM	Low manure,
MM	Medium manure,
MF	Medium fertilizer,
N	Nitrogen
N_2O	Nitrous oxide
PAW	Plant available water
PSD	Pore size distribution
SOC	Soil organic carbon
SOM	Soil organic matter
SWR	Soil water retention
TN	Total nitrogen
XCT	X-ray computed tomography
τ	Tortuosity
Ψ_m	Matric potential

ABSTRACT

SOIL PROFILE PROPERTIES AND GREENHOUSE GAS EMISSIONS AS INFLUENCED BY LONG-TERM CATTLE MANURE AND INORGANIC FERTILIZER APPLICATIONS UNDER CORN-SOYBEAN-SPRING WHEAT ROTATION IN EASTERN SOUTH DAKOTA

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2023

The application of manure and inorganic fertilizer in row crops may significantly influence soil and greenhouse gas (GHG) emissions. Understanding the long-term influence of these management practices on soil pore characteristics, hydro-physical properties and greenhouse gas emission is essential in developing proper conservation practices. However, there is limited information on the impact of cattle manure and inorganic fertilizer application on soil hydro-physical properties, soil pore characteristics at lower depths and surface GHGs emissions. Therefore, the objectives of this study were to; (i) utilize X-ray computed tomography (XCT) technique to quantify the impact of manure and fertilizer amendments under a corn (*Zea mays* L.)-soybean (*Glycine max* L.)-spring wheat (*Triticum aestivum)* rotation system on soil pore characteristics to the depth of 40 cm; (ii) assess the impact of different manure and inorganic fertilizer application rates on soil profile organic carbon and hydro-physical properties under corn-soybean-spring wheat rotation; (iii) to investigate the impacts of cattle manure and inorganic fertilizer on soil surface greenhouse gases (GHG) [carbon dioxide (CO_2), nitrous oxide (N_2O), and methane (CH_4)] fluxes from soils managed under corn-soybean- spring wheat rotation. The study was conducted at Brookings (initiated in 2008) and Beresford (2003) in South Dakota. Treatments included: low manure (LM), medium manure (MM), high manure (HM), medium fertilizer (MF), high fertilizer (HF), and control (CK). Four replicated intact cores were collected from all the treatments at 0-10, 10-20, 20-30, and 30-40 cm depths.

Data showed that treatments by depth interactions were mainly significant for soil organic carbon (SOC) content at 0-20 cm. The HM treatment increased the SOC by 8 to 68% compared to the CK and MF at 0-20 cm for either site. However, treatments did not always impact these parameters beyond 20 cm depth. The HM increased the SOC and TN stocks by 16-62% as compared to the CK, MF, and HF at 0-10 cm for either site. However, treatments did not always impact these parameters beyond 20 cm depth. Considering treatment as the main effect, the MM, HM, and HF increased the total number of pores (TP) compared to the CK at Beresford site. Soil depth impacted the TP and the total number of macropores (Tmacro), where more Tmacro was observed at 0-10 cm compared to the 30-40 cm depth at Beresford site. The MM and HM increased the SWR at 0, -5 kPa compared to MF, HF, and CK in Brookings, and the MM increased the SWR at -30 kPa as compared to the MF in Beresford at 0-40 cm depths. The cumulative soil surface N_2O, and CH_4 fluxes were increased by the MF treatment compared to the MM and CK in 2020. In general, the MF treatment on average resulted in higher cumulative GHG emissions compared to the MM and CK in both years. Also the global warming potential rate increased in 2020 by inorganic fertilizer rate as compared to the MM and CK.

This study illustrates the improvement in the XCT-derived pore characteristic with the long-term application of manure to a greater depth in the soil and highlighted the importance of the XCT technique in quantifying soil pore characteristics. For the soil

hydro-physical study, it was observed that the continuous manure application may enhance organic carbon and hydro-physical properties at deeper depths. The study also concluded that long-term manure application is more beneficial when you consider the application rate and its timing. It can improve hydro-physical properties, thereby stabilizing the soil structure and improving water retention at deeper depths. For the greenhouse gas study, data showed that inorganic fertilizer application in crops can be harmful to the environment by emitting higher GHG emissions, therefore, sustainable management practices needs to be explored to mitigate GHG emissions and reduce or eliminate the negative environmental impacts.

CHAPTER 1

INTRODUCTION

Soil management practices can impact soil organic carbon, which can affect soil health, crop yields, and sustainability (Aziz et al., 2009). Inorganic fertilizers and manure application affect soil hydro-physical environment by increasing the above-ground and root biomass due to the immediate supply of nutrients in sufficient quantities to crops (López Pérez et al., 1990).

Manure when added to the soil can also directly improve C and N fractions in the soil (Abagandura et al., 2023). This can be due to the increased organic carbon content in the manure. Therefore, the composition of manure plays an important role in the amount of C and N fractions in the soil. For instance, when there is a high nitrogen content or low C/N ratio in the manure, organic matter becomes easily degraded (Gross & Glaser, 2021). The application of manure to croplands, when used appropriately, can be a sustainable management strategy to improve productivity while enhancing soil organic carbon (SOC) and other hydro-physical properties of soils. Manure is also used for soil amelioration and to improve soil health (Ansari et al., 2020). The addition of cattle manure to the soils can improve soil physical attributes such as soil aggregate stability and reduce soil compaction by increasing the SOC content and total nitrogen content (Blanco-Canqui et al., 2015; Ozlu et al., 2019; Sekaran et al., 2020). It is a cost-effective way to dispose of and reprocess waste from livestock production. However, the over-application of manure as a result of high animal density is unacceptable from many points of view including greenhouse gas emissions (Schröder, 2005), and can be detrimental to the environment.

Therefore, it is interesting to observe the changes in soil and greenhouse gas emissions due to long-term manure application at different application rates. Studies showed that the incorporation of manure into the soils can reduce mineral fraction density, and thereby lowering soil bulk density (ρb) (Zhou et al., 2016). Others documented the application of inorganic fertilizer can increase the soil ρb (Pahalvi et al., 2021) which can adversely affect soil structure, reduce soil water retention, and thereby reduce plant available water (Agegnehu et al., 2014). Manure application as a soil amendment has been used globally to improve soil physical and hydrological properties (Ozlu et al., 2019). The application of manure can enhance soil aggregation, thereby promoting the formation of pores which can improve the water holding capacity of the soils (Usowicz & Lipiec, 2020). Yagüe et al. (2016) also revealed that manure application improved soil water infiltration regardless of the tillage practices. Manure acts as a binding agent in soil particles to improve soil aggregation (Hoover et al., 2019). Several studies reported the application of manure can enhance SOC, total nitrogen (TN) contents, and soil nutrients (Yang et al., 2014; Zhao et al., 2014). However, the effectiveness of manure application in increasing the SOC depends on the manure application rate (Abagandura et al., 2023), duration of continuous manure application, and manure quality (Ozlu et al., 2019).

Over several decades, large-scale production and application of inorganic fertilizers have tremendously increased and surpassed the use of organic manure to accomplish the goal of crop yield enhancement (Blanco-Canqui et al., 2015). However, the use of inorganic fertilizers is generally associated with the reduction in soil aeration, reducing SOC content, aggregate stability, movement of water through the soils, structural stability, and increases the risk of nutrient losses (Blanco-Canqui et al., 2013;

Zhou et al., 2016). The physical and chemical effects of mineral fertilizers on soil structure further depend on fertilizer type. For example, a large amount of ammonium fertilizers can disperse clays, adversely affecting soil aggregation (Paradelo et. al., 2013)

. Therefore, the long-term application of inorganic fertilizer alone can be detrimental to the soil physical properties, and the changes in soil pore characteristics because of its application should be quantified. In addition, continuous application may impact the soil to a larger depth. Therefore, it is interesting to observe the changes in soil pore-characteristics to a deeper depth due to long-term application of manure and inorganic fertilizer in row crops. X-ray computed tomography (XCT) is an innovative technique for visualizing soil pore structure (Singh et al., 2021). Results can be achieved more accurately and faster when compared to using soil water retention data to quantify porosity. It is an efficient method to evaluate the effect of productive rejuvenation on aggregate stability and to visualize and quantify the 3-D soil pore network of different soil types (Zhao et al., 2017; Zhou et al., 2012). For instance, XCT can be used to identify the soil macroporosity and mesoporosity, which are more sensitive to management practices such as the application of manure and fertilizer. Inorganic fertilizer application can indirectly improve the carbon content in the soil by increasing crop dry matter production which improves the biomass inputs into the soil from roots and crop residues (Jiang et al., 2014). This supports the studies that suggest moderate manure application is beneficial for long-term use, soil C and N fractions form an easily decomposable sub-pool of SOC, and this is a sensitive indicator of the effects of management practice on the soil. Based on this, C and N fractions are early indicators of changes in soil health (Bongiorno et al., 2019). Therefore, the changes in C and N fractions can reveal the effects of manure and inorganic fertilizer, tillage, and other management practices on the soil, which makes active organic carbon an important indicator for soil organic matter, soil fertility, and crop productivity (Nie et al., 2019). Inorganic fertilizers, primarily nitrogen-based fertilizers, contribute to emissions of nitrous oxide (N_2O) from agricultural soils (Hui et al., 2017). Soil N_2O is primarily produced from manure application, directly by nitrification–denitrification processes or indirectly when N is lost through NH_3 volatilization or nitrate leaching and then converted to N_2O (Sun et al., 2014). Despite the high nutrient bioavailability of the application of inorganic fertilizer, continuous application may lead to a decline in SOC content (Jiang et al., 2014). Although, inorganic fertilizer application can also improve the SOC content and enhance the soil structure (Hati et al., 2007). The long-term application of inorganic fertilizers can also lead to the deterioration of soil health in terms of soil physical, chemical, and biological properties (Agegnehu et al., 2014). When compared with a moderate application of manure, the latter has been found to improve the soil structure and infiltration rate in the long term. Therefore, long-term application of manure can reduce the incurred cost of production by reducing or eliminating the application of inorganic fertilizer, and hence in turn protecting the environment by reducing or eliminating negative impacts of the continuous use of inorganic fertilizer. However, it is also important to know the optimal rate of manure application as a high rate of manure and inorganic fertilizer application on a long-term basis can lead to nitrogen and phosphorus pollution, surface runoff, and decreased soil aeration (Zhang et al., 2017), thereby decreasing SOC and TN stock.

Study Objectives

The purpose of this study was to evaluate the impact of long-term manure and inorganic fertilizer application on soil hydro-physical properties, X-ray computed tomography soil pore characteristics, and surface GHG emissions to determine whether the long-term application of manure or inorganic fertilizer can be adopted as a sustainable management practice to benefit the soils and environment. The objectives of this study were evaluated in three sub-studies as outlined below. Specific objectives were evaluated individually for each study.

Study 1. This study was entitled “cattle manure application for 12-17 years enhanced depth distribution of x-ray computed tomography-derived soil pore characteristics” with the specific objective being to (i) assess the impacts of different rates of manure and inorganic fertilizer on soil pore characteristics, SOC, and total nitrogen (TN) to a depth of 40 cm (ii) examine the correlations between SOC and various XCT-derived soil pore characteristics.

Study 2. This study was entitled “changes in soil profile organic carbon and hydro-physical properties as impacted by long-term manure and inorganic fertilizer rates under a corn-soybean rotation system” with the specific objectives being to (i) assess the impacts of different rates of manure and inorganic fertilizer on soil water retention (SWR), ρ_b, and plant available water (PAW) content to a depth of 40 cm, (ii) examine the effects of different rates of manure and inorganic fertilizer on SOC and TN stock, and cold and hot-water extractable C and N to a depth of 40 cm, and (iii) correlate the SOC and TN stock to PAW content across different treatments.

Study 3. This study was entitled “long-term manure and inorganic fertilizer impacts on soil greenhouse gas fluxes under corn-soybean-spring wheat rotation system” with the specific objective being measurement and comparison of soil surface carbon dioxide. (CO_2), nitrous oxide (N_2O) and methane (CH_4) fluxes among medium manure (MM), medium fertilizer (MF), and control (CK) treatments.

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CHAPTER 2

LITERATURE REVIEW

In recent times, several soil scientists have mentioned concerns about developments in soil science and the possible consequences on food production, biodiversity degradation, and water quality which could influence the capacity of these soils to retain organic carbon (Mol & Keesstra, 2012).

2.1. Inorganic fertilizer

Fertilizer use by U.S. agriculture has increased over the past few decades. Historically, fertilizer demand has been affected by population and economic growth, agricultural production, fertilizer prices, and government policies (Food & Agriculture Organization, 2011). Because of this, fertilizer usage has increased in the agriculture sector in recent times. Nitrogen (N), phosphorus (P), and potassium (K) are primary nutrients and are supplied either individually or in various combinations and ratios to the crops (Amenumey & Capel, 2014). This nutrient can be directly supplied to crops as an inorganic fertilizer. Inorganic fertilizers are good for enhancing plant growth because the nutrients are water-soluble. Therefore, the effects of inorganic fertilizer are immediate with nutrients supplied. Appropriate applications of inorganic fertilizer can increase soil organic matter through higher levels of root mass and crop residues (Han et al., 2016)

2.1.1. Harmful Effects of Inorganic Fertilizer and Manure

Inorganic fertilizers and manure application can increase crop yields and improve food security. However, long-term inorganic fertilizer use or high application of inorganic fertilizer can be associated with environmental problems like soil degradation, water pollution, and greenhouse gas emissions (Savci, 2012). It has been observed that without crop rotations and animal manures to maintain soil productivity, farmers have increased the use of inorganic fertilizers (Parr et al., 1994). Also, over-application of inorganic fertilizer can cause harmful effects such as leaching, pollution of water, and reduces the availability of the trace element (Schubert, 2009). Inorganic fertilizer enhances soil organic matter decomposition, which leads soil degradation and decrease in soil aggregation. All these results in nutrients loss through leaching, gas emission can lead to diminish fertilizer efficiency (Alimi et al., 2007). Over-application of inorganic fertilizer can destroy soil organisms, reduce the colonization of plant roots with mycorrhizae and inhibit symbiotic N-fixation by rhizobia due to high N-fertilization and also hazardous to the soil environment. The problem over-application of inorganic fertilizer causes are not only limited to the soil health but also to the environment and human health (Savci, 2012). Over-application of manure as a result of high animal density is unacceptable from many points of view including greenhouse gas emissions (Schröder, 2005). In some cases, over-application of manure can lead to over-supplication of phosphorus on production sites and at field manure application sites. This is associated with the fact that when manure has been applied to meet the nitrogen requirement of plants, it may over-supply P, because of the low nitrogen to phosphorus ratio of most manures (Mikkelsen, 2000).

2.2. Impacts of Manure and Inorganic Fertilizer on Soil Porosity

The differences observed in soil pore structure may influence the soil microbial activity and this may enhance or adversely impact soil organic carbon (SOC) content (Kravchenko et al., 2015) which may in the long run impact the soil structure. The arrangement of soil pore space is responsible for crucial processes like; transportation and storage of water and nutrients, and gas exchange with the atmosphere (Vogel et al., 2019). The changes in the soil pore network may influence solute transport (Soto-Gómez et al., 2018). Therefore, it is important to understand the effects of different soil amendments on the soil structure. Manure is used for soil amelioration and soil health improvement (Ansari et al., 2020). The addition of cattle manure to the soils can improve soil physical attributes such as soil aggregate stability and reduce soil compaction by increasing the SOC content and total nitrogen content (Blanco-Canqui et al., 2015; Ozlu & Kumar, 2018; Ozlu et al., 2019; Sekaran et al., 2020). Also, with the presence of earthworms in manure, which are the major decomposers of organic matter, there is a release of binding agents that increase aggregate stability and improve soil structure (Akhila & Entoori, 2022). It is a cost-effective way to dispose of and reprocess waste from livestock production. However, the over-application of manure is unacceptable from many points of view including greenhouse gas emissions, and can be detrimental to the environment (Centner, 2023). For instance, Manure-derived Na^+ has also been proposed to act as a dispersion agent which may lower soil aggregate stability (Guo et al., 2019), there deteriorating the soil structure. Therefore, the effectiveness of manure application in increasing the SOC depends on the manure application rate (Abagandura et al., 2023), duration of continuous manure application, and manure quality (Ozlu et al., 2019).

Inorganic fertilizers and manure application affect the soil physical environment by increasing the above-ground and root biomass due to the immediate supply of nutrients in sufficient quantities to crops (Meena et al., 2018; Moharana et al., 2012). Over several decades, large-scale production and application of inorganic fertilizers have tremendously increased and surpassed the use of manure to achieve the goal of grain yield enhancement (Blanco-Canqui et al., 2015). However, the use of inorganic fertilizers is generally associated with the reduction in soil aeration, reduction of SOC content, lower aggregate stability, decreased movement of water through the soils, decreased structural stability, and increases the risk of nutrient losses (Zhou et al., 2016). The physical and chemical effects of mineral fertilizers on soil structure further depend on fertilizer type. For example, a large amount of ammonium fertilizers can disperse clays, adversely affecting soil aggregation, while a phosphatic fertilizer may enhance aluminum, and aluminum phosphate fertilizer can promote aggregate stability (Yan et al., 2016). The long-term application of inorganic fertilizer alone can be detrimental to the soil physical properties. Therefore, quantification is crucial as these changes may occur from changes in the soil pore characteristics. To better understand the effect of the continuous application of different manure and inorganic fertilizer rate on the soil structure, the extent to which the soil pore characteristic is improved depth-wise is crucial. Therefore, it is interesting to observe the changes in soil pore characteristics to a lower depth because of the long-term application of manure and inorganic fertilizer in row crops.

2.2.1. Impacts of Manure and Fertilizer on soil organic carbon and soil hydro-physical properties

Soil organic carbon is important in the improvement of soil physical, chemical and biological properties (Ouédraogo et al., 2007). Conserving the soil organic carbon is crucial enhancing sustainable soil management (Doran, 1996). Manure and inorganic fertilizer are majorly used to improve soil quality and crop productivity (Verma & Sharma, 2007). However, soil organic carbon is not sensitive to short-term changes of soil quality (Haynes, 2005). Manure generally improves soil organic carbon (SOC) content, as reported in many individual studies. For example, Sainju et al. (2008) observed an increase of soil surface (0–20 cm) SOC stock of about 3.2 Mg C ha^{-1} after 10 years of poultry litter application in comparison to inorganic fertilizer treatment (Sainju et al., 2008). In Nepal, after 25 annual cattle manure applications, surface (0–30 cm) SOC stocks were higher by about 19.1 Mg C ha^{-1} than control (unfertilized) plots (Gami et al., 2009). In China, after 22 years of pig manure application, the surface soil layer (0–15 cm) accumulated 3.8 Mg C ha^{-1} more than inorganic fertilizer (Huang et al., 2010). However, some studies report no significant or even negative change of SOC stocks following manure application (Angers et al., 2010). However, the effectiveness of manure application in increasing the SOC depends on the manure application rate (Abagandura et al., 2023), duration of continuous manure application, and manure quality (Ozlu et al., 2019).

Soil aggregation is a key indicator of soil quality that determines water, gas and nutrient retention and transport in soils, and creates a conducive environments for microbial communities (Feeney et al., 2006). Zhang et al. (2014) noted that the incorporation of manures would significantly improve the root growth by modifying the soil physical properties (Zhang et al., 2014). Improving soil physical properties may also improve soil pore characteristics. Bulk density is closely linked to soil compaction, which modifies the interactions between the air, water, and soil and subsequently has an impact on microbial activity, nutrient uptake, and water retention (Martınez & Zinck, 2004). The application of manure can improve soil microbial activity, increase microbial metabolites such as polysaccharides by the process of decomposition, and acts as the source of binding soil aggregate (Lin et al., 2019; Ji et al., 2014). These products can promote the formation of soil aggregates and improve soil structure.

2.2.2. X-ray computed tomography (CT) on soils.

It is well known that the composition, distribution, and orientation of soil constituents reflect soil development. Therefore, X-ray CT can be considered an important tool in obtaining qualitative and quantitative data on soils in a spatial manner, respecting limitations that are imposed by the energy levels utilized for image acquisition and by the resolution of imagery. Understanding the information about the soil, size, shape, and arrangement can show the soil weathering process and nutrient release (Taina et al., 2008).

X-ray computed tomography (XCT) is an innovative technique for visualizing soil pore structures (Singh et al., 2021). Results can be achieved more accurately and faster when compared to using soil water retention data to quantify porosity. It is an efficient method to evaluate the effect of productive rejuvenation on aggregate stability and to visualize and quantify the 3-D soil pore network of different soil types (Zhao et al., 2017; Zhou et al., 2012). For instance, XCT can be used to identify soil macroporosity and mesoporosity, which are more sensitive to management practices such as the application of manure and fertilizer. The use of XCT to identify the complex geometry of soil macropores in the order of a few micrometers allows correlating pore characteristics to water transport, as well as other physical properties of soils. The XCT scanning technique has been used to compare the pore structure of manure and inorganic fertilizer treated soils. Zhou et al. (2016) used the micro-CT scanning technique to quantify soil porosity and found that the soils treated with the combination of inorganic fertilizer and organic manure had greater intra- and inter-aggregate pores which significantly improved their saturated hydraulic conductivity, compared to that of the non-fertilized soils. Despite the application of the XCT technique in studying soil pores under diverse management scenarios, studies involving the use of XCT to study soil pore systems to a deeper soil depth because of long-term manure and fertilizer applications at different rates in row-crop are limited.

2.3. Agricultural emissions

Global agricultural GHG emissions increased from 3.26 billion tons in 1961 to 5.96 billion tons in 2019 (FAO. FAOSTAT Climate Change, 2021). GHG emissions have attracted broad interest because of the various potential consequences of climate change (Lal et al., 2018). Of the several GHGs, carbon dioxide (CO_2) is recognized as the largest contributor to the greenhouse effect it contributes about 60% of total radioactive forcing among the longstanding GHG (Forster et al., 2007). Agriculture has been observed to contribute around 10-12% of global anthropogenic GHG emissions (Smith et al., 2014). According to the Intergovernmental Panel on Climate Change, a positive GHG value indicates an increase or gas emission into the atmosphere, while a negative value indicates greenhouse gases sinking into the soil (Houghton, 1996). Global warming potential (GWP) has been used to quantitatively compare the greenhouse effect of different greenhouse gases. Global warming potential is similar to the ozone depleting potential. However, the global warming potential is subject to major conceptual difficulties arising from the fact that the atmospheric lifespan for part of the emitted CO_2 is, for all practical purposes, infinite. Measuring the GWP can be used to shift attention away from short lived gases such as methane and toward CO_2. As this is calculated in CO_2 equivalent (Harvey, 1993). Another thing to understand is that several naturally produced greenhouse gases trap heat, including water vapor, carbon dioxide (CO_2), ozone (O_3), methane (CH_4) and nitrous oxide (N_2O). Which can contribute to the GHG emission. The CO_2, CH_4, and N_2O gases are long-lasting in the atmosphere and are the major contributors to positive increases in GHG emission. Agricultural activities are significant producers of CH_4 and N_2O gas. (Houghton, 1996).

2.3.1. Impacts of fertilizer and manure on greenhouse gas (GHG) emissions

Over the past centuries, the human population has nearly increase by 500% from about 1.6 billion people in 1900 to nearly 7.6 billion in 2017 (Nations, 2017). And is predicted to increase to 9.8 billion in 2050 (Dorling, 2021), which at this growth rate might surpass the estimated increase. This has led to an increase in crop production, therefore increasing inorganic fertilizer usage by 200–300% increase between 1970 and 2010 (Change, 2014). Subsequently, it has been estimated that nearly 50% of the world's population is now dependent on nitrogen fertilizers for their sustenance (Erisman et al., 2008). Furthermore, the International Fertilizer Association (IFA) reported a 46%

increase in urea production between 2003 and 2013 (Heffer & Prud’homme, 2016) and the Food and Agriculture Organization of the United Nations (FAO) predicted an annual increase in fertilizer nutrient demand of 1.5, 2.2 and 2.4% for nitrogen, phosphorus (P) and potassium (K), respectively, between 2016 and 2020 (FAOSTAT, 2017). Although, fertilizers have assisted us in keeping up with the growing demand for agricultural products, their historical overuse (Byrareddy et al., 2019; Sun et al., 2019) has introduced harmful effects to the environments. Indeed, improper fertilizer usage can have a detrimental effect on ecosystem by causing soil nutrient depletion, nutrient run-off, reduced biological diversity and greatly increased greenhouse gas (GHG) emissions from agricultural practices (Sutton et al., 2013). Agriculture has been observed to contribute around 10-12% of global anthropogenic GHG emissions (Smith et al., 2014). Production of inorganic nitrogen fertilizers alone accounts for approximately 2% of the world's energy use (Sutton et al., 2013). This alone can significantly impact the GHG emission rate.

Inorganic fertilizer or manure application can impact soil N_2O emission as it is formed from microbial processes through denitrification and nitrification under dry and wet conditions (Smith & Conen, 2004). Nitrous oxide is a more potent GHG with a radiative forcing potential approximately 12 times larger than CH_4 (Alyea et al., 1996). Transformation from ammonium to nitrate via nitrification is a source of N_2O and produces NO_3^- which is a source of N for denitrification, the biological reduction of nitrate to N_2 gas, where N_2O is an important product of incomplete denitrification (Chadwick et al., 2011). Methane from manure is generated during anaerobic decomposition of organic matter in the feces and bedding material (Møller et al., 2004).

The absence of oxygen is a precondition for production of CH_4 via microbial metabolism of organic material in livestock manure. Methane production from manure is affected by environmental factors such as temperature (Sommer et al., 2007), biomass composition and manure management (Ni et al., 2008). Soil management practices that inserts organic wastes and incorporate carbon have been evaluated as important way for increasing the capacity of atmospheric carbon sinks and mitigating global warming (Johnson et al., 2011; Tian et al., 2009). The application of inorganic fertilizers and manure can alter soil GHG emissions, although the response varies in function of several factors such as changes in temperature, precipitation and waste composition (Scott et al., 2000).

2.4. Research Gap

The literature reviewed reveals that previous studies have evaluated the impacts of manure and inorganic fertilizer on soil hydro-physical properties, x-ray computed tomography-derived soil pore characteristics and GHG emissions separately under diverse environmental conditions. However, there are some research gaps among the studies those are mentioned below as.

1. Previous studies have explored the impacts of manure and inorganic fertilizer separately on soil quality. However, studies that assessed the impacts of the different rates of long-term manure and inorganic fertilizer application on soil hydro-physical properties to lower depths are very limited.

2. Fewer studies on observing the soil pore characteristics managed under different rates of long-term manure and inorganic fertilizer application using X-ray computed

tomography scanning technique, that can provide 3-D visualization and analysis of soil pores.

3. Limited information is available on impact of long-term manure and inorganic fertilizer application on greenhouse gas (GHG) emission fluxes, cumulative GHG emission, and global warming potential.

Therefore, this study will try to fill the above-mentioned research gaps with the major goal of the study was to assess the impacts of different rates of manure and inorganic fertilizer application under crop rotation system on soil hydro-physical (e.g., soil organic carbon, water retention, bulk density), soil pore characteristics and GHG emissions.

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CHAPTER 3

Cattle Manure Application for 12 and 17 Years Enhanced Depth Distribution of X-Ray Computed Tomography-Derived Soil Pore Characteristics

Abstract

Long-term fertilizer application in row crops may influence soil pore characteristics, thereby impacting soil aggregation and structure. Understanding these influences on soil pore characteristics is useful in adopting suitable conservation practices. However, there is limited information on the impact of cattle manure and inorganic fertilizer application at various rates on soil pore characteristics at a microscale level in the soil profile. Therefore, in this study, the X-ray computed tomography (XCT) technique was used to quantify the impact of manure and inorganic fertilizer amendments under a corn (*Zea mays* L.)-soybean (*Glycine max* L.)-spring wheat (*Triticum aestivum)* rotation system on soil pore characteristics to a depth of 40 *cm* at two experimental sites i.e., Brookings (initiated in 2008) and Beresford (2003) in South Dakota. Treatments included: low manure (LM), medium manure (MM), high manure (HM), medium fertilizer (MF), high fertilizer (HF), and unfertilized control (CK). Four replicated intact soil cores were collected from each treatment at 0-10, 10-20, 20-30, and 30-40 cm depths. Image visualization and processing were performed using ImageJ software at a voxel resolution of (0.26×0.26×0.28) mm^3. Overall, the HM treatment increased the SOC as compared to all other treatments at both study sites at 0-20 cm of soil depth. For example, the HM treatment increased the SOC by 8 to 68% compared to the CK and MF at the study sites. However, both high and medium manure treatments were able to increase the

macroporosity and mesoporosity in the 0-10 and 10-20 cm soil depths at both study sites. The HM, MM, and HF also increased the total number of pores (TP) compared to the CK at the Beresford site. In addition, the XCT-derived macroporosity and the total number of macropores showed a linear increase with the increase in SOC across all the treatments, suggesting an improvement in soil pore structure with an increase in SOC using the long-term manure treatments. This study illustrates the importance of the XCT technique in quantifying soil pore characteristics and suggests a long-term medium manure application to enhance soil structure as compared to an equivalent inorganic fertilizer application.

3.1. Introduction

The changes in soil pore structure may influence the soil microbial activity and this may enhance or adversely impact soil organic carbon (SOC) content (Kravchenko et al., 2015) which in the long run impacts the overall soil structure. The arrangement of soil pore space is responsible for crucial processes like water storage, nutrient cycling, gas exchange with the atmosphere, and solute transport (Soto-Gómez et al., 2018; Vogel et al., 2019). Therefore, it is important to understand the effects of different soil amendments on the soil structure. Manure is used for soil amelioration and soil health improvement (Ansari et al., 2020). The addition of cattle manure to the soils can improve soil physical attributes such as soil aggregate stability and reduce soil compaction by increasing the SOC content and total nitrogen content (Blanco-Canqui et al., 2015; Ozlu & Kumar, 2018a; Ozlu et al., 2019; Sekaran et al., 2020). In addition, with the presence of earthworms in manure, which are the major decomposers of organic matter, there is a release of binding agents that increase aggregate stability and improve soil structure

(Akhila & Entoori, 2022). It is a cost-effective way to dispose of and reprocess waste from livestock production. However, the over-application of manure is unacceptable from many points of view including greenhouse gas emissions, and can be detrimental to the environment (Centner, 2023). For instance, Manure-derived Na^+ has also been proposed to act as a dispersion agent which may lower soil aggregate stability (Guo et al., 2019), there deteriorating the soil structure. Therefore, the effectiveness of manure application in increasing the SOC depends on the manure application rate (Abagandura et al., 2023), duration of continuous manure application, and manure quality (Ozlu et al., 2019).

Inorganic fertilizers and manure application affect the soil physical environment by increasing the above-ground and root biomass due to the immediate supply of nutrients in sufficient quantities to crops (Meena et al., 2018; Moharana et al., 2012). Over several decades, large-scale production and application of inorganic fertilizers have tremendously increased and surpassed the use of manure to achieve the goal of grain yield enhancement (Blanco-Canqui et al., 2015). However, the use of inorganic fertilizers is generally associated with the reduction in soil aeration, reduction of SOC content, lower aggregate stability, decreased movement of water through the soils, decreased structural stability, and increased risk of nutrient losses (Zhou et al., 2016). The physical and chemical effects of mineral fertilizers on soil structure further depend on fertilizer type and their rates of application. For example, a large amount of ammonium fertilizers can disperse clays, adversely affecting soil aggregation, while a phosphatic fertilizer may enhance aluminum while aluminum phosphate fertilizer can promote aggregate stability (Yan et al., 2016). Therefore, it is important to quantify the impacts of long-term application of inorganic fertilizers on the soil pore characteristics. Furthermore, it was observed that the impacts of soil amendments (fertilizer and manure) on SOC were observed to greater depths in soil (Liu et al., 2013). Therefore, to better understand the effect of the continuous and long-term application of manure and inorganic fertilizer at various rates on the soil structure, it is crucial to quantify the impact to a lower depth in the soil.

X-ray computed tomography (XCT) is an innovative technique for visualizing soil pore structures (Singh et al., 2021). Results can be achieved more accurately and faster when compared to using soil water retention data to quantify porosity. It is an efficient method to evaluate the effect of productive rejuvenation on aggregate stability and to visualize and quantify the 3-D soil pore network of different soil types (Zhao et al., 2017; Zhou et al., 2012). For instance, XCT can be used to identify soil macroporosity and mesoporosity, which are more sensitive to management practices such as the application of manure and fertilizer. The use of XCT to identify the complex geometry of soil macropores in the order of a few micrometers allows correlating pore characteristics to water transport, as well as other physical properties of soils. The XCT scanning technique has been used to compare the pore structure of manure and inorganic fertilizer-treated soils. Zhou et al. used the micro-CT scanning technique to quantify soil porosity and found that soils treated with the combination of inorganic fertilizer and organic manure had greater intra- and inter-aggregate pores which significantly improved their saturated hydraulic conductivity, compared to that of the non-fertilized soils (Zhou et al., 2016). Despite the application of the XCT technique in studying soil pores under diverse management scenarios, studies involving the use of XCT to study soil pore systems to a lower soil depth after long-term manure and fertilizer applications at different rates in row crops are limited.

Studies have reported an improvement in soil physical attributes with manure application in combination with inorganic fertilizers as compared to unfertilized soils (Schjønning et al., 1994; Yang et al., 2011; Zhengchao et al., 2013). However, comparatively limited information is available on the long-term impact of only manure or only inorganic fertilizers at different rates on XCT-derived soil pore characteristics especially unique properties like tortuosity, fractal dimension, and average branch length. Two long-term (>10 years) field experiments were utilized in this study to observe the changes in pore characteristics to 40 cm depth following the long-term application of manure and inorganic fertilizer at different rates. The present study is based on the hypothesis that long-term manure application may improve soil pore characteristics to a depth of 40 cm as compared to long-term inorganic fertilizer application. The specific objectives of the study are to (1) assess the impacts of different rates of only manure and only inorganic fertilizer on XCT-derived soil pore characteristics, soil organic carbon (SOC), and total nitrogen (TN) to a depth of 40 cm (2) examine the correlations between SOC and various XCT-derived soil pore-characteristics.

3.2. Materials and Methods

3.2.1. Study site

Sampling was conducted from two existing long-term studies located at South Dakota State University's research farms. The first site was located at Felt Research Farm

(44˚ 22’ 07.15” N and 96˚ 47’ 26.45” W) near Brookings, South Dakota on a well-drained Vienna soil (Fine-loamy, mixed, frigid Udic Haploborolls), and the second site was at Southeast Research Farm (43˚ 02’ 33.46” N and 96˚ 53’ 55.78” W) in Clay County, near Beresford, South Dakota, USA, on Egan soil (Fine-silty, mixed, mesic Udic Haplustolls). The long-term research was initiated in 2008 at Brookings and in 2003 at Beresford to study the effect of organic and inorganic fertilizer application rates on crop production and soil properties. The plot dimensions for Brookings site were 6m by 18 m. The plots were nearly flat with a slope of <1% and an elevation of 518 m. The experimental areas are in a humid continental climate having relatively humid summers and cold, snowy winters with a mean air temperature of 27.8°C in the summer and -15.8°C in the winter, respectively. The mean annual precipitation was about 638 mm. The plots at Beresford site were established in nearly flat areas with a slope of <1%, and an elevation of 390 m. The plot dimensions at Beresford were 5m by 20 m. This experimental site was observed with a humid continental climate having relatively humid summers and snowy winters with a mean air temperature of 29.5°C in the summer and -13.6°C in the winter, respectively. The mean annual precipitation was about 678 mm.

3.2.2. Study Treatments

Each study site included six treatments: The three manure application rates included were: low manure (LM) contained a quantity of manure rate based on the recommended phosphorous requirement, medium manure (MM) contained a quantity of manure rate based on recommended nitrogen requirement, and high manure (HM) contained a quantity of manure-based on double the recommended nitrogen requirement. The two inorganic fertilizer application rates included were: medium fertilizer (MF) contained the recommended fertilizer rate, high fertilizer (HF) contained a high fertilizer rate, and a control treatment (CK) which did not receive manure and fertilizer. Treatments were arranged in a randomized complete block design with four replicates. The cropping system was corn (*Zea mays* L.)-soybean [*Glycine max* (L.) Merr] rotation at both sites, until 2019. In 2020, spring wheat (*Triticum aestivum* L.) and cover crops (radish) were added to the cropping system, and it became a corn-soybean-spring wheat rotation system.

The manure and fertilizer application rates followed the South Dakota Fertilizer Recommendation Guide for the 3.7 Mg ha^{-1} spring wheat yield goal in 2020. In 2020, the nutrient application target rate for both sites was 136 kg N ha^{-1}, 49 kg P_2O_5 ha^{-1}, and 91.5 kg K_2O ha^{-1}. Additional treatment details used in this study since 2003 can be found in,(Gautam et al., 2020; Ozlu & Kumar, 2018b). Beef dry and dairy solid manure were used at the Beresford and Brookings sites, respectively, to apply the target nutrient rates according to the treatments. The manure used was analyzed by certified commercial laboratories for nutrient concentration to determine application rates. No manure or fertilizer was applied before the soybean. The amount of manure and fertilizer applied was based on the soil test levels and target yield goal in each year applied. On average, dairy manure with approximately 31% and beef manure with 22% moisture for Brookings and Beresford sites, respectively, were used from 2003 to 2020. Urea, mono ammonium phosphate, and potash fertilizer were used for the inorganic fertilizer. The study sites were tilled to a soil depth of 20 cm about 1 to 3 days before planting, to incorporate all the treatments added and residue left on the field.

3.2.3. Soil Sampling and Sample Preparation

A total of 192 (96 from each site) intact cores from six treatments, four soil depths (0 to 10, 10 to 20, 20 to 30, and 30 to 40 cm), and four replications from each site were collected in July 2020. Plexiglass cores (76.2 mm long and 76.2 mm in diameter, with a 3.2-mm-thick wall) were used for the sampling. These cores were extracted from the soil manually using a core sampler vertically inserted in the soil (Figure 5). For each depth, the plexiglass cores were inserted leaving 11.9 mm of soil at the top and bottom to minimize disturbance to the intact soil sample. Soil cores were then trimmed using a serrated knife, sealed with plastic caps at both ends, labeled, and stored in plastic bags at 4°C pending analysis. In the laboratory, soil cores were slowly saturated from the bottom and then drained at −4.0 kPa using a low-tension table to remove water from macropores to improve image contrast for XCT scanning. Samples were secured at both ends with wooden caps and masking tape and stored in a cold room in preparation for scanning. The cores were transported in a cooler to the University of Missouri Veterinary Health Center in Columbia, MO for XCT scanning.

3.2.4 X-ray Computed Tomography Scanning and Image Analysis

Intact core samples were scanned using a Toshiba Aquilion 64 X-ray CT scanner to acquire images. About ten cores were placed horizontally on the scanner bench per time. To perform a spiral scanning with a peak voltage current of 135 kV and an X-ray tube current of 200 mA. The slice thickness was 0.28 mm, producing a resultant voxel size of $(0.26 \times 0.26 \times 0.28)\ \mathrm{mm}^3$. The entire sample was imaged with a field of view of 512 by 512 pixels. The captured XCT scanned data were exported as a stack of TIFF images and 3D visualization, image cropping, and segmentation were performed using the public domain processing software FIJI (ImageJ software)'(Abràmoff et al., 2004; Rasband, 2015; Schneider et al., 2012). Image slices that were subject to interference from the beam hardening were removed from the stack (Singh et al., 2020). Stacks were then cropped to obtain a region of interest (ROI) (60 mm in diameter and 60 mm in height). Stacks were pre-processed with a median three-dimensional filter (radius = 2.0 voxels) to reduce the possibility of reading noise, and contrast enhancement with saturated pixels of 0.4% was used to improve the contrast between the soil matrix and pores in the image. The images were converted to eight-bit and the segmentation process was carried out by the auto-local threshold algorithm of Phansalkar taking the parameter radius as 10, parameter 1(k) as 0.3, and parameter 2 (r) as 0.2 in ImageJ (Phansalkar et al., 2011). The Phalsankar algorithm is a modification of Sauvola's thresholding method to deal with low-contrast images (Sauvola & Pietikäinen, 2000). This procedure resulted in a binary image in which pores and soil matrix were represented by white and black pixels, respectively. The binary image obtained from segmentation was then visually inspected to check the image quality and the presence of artifacts, and features made up of one voxel were removed to avoid classification of noise in further analysis (Singh et al., 2020). Several definitions of macropores are available in the literature. For example, pores with an equivalent cylindrical diameter (ECD) larger than 1 mm (Luxmoore, 1981), larger than 1.2 mm (Katuwal et al., 2015), and larger than 3 mm (Germann & Beven, 1981) were considered as macropores. In this study, pores with ECD ≥ 1000 μm were considered as macropores. Macroporosity (>1,000 μm ECD) and coarse mesoporosity (150 to 1,000 μm ECD) and total porosity, which is macroporosity plus coarse mesoporosity, were obtained as the ratio of the total volume of macropores and coarse mesopores, and all pores, respectively, to the volume of ROI.

3.2.4.1 Quantification of pore characteristics

To measure the pore characteristics, the “Particle Analyser” and “Skeletonize-3D” plugins available with BoneJ in ImageJ (Doube et al., 2010) were used. ImageJ was used to estimate the total number of pores (TP), total number of macropores (MP), and total number of mesopores. The skeletonized binary stack provided a summary of the tortuosity (τ), average branch length (BL, mm), fractal dimension (FD), and degree of anisotropy (DA) for each of the 192 intact soil cores.

The mean tortuosity (τ) for the entire soil core was calculated as the ratio of the total actual pore length (L_t) to the total Euclidean distance (L_l) of all the pores in the soil core:

$$\tau = \frac{\sum_{i=1}^{n} L_t}{\sum_{i=1}^{n} L_l}$$

where, i is the index of a pore branch and n is the total number of pore branches in a soil core. In this study, average branch length and τ, were calculated only in the z (vertical) direction. The 3D binary images were used to estimate the FD using the box-counting algorithm in ImageJ. A schematic diagram of the various steps used in XCT analysis is shown in Figure 6. The image-based soil porosity ($\mathrm{cm^3\ cm^{-3}}$) was determined as follows:

$$\text{Porosity } (\mathrm{cm^3\ cm^{-3}}) = \frac{\text{Total volume of pores}}{\text{Volume of ROI}}$$

where ROI is the region of interest, that is the cropped CT image used in our analysis.

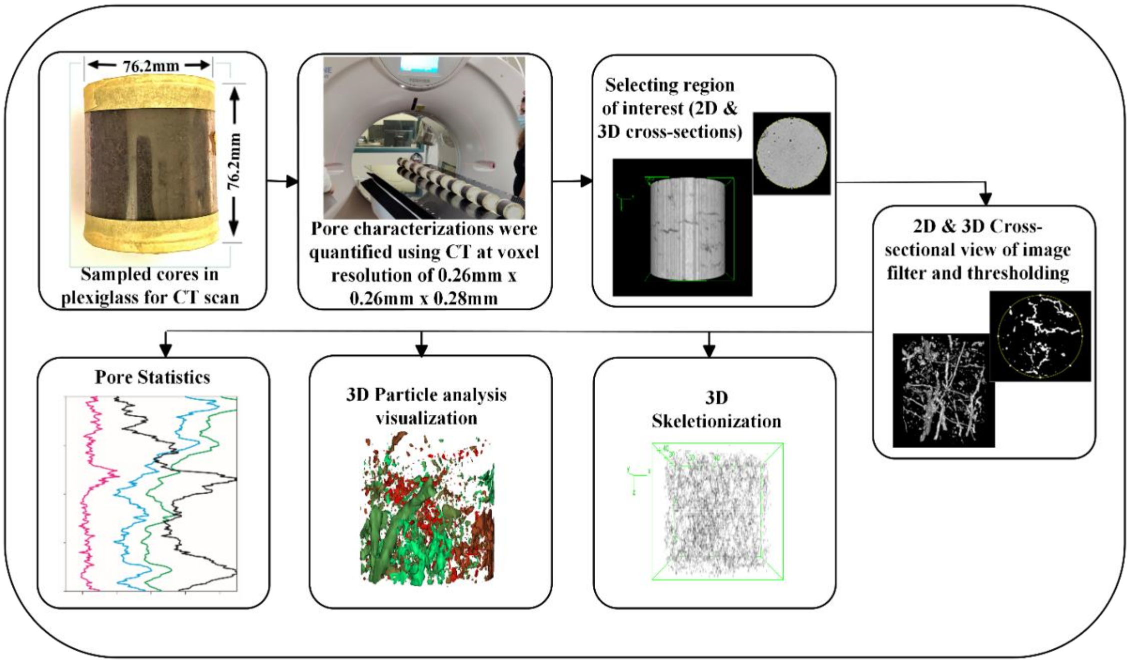

Figure 6. Workflow showing the procedures used in this study for image processing and pore quantification from X-ray computed tomography scanned data.

3.2.5 Soil organic carbon and nitrogen assay

Soil samples were sieved through a 0.5 mm sieve for SOC and TN analysis. All visible residues were removed before grinding using a planetary mill pulverisette (Fritsch International). The SOC and TN were determined by dry combustion method (Schumacher, 2002) using a Tru-Spec- carbon/hydrogen/nitrogen analyzer (TruSpec; LECO Corporation, St. Joseph, MI, USA).

3.2.6 Statistical Analysis

Analysis of variance (ANOVA) was conducted to compare the effects of different treatments and soil depth on the soil pore characteristics, soil organic carbon, and total nitrogen in the R modeling environment RStudio (RStudio, Inc., Boston, MA, USA). Treatment × Depth interactions were also observed using two-way ANOVA. Data were transformed when necessary, using the Box-Cox method. Significance was determined at $\alpha = 0.05$ level for all statistical analyses in this study. The relationships between XCT-derived soil pore characteristics, SOC, and TN content were analyzed by Pearson's correlation. The statistical significance of Pearson's correlation coefficient was determined at the $p = .05$ and $p = .01$ levels. Principal component analysis (PCA) of the full dataset was analyzed using the multiple factor analysis (MFA) procedure in RStudio. The PCA was used to subgroup experimental treatments based on the measured XCT-derived soil pore characteristics by generating the eigenvectors of the parameters and component scores for each unit. Each eigenvector loading indicates the direction and magnitude of association between XCT-derived soil pore characteristics and treatments.

3.3. Results and Discussion

3.3.1. Impact of manure and inorganic fertilizer application on Soil Organic Carbon and Total Nitrogen content (SOC and TN)

Treatments by depth interactions were mainly significant for the SOC content for both sites and were significant for the TN content for Beresford site (Table 3.1 and Figure 3.1). Where the HM treatment increased the SOC compared to the CK and MF at 0 to 20 cm in either site. However, treatments did not impact SOC beyond 20 cm depth at Beresford site. Considering treatment as the main effect, the HM increased SOC and TN content compared to the MF at 0 to 40 cm depths in Beresford site. Considering depth as the main effect, the SOC values were the highest at the surface (10 cm) depth as compared to the other depths at both sites (Table 3.1).

At Brookings site, significant treatment × depth interaction was observed for the SOC contents (Figure 3.1A). The LM, MM, and HM treatments (29.3, 27.4, and 31.7 g kg^{-1}, respectively) increased the SOC content as compared to the MF (24.5 g kg^{-1}) by 19.6, 11.8, and 29.4%, respectively, at 0 to 10 cm. At 10 to 20 cm, the HM increased SOC content by 7.8 to 10.9% as compared to CK, MF, HF, and LM. At 20 to 30 cm, the LM treatment increased SOC content by 8.6, 9.2, and 16.9% as compared to the CK, MF, and HM, respectively. At 30 to 40 cm, the LM and MM increased SOC contents by 22.1 to 27.8% when compared to the MF, HF, and HM. At Brookings site, the TN contents were influenced by the soil depth (Table 3.1). The TN content at 0 to 10 cm was 1.4, 1.9, and 2.6 times higher than TN contents at 10 to 20 cm, 20 to 30 cm, and 30 to 40 cm, respectively. In addition, the TN content at 10 to 20 cm was 1.3 times higher than the 20 to 30 cm depth and 1.4 times higher at 20 to 30 cm when compared to 30 to 40 cm, at Brookings site.

At Beresford site, significant treatment × depth interactions were observed for both SOC and TN contents (Figure 3.1E and 3.1F). The HM increased SOC and TN content by 40.1 to 93.8% as compared to other treatments, at 0 to 10 cm. At 10 to 20 cm, the HM increased SOC content by 25 and 27.7% as compared to the CK and MF, respectively. In addition, the HM treatment increased TN content as compared to the CK, MF, and MM treatments at 10 to 20 cm soil depth. There were no treatment impacts at 20 to 30 and 30 to 40 cm depth for SOC and TN contents at Beresford site.

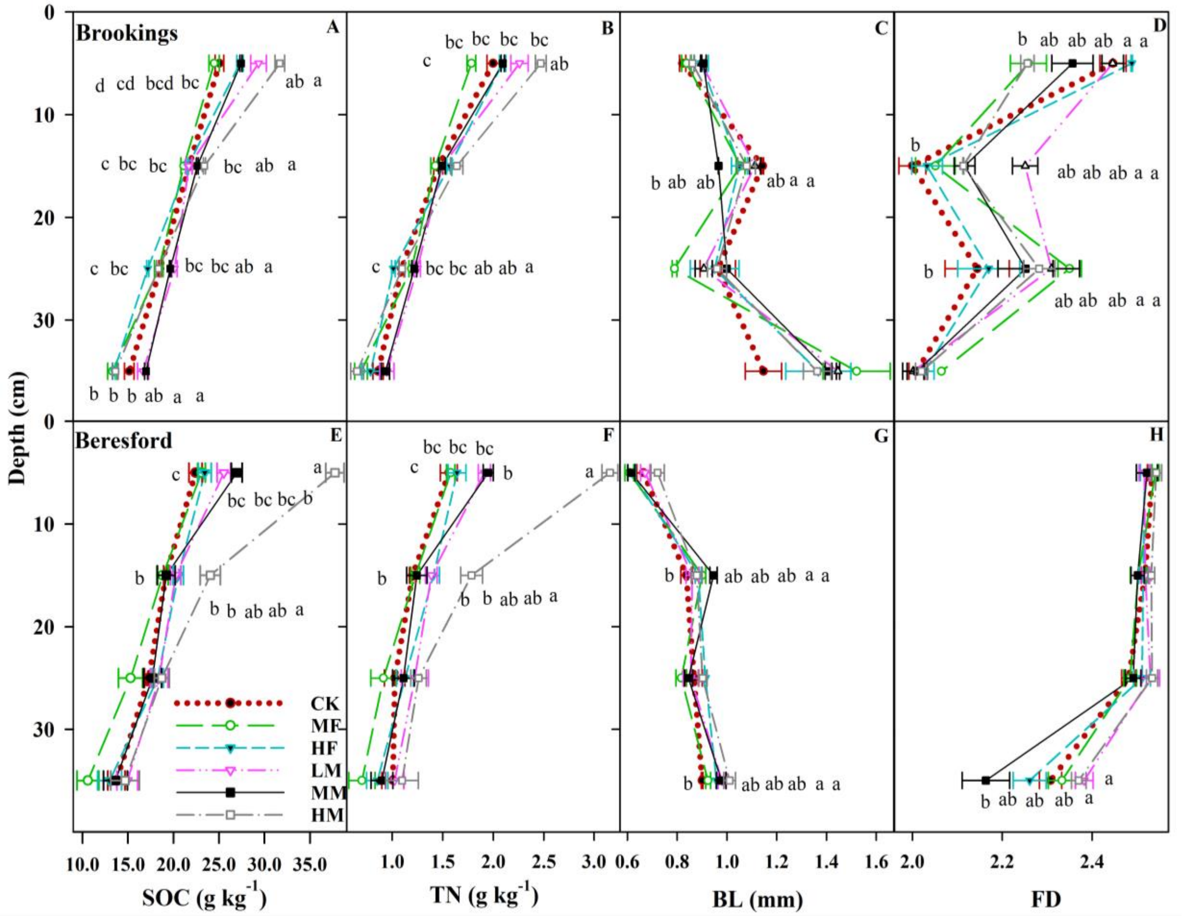

Figure 3.1. Soil depth and long-term nutrient application effects on soil organic carbon (SOC), total nitrogen (TN), branch length (BL), and fractal dimension (FD). Nutrient applications include medium fertilizer (MF), high fertilizer (HF), low manure (LM), medium manure (MM), high manure (HM) rate applications, and control (CK) at Brookings (Figure 3.1A – D) and Beresford sites (Figure 3.1E – 1H). Different lower-case letters indicate statistically significant different treatments at *p*=0.05 level at each depth.

Table 3.1. Soil organic carbon (SOC), total nitrogen (TN), XCT-derived average branch length (BL), tortuosity (*τ*), degree of anisotropy (DA), and fractal dimension (FD) as influenced by long-term medium fertilizer (MF), high fertilizer (HF), low manure (LM), medium manure (MM), high manure (HM) rate applications, and control (CK) as a function of treatment and soil depths.

	SOC **($g\ kg^{-1}$)**	**TN** **($g\ kg^{-1}$)**	**BL** **(*mm*)**	**Tortuosity** **(τ)**	**DA**	**FD**
			Brookings Site			
Treatment						
CK	20.1^{a}	1.4^{a}	1.019^{a}	1.284^{a}	0.306^{a}	2.131^{a}
MF	19.3^{a}	1.3^{a}	1.051^{a}	1.285^{a}	0.245^{a}	2.121^{a}
HF	19.9^{a}	1.4^{a}	1.066^{a}	1.288^{a}	0.261^{a}	2.142^{a}
LM	21.9^{a}	1.5^{a}	1.091^{a}	1.273^{a}	0.293^{a}	2.158^{a}
MM	21.7^{a}	1.4^{a}	1.070^{a}	1.281^{a}	0.320^{a}	2.197^{a}
HM	21.8^{a}	1.5^{a}	1.059^{a}	1.281^{a}	0.286^{a}	2.166^{a}
Depth (cm)						
0 – 10	27.5^{a}	2.1^{a}	0.870^{c}	1.271^{b}	0.294^{a}	2.375^{a}
10 – 20	22.0^{b}	1.5^{b}	1.068^{b}	1.265^{b}	0.323^{a}	2.090^{c}
20 – 30	18.7^{c}	1.1^{c}	0.928^{c}	1.298^{a}	0.229^{b}	2.251^{b}
30 – 40	14.8^{d}	0.8^{d}	1.371^{a}	1.295^{a}	0.294^{a}	2.019^{c}
			Analysis of Variance (P>F)			
Treatments	0.532	0.861	0.978	0.779	0.188	0.943
Depth	<0.001	<0.001	<0.001	<0.001	0.002	<0.001
Trt × Depth	0.033	0.308	0.030	0.025	0.042	0.003
			Beresford Site			
Treatment						
CK	$18.2^{ab\dagger}$	1.2^{b}	0.818^{a}	1.209^{a}	0.644^{a}	2.460^{a}
MF	16.9^{b}	1.1^{b}	0.812^{a}	1.207^{a}	0.654^{a}	2.466^{a}
HF	18.8^{ab}	1.3^{b}	0.844^{a}	1.210^{a}	0.583^{a}	2.451^{a}
LM	19.8^{ab}	1.4^{ab}	0.842^{a}	1.216^{a}	0.623^{a}	2.487^{a}
MM	19.4^{ab}	1.3^{b}	0.846^{a}	1.211^{a}	0.650^{a}	2.420^{a}
HM	23.8^{a}	1.8^{a}	0.878^{a}	1.211^{a}	0.631^{a}	2.495^{a}
Depth (cm)						
0 – 10	26.5^{a}	2.0^{a}	0.650^{c}	1.228^{a}	0.684^{a}	2.529^{a}
10 – 20	20.3^{b}	1.4^{b}	0.884^{b}	1.221^{a}	0.704^{a}	2.515^{a}
20 – 30	17.6^{b}	1.1^{bc}	0.867^{b}	1.200^{b}	0.697^{a}	2.505^{a}
30 – 40	13.4^{c}	0.9^{c}	0.958^{a}	1.195^{b}	0.439^{b}	2.303^{b}
			Analysis of Variance (P>F)			
Treatments	<0.001	0.002	0.752	0.880	0.669	0.452
Depth	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Trt × Depth	<0.001	<0.001	0.009	0.370	0.193	0.007

[†] Mean values within the same column followed by different small letters for each site are significantly different at p<0.05 for treatment

In this study, high manure application rate was found to have the largest impact on the SOC and TN contents as compared to the medium inorganic fertilizer application rate at the surface depth (0 to 10 cm) of both sites. It is likely due to the direct addition of larger amounts of organic matter and total nitrogen in the form of animal manure, and indirectly through increased stubbles and root residues (Mallory & Griffin, 2007; Merbach & Schulz, 2013). Ozlu & Kumar, (2018a) found that a higher manure application rate increases the soil organic matter and total nitrogen when compared to a lower manure application rate. Therefore, an increasing amount of manure would increase SOC and TN content, but a moderate application of manure would be more beneficial for the long term over a high manure application rate (Wei et al., 2022). On the other hand, inorganic fertilizers indirectly influence SOC contents by increasing crop yields, thereby impacting biomass inputs, and increasing the return of crop residues to the soil (Jiang et al., 2014). This might be the reason for the observed high values of SOC and TN for HF plots at both sites in our study. However, the long-term use of inorganic fertilizer and high manure application rate may increase the risk of environmental pollution and lower sustainability (Yagmur et al., 2017; Zhang et al., 2013). The highest SOC content was measured at the surface depth. This may be because of supplied nutrients at the surface depth and decreasing root biomass with depth increase (Li et al., 2011; Liu et al., 2013). We also observed that the different manure applications improved the SOC at lower soil depths at Brooking site (Figure 3.1). This may be due to earthworms burrowing through the soil causing the SOC content to move to lower depths (Lorenz & Lal, 2005).

3.3.2. Impact of manure and inorganic fertilizer application on XCT-derived pore characteristics

Treatments by depth interactions were significant for the total number of pores (TP), the total number of macropores (Tmacro), and the total number of mesopores (Tmeso) in Brookings site (Table 3.2 and 3.3). Where the MM treatment increased the TP, Tmacro, and Tmeso compared to the MF, and HF at 0 to 20 cm. At 20 to 30 cm, the MF treatment increased TP, Tmacro, and Tmeso compared to the MM. Treatment did not impact the TP, Tmacro, and Tmeso at 30 to 40 cm in Brookings site. Considering treatment as the main effect, the MM, HM, and HF treatment increased the TP compared to the CK treatment at Beresford site. Considering depth as the main effect, the TP and Tmacro values were higher at 0 to 10 cm compared to the 30 to 40 cm in Beresford site (Table 3.2).

At Brookings site, significant treatment × depth interactions were observed for TP, Tmacro, and Tmeso (Table 3.3). For the TP, the HF treatment had 2.3 and 2 times lower TP than the MM and LM at 0 to 10 cm. At 10 to 20 cm, the MM and HM increased the TP 1.4 to 2.0 times more than the CK and HF. At 20 to 30 cm, the HM and MF increased the TP 1.4 to 2.0 times more than the CK, HF, and MM. However, treatment did not impact TP at 30 to 40 cm. For the Tmacro, the LM and MM increased Tmacro 1.7 to 2.0 times more than the MF and HF, at 0 to 10 cm. At 10 to 20 cm, the MM increased Tmacro was 1.5 to 1.9 times more than the CK, MF, and HF. In addition, the HM increased the Tmacro 2.7, 1.6, and 2.5 times more than the CK, LM, and MM, respectively, at 20 to 30 cm, at Brookings site. However, treatment did not impact Tmacro at 30 to 40 cm. For the Tmeso, the MM increased the Tmeso by 1.7, 1.8, 2.3, and

1.7 times more than the CK, MF, HF, and HM, respectively, at 0 to 10 cm at Brookings site. At 10 to 20 cm, the MM increased the Tmeso by 1.5 to 2.0 times more than the CK, MF, HF, LM, and HM at 10 to 20 cm. At 20 to 30 cm, the MF and HF increased the Tmeso by 1.5 to 2.0 times more than the CK, LM, and MM at Brookings site. However, treatment did not impact Tmeso at 30 to 40 cm, in Brookings site.

At Beresford site, when considering treatment as the main effect, the MM, HM, and HF increased TP compared to the CK for Beresford site. Considering depth as the main effect, significant differences were observed for the TP and Tmacro (Table 3.2). The TP at 30 to 40 cm was 1.5, 1.32, and 1.27 times lower than at 0 to 10 cm, 10 to 20 cm, and 20 to 30 cm, respectively. The Tmacro at 0 to 10 cm was 1.3 and 1.4 times higher than the Tmacro at 20 to 30 cm and 30 to 40 cm, respectively. The Tmacro at 10 to 20 cm was 1.3 times higher than the Tmacro at 30 to 40 cm.

Table 3.2. XCT-derived macroporosity, mesoporosity, porosity total number of pores, macropores, and mesopore as influenced by long-term medium fertilizer (MF), high fertilizer (HF), low manure (LM), medium manure (MM), high manure (HM) rate applications, and control (CK) as a function treatment and soil depth.

	Total no. of pores	**Total no. of macropores**	**Total no. of mesopores**	**Porosity (*cm^{3}cm^{-3}*)**	**Macroporosity (*cm^{3}cm^{-3}*)**	**Mesoporosity (*cm^{3}cm^{-3}*)**
			Brookings Site			
Treatment						
CK	858^{a}	113^{a}	745^{a}	0.010^{a}	0.007^{ab}	0.003^{a}
MF	977^{a}	116^{a}	861^{a}	0.009^{a}	0.005^{b}	0.002^{a}
HF	839^{a}	113^{a}	726^{a}	0.012^{a}	0.008^{ab}	0.002^{a}
LM	1256^{a}	166^{a}	1089^{a}	0.013^{a}	0.010^{ab}	0.003^{a}
MM	1421^{a}	168^{a}	1253^{a}	0.017^{a}	0.010^{a}	0.004^{a}
HM	1111^{a}	152^{a}	978^{a}	0.014^{a}	0.009^{ab}	0.003^{a}
Depth (cm)						
0 - 10	2411^{a}	299^{a}	2111^{a}	0.020^{a}	0.013^{a}	0.007^{a}
10 – 20	941^{b}	125^{b}	815^{b}	0.010^{b}	0.010^{b}	0.002^{bc}
20 – 30	528^{c}	61^{c}	466^{c}	0.015^{b}	0.006^{c}	0.002^{b}
30 – 40	428^{c}	66^{c}	375^{c}	0.005^{c}	0.003^{d}	0.001^{c}
			Analysis of Variance (P>F)			
Treatments	0.4170	0.4398	0.4017	0.1489	0.0358	0.4757
Depth	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Trt × Depth	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
			Beresford Site			
Treatment						
CK	2176^{b}[†]	190^{a}	1986^{b}	0.020^{c}	0.014^{b}	0.006^{c}
MF	2609^{ab}	188^{a}	2421^{ab}	0.024^{bc}	0.016^{ab}	0.008^{bc}
HF	2911^{a}	235^{a}	2676^{a}	0.024^{bc}	0.014^{b}	0.010^{ab}
LM	2703^{ab}	214^{a}	2488^{ab}	0.028^{ab}	0.019^{a}	0.009^{abc}
MM	2888^{a}	237^{a}	2652^{a}	0.025^{bc}	0.017^{ab}	0.008^{abc}
HM	2965^{a}	242^{a}	2723^{a}	0.031^{a}	0.020^{a}	0.011^{a}
Depth (cm)						
0 - 10	3128^{a}	258^{a}	2869^{a}	0.029^{a}	0.020^{a}	0.010^{a}
10 – 20	2836^{a}	230^{ab}	2606^{a}	0.025^{ab}	0.018^{ab}	0.008^{a}
20 – 30	2725^{a}	199^{bc}	2525^{a}	0.026^{ab}	0.015^{bc}	0.008^{a}
30 – 40	2145^{b}	182^{c}	1963^{b}	0.023^{b}	0.014^{c}	0.008^{a}
			Analysis of Variance (P>F)			
Treatments	0.0036	0.0318	0.0051	<0.0001	<0.0001	<0.0001
Depth	<0.0001	<0.0001	<0.0001	0.0035	<0.0001	0.2291
Trt × Depth	0.9915	0.9610	0.9810	<0.0001	0.0062	0.0037

[†] Mean values within the same column followed by different small letters for each site are significantly different at p<0.05 for treatment

Table 3.3. XCT-derived total number of pores, macropores, and mesopores as influenced by long-term medium fertilizer (MF), high fertilizer (HF), low manure (LM), medium manure (MM), high manure (HM) rate applications, and control (CK) across 0-40 *cm* depth.

	Total no. of pores				**Total no. of macropores**				**Total no. of mesopores**			
	0 - 10	10 – 20	20 - 30	30 - 40	0 - 10	10 – 20	20 - 30	30 - 40	0 - 10	10 – 20	20 - 30	30 - 40
	--------------*Depth (cm)*-------------				--------------*Depth (cm)*-------------				--------------*Depth (cm)*-------------			
					Brookings Site							
Treatment												
CK	2075^{bc}	672^{c}	345^{c}	341^{a}	279^{ab}	89^{c}	35^{d}	49^{a}	1796^{bc}	583^{c}	310^{c}	292^{a}
MF	2005^{bc}	826^{bc}	691^{a}	387^{a}	234^{b}	89^{c}	76^{ab}	64^{a}	1778^{bc}	737^{bc}	615^{a}	323^{a}
HF	1555^{c}	778^{c}	626^{ab}	401^{a}	205^{b}	115^{bc}	66^{abc}	65^{a}	1349^{c}	661^{c}	559^{a}	337^{a}
LM	3144^{ab}	928^{bc}	454^{bc}	498^{a}	389^{a}	138^{ab}	59^{bcd}	81^{a}	2755^{ab}	790^{bc}	396^{bc}	417^{a}
MM	3528^{a}	1358^{a}	405^{c}	395^{a}	407^{a}	170^{a}	38^{cd}	59^{a}	3121^{a}	1187^{a}	367^{c}	337^{a}
HM	2159^{bc}	1088^{ab}	646^{a}	551^{a}	284^{ab}	152^{ab}	94^{a}	79^{a}	1875^{bc}	936^{b}	552^{ab}	547^{a}
					Analysis of variance ($p > F$)							
	0.0009	<0.0001	<0.0001	0.1279	0.0010	<0.0001	<0.0001	0.2465	0.0009	<0.0001	<0.0001	0.0808
					Beresford Site							
Treatment												
CK	$2732^{a\dagger}$	2372^{a}	2303^{a}	1297^{b}	233^{a}	195^{a}	168^{a}	164^{a}	2499^{a}	2177^{a}	2135^{a}	1133^{b}
MF	2997^{a}	2859^{a}	2489^{a}	2091^{ab}	245^{a}	189^{a}	167^{a}	153^{a}	2752^{a}	2670^{a}	2323^{a}	1938^{ab}
HF	3327^{a}	3096^{a}	2979^{a}	2240^{ab}	253^{a}	262^{a}	232^{a}	192^{a}	3074^{a}	2834^{a}	2748^{a}	2048^{ab}
LM	3133^{a}	2782^{a}	2669^{a}	2226^{ab}	244^{a}	219^{a}	210^{a}	182^{a}	2889^{a}	2562^{a}	2450^{a}	2043^{ab}
MM	3125^{a}	2938^{a}	2925^{a}	2565^{a}	303^{a}	273^{a}	191^{a}	178^{a}	2822^{a}	2665^{a}	2374^{a}	2387^{a}
HM	3453^{a}	2970^{a}	2983^{a}	2452^{a}	272^{a}	243^{a}	229^{a}	222^{a}	3810^{a}	2727^{a}	2754^{a}	2230^{a}
					Analysis of variance ($p > F$)							
	0.5445	0.1575	0.5311	0.0251	0.5733	0.4459	0.336	0.1420	0.5699	0.2304	0.5577	0.0268

†Mean values within the same column followed by different small letters for each site are significantly different at $p < 0.05$.

Treatments by depth interactions were mainly significant for porosity, macroporosity, and mesoporosity in both sites (Figure 3.2). Where at Brookings site, the MM increased porosity, macroporosity, and mesoporosity compared to the MF at 0 to 20 cm. However, treatment did not impact porosity and macroporosity below 20 cm in Brookings site. At Beresford site, the HM increased porosity, macroporosity, and mesoporosity compared to the CK, MF, and MM at 0 to 10 cm. At 20 to 30 cm, the HM increased porosity and macroporosity compared to the HF. However, treatment did not impact porosity and macroporosity at 10 to 20 and 30 to 40 cm. Also, treatment did not impact mesoporosity below 10 cm in Beresford site. Considering treatment as the main effect, the HM increased the macroporosity compared to the CK at 0 to 40 cm in the Beresford site. At Brookings site, the MM increased macroporosity when compared to MF at 0 to 40 cm. Considering depth as the main effect, the porosity, macroporosity, and mesoporosity were the highest at the surface (10 cm) depth as compared to the 30 to 40 cm in both sites (Table 3.2).

At Brookings site, the porosity for the LM, MM, and HM treatments was 1.6 to 2.6 times higher than the MF and HF at 0 to 10 cm (Figure 3.2A). At 10 to 20 cm, the MM treatment increased soil porosity by 3.1 to 4.4 times more than the CK, MF, and LM. Treatment × depth interaction was not significant for porosity at lower depths (20 to 40 cm). At Brookings site, the macroporosity values of the LM treatment were 1.7, 3.3, and 2.0 times higher than the CK, MF, and HF treatments, respectively, at 0 to 10 cm (Figure 3.2B). In addition, the MM and HM treatments showed 2.3 and 2.0 times higher macroporosity than the MF treatment, respectively, at 10 to 20 cm. Treatment × depth interaction was not significant for macroporosity at lower depths (20 to 40 cm). The MM for mesoporosity at Brookings site was 2.0 and 2.5 times higher than MF and HF, respectively, at 0 to 10 cm (Figure 3.2C). At 10 to 20 cm, the MM treatment increased the soil mesoporosity compared to the CK, MF, and LM. At 20 to 30 cm, the MF and HM increased the soil mesoporosity by 50% compared to the CK. In addition, at 30 to 40 cm, the LM and HM treatment increased the soil mesoporosity as compared to the MF treatments at Brookings site.

At Beresford site, the HM increased soil porosity as compared to the CK, MF, HF, LM, and MM at 0 to 10 cm; no differences were observed at 10 to 20 cm (Figure 3.2D). At 20 to 30 cm, the LM and HM treatments increased the soil porosity by 53 and 47% as compared to the HF; no differences were observed at 30 to 40 cm. At Beresford site, the HM treatment showed 1.4 to 2.1 times higher macroporosity than other treatments at 0 to 10 cm (Figure 3.2E). At 20 to 30 cm, the HM increased macroporosity 1.6 times more than the HF. Treatment × depth interaction was not significant for macroporosity at 10 to 20 cm and 30 to 40 cm. The HM for mesoporosity was 2.1, 2.4, and 3.4 times higher than the MM, MF, and CK, respectively, at 0 to 10 cm (Figure 3.2F). Treatment × depth interaction was not significant for mesoporosity at lower depths (10 to 40 cm).

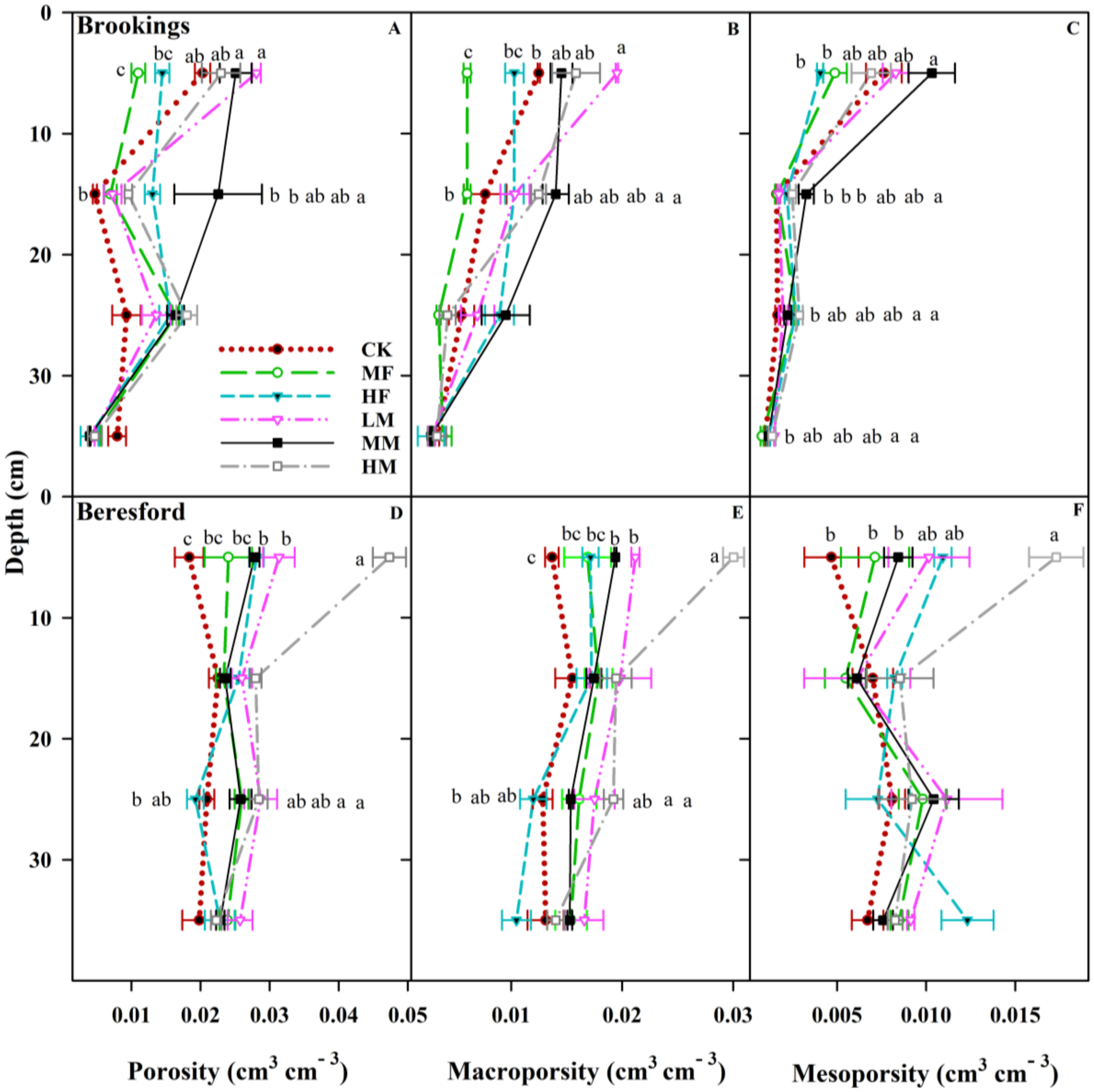

Figure 3.2. Soil depth and long-term nutrient application effects on porosity (cm cm^{-3}), macroporosity (cm cm^{-3}), and mesoporosity (cm cm^{-3}). Nutrient applications include medium fertilizer (MF), high fertilizer (HF), low manure (LM), medium manure (MM), high manure (HM) rate applications, and control (CK) at Brookings (Figure 3.2A – 3.2C) and Beresford sites (Figure 3.2D – 3.2F). Different lower-case letters indicate statistically significant different treatments at p=0.05 level at each depth.

Treatments by depth interactions were mainly significant for the average branch length (BL), tortuosity (τ), degree of anisotropy (DA), and fractal dimension (FD) for Brookings site (Table 3.4 and Figure 3.1C and 3.1D) and were significant for the BL and

FD values in Beresford site (Figure 3.1G and 3.1H). At Brookings site, varying treatment impacts were observed at 10 to 20 cm. There were little to no significant differences observed at other depths. At Beresford site, the HM increased the BL compared to the CK and MF at 30 to 40 cm. There were little to no significant differences observed at 0 to 30 cm depths. Considering depth as the main effect, the τ, and DA showed varying impacts across the soil depths in the Beresford site (Table 3.1).

At Brookings site, the BL, tortuosity (τ), degree of anisotropy (DA), and FD showed significant treatment × depth interactions (Figure 3.1C and 3.1D and Table 3.4). The LM treatment for BL was 14.4% higher than the MM, at 10 to 20 cm soil depth. However, there were no significant differences observed at other depths. The MF increased tortuosity (τ) more than CK and LM by 4.8 and 5.7%, respectively at 10 to 20 cm. However, treatment did not impact other depths. In addition, the LM, MM, and CK increased the DA by 8.4 to 10.2% as compared to the MF and HF at 0 to 10 cm. At 10 to 20 cm, the MM increased the DA by 9.8 to 13.6% as compared to the CK, MF, and LM. However, treatment did not impact 20 to 40 cm depths. Also, the FD for the MM was 2 times higher than the MF at 20 to 30 cm soil depth. However, treatment did not impact other depths (Table 3.4).

At Beresford site, significant treatment × depth interactions were observed for the BL and FD (Figure 3.1G and 3.1H). The MM increased the BL by 13.1% as compared to the CK at 10 to 20 cm. At 30 to 40 cm, the HM increased BL by 9.8 and 12.2% as compared to the MF and CK, respectively. However, treatment did not impact the BL at 0 to 10 and 20 to 30 cm (Figure 3.1G). The LM and HM treatments for FD were 10.2% and 9.7% higher than the MM at Beresford site for 30 to 40 cm depth. However, treatment did not impact the FD at 0 to 30 cm (Figure 3.1H). The τ and DA showed significant depth effect and the τ values for 0 to 10 cm and 10 to 20 cm were higher than the τ values for 20 to 30 cm and 30 to 40 cm by 1.8 to 2.8%, respectively. Also, the DA at 30 to 40 cm was lower than 0 to 10, 10 to 20, and 20 to 30 cm, by 8.8 to 60.4% at Beresford site.

Table 3.4. XCT-derived tortuosity (τ), degree of anisotropy (DA) as influenced by long-term medium fertilizer (MF), high fertilizer (HF), low manure (LM), medium manure (MM), high manure (HM) rate applications, and control across 0 to 40 *cm* depth.

	Tortuosity (τ)				**DA**			
	0-10	10–20	20-30	30-40	0-10	10–20	20-30	30-40
	-------------*Depth(cm)*-------------				-------------*Depth(cm)*------------			
				Brookings Site				
Treatment								
CK	1.28^{a}	1.24^{b}	1.29^{a}	1.32^{a}	0.68^{ab}	0.75^{a}	0.72^{a}	0.44^{ab}
MF	1.25^{a}	1.30^{a}	1.31^{a}	1.29^{a}	0.70^{a}	0.68^{a}	0.70^{a}	0.41^{ab}
HF	1.27^{a}	1.28^{ab}	1.30^{a}	1.31^{a}	0.60^{b}	0.66^{a}	0.70^{a}	0.37^{b}
LM	1.28^{a}	1.23^{b}	1.31^{a}	1.28^{a}	0.72^{a}	0.72^{a}	0.69^{a}	0.48^{ab}
MM	1.27^{a}	1.27^{ab}	1.29^{a}	1.29^{a}	0.70^{a}	0.71^{a}	0.66^{a}	0.52^{a}
HM	1.28^{a}	1.27^{ab}	1.30^{a}	1.28^{a}	0.70^{ab}	0.70^{a}	0.72^{a}	0.40^{b}
				Analysis of variance ($p > F$)				
	0.061	0.016	0.333	0.549	0.001	0.578	0.817	0.009
				Beresford Site				
Treatment								
CK	1.22^{a}	1.22^{a}	1.19^{a}	1.19^{b}	0.30^{a}	0.35^{a}	0.25^{ab}	0.32^{a}
MF	1.22^{a}	1.22^{a}	1.19^{a}	1.19^{b}	0.27^{a}	0.23^{a}	0.16^{b}	0.33^{a}
HF	1.23^{a}	1.22^{a}	1.18^{a}	1.20^{a}	0.23^{a}	0.33^{a}	0.18^{ab}	0.30^{a}
LM	1.22^{a}	1.23^{a}	1.20^{a}	1.20^{a}	0.36^{a}	0.37^{a}	0.24^{ab}	0.21^{a}
MM	1.23^{a}	1.21^{a}	1.21^{a}	1.20^{ab}	0.37^{a}	0.29^{a}	0.32^{a}	0.30^{a}
HM	1.23^{a}	1.22^{a}	1.19^{a}	1.21^{a}	0.24^{a}	0.37^{a}	0.22^{ab}	0.31^{a}
				Analysis of variance ($p > F$)				
	0.966	0.212	0.533	0.001	0.078	0.253	0.037	0.333

†Mean values within the same column followed by different small letters for each site are significantly different at $p < 0.05$.

The application of manure was found to improve the soil pore characteristics compared to the application of inorganic fertilizer. Similar results were observed by (Fang et al., 2018; Zhou et al., 2016). Manure can enhance soil microbial activity and decomposition can increase microbial metabolites such as polysaccharides. The release of polysaccharides and bacterial gum will help bind soil particles and improve soil aggregation (Ji et al., 2014; Lin et al., 2019). Therefore, promoting the formation of soil aggregate increases the total number of macropores and mesopores. Also, the presence of earthworms in manure will help decompose organic matter, and release binding agents which increase aggregate stability and improve soil structure (Akhila & Entoori, 2022). Macropores play an important role in crucial soil physical processes, like, water infiltration and solute transport (Katuwal et al., 2015). In addition, macropores provide a faster route for root growth (Fang et al., 2018). Manure application helped improve the number of macropores in our research, which is in agreement with the finding of Singh et al. (Singh et al., 2021). According to Zhang et al., (2017) Zhang et al. there is a high correlation between the total number of macropores and macroporosity. Therefore, treatments that improve the total number of macropores likely will improve macroporosity. The same might be true for the total number of mesopores and mesoporosity. In observing the depth impact, a decrease in the number of pores with an increase in soil depth was observed in this study which was also reported by (Kim et al., 2010). This could be because the impacts of the application of manure and inorganic fertilizer decrease with increasing depth.

The FD ranged from 2.0 to 2.5, where the FD value at 30 to 40 cm soil depth was lower than the other depths (0 to 30 cm). In general, the various rates of inorganic fertilizer and manure application had little to no impact on soil FD at different depths. Fang et al., (2021) concluded similar effects as well. Rivier et al. reported an increased FD with manure application which was similar to our findings in the Brookings site, where the application of MM increased FD as compared to MF treatments at 20 to 30 cm soil depth (Rivier et al., 2022). Higher FD values can explain a more complex pore structure (Peng et al., 2011) which can contribute to soil fertility (Xu et al., 2019).

Therefore, our study shows that MM application improved the formation and stabilization of soil structure.

According to Arora et al., (2012) higher soil τ values indicate lower soil pore connection and vice versa. In addition, Pires et al. suggested that a higher value of τ with an increased number of pores at lower depth favors the formation of pores of irregular shape (Pires et al., 2017). Thereby influencing pore connectivity. In this study, there was no overall difference in soil τ among the different treatments regardless of the little impacts found at lower depths. This can be because when τ was lower in inorganic fertilizer than manure application, the total number of pores was higher, resulting in increased interconnected pores.

To better understand the impacts of the various soil pore characteristics on the soil structure, observing the combined effects is necessary. For instance, as previously discussed, a decrease in tortuosity values indicates increased pore interconnectivity which may indicate soil degradation. Also, as shown by Hu et al., (2015) high porosity and tortuosity may be related to a well-developed root system. In this study, medium manure application impacted soil τ. Also, the MM and HM treatment improved the soil porosity at 0 to 10 cm soil depth when compared to the MF at Brookings and Beresford sites, respectively. Therefore, indicating an improved root system.

3.3.3. Correlations between SOC, TN content, and various XCT-derived soil pore characteristics.

The SOC content is positively correlated with the TN content, macroporosity, and total number of macropores (Tmacro) and is negatively correlated to the average branch length (BL) (Figures 3.3 and 3.4). Therefore, the differences observed in SOC content amongst LM, MM, HM, MF, HF, and CK treatments might indicate SOC content can directly impact the XCT-derived soil pore characteristics. Principal component analysis (PCA) was performed to evaluate how the measured XCT-derived parameters and SOC and TN values were distributed among the six treatments (Figure 3.5**.** The PC1 and PC2 explained 12% and 43% of the variation, accounting for 55% of the total variance. Different groups formed across the two principal component axes indicated the effects of different manure and fertilizer application rates on soil pore characteristics. Specifically, the TP, Tmacro, Tmeso, porosity, macroporosity, mesoporosity, and FD were more relevant to the PC1, whereas SOC, TN, and tortuosity values were more important to the PC2. The PC1 differentiated the control and fertilizer treatments from the manure treatments. The PC2 better differentiated the three manure treatments (LM, MM, and HM) probably because of higher SOC and TN in HM. The contribution of XCT-measured soil parameters to PC1 emphasized the positive influence of manure treatments on soil pore characteristics.

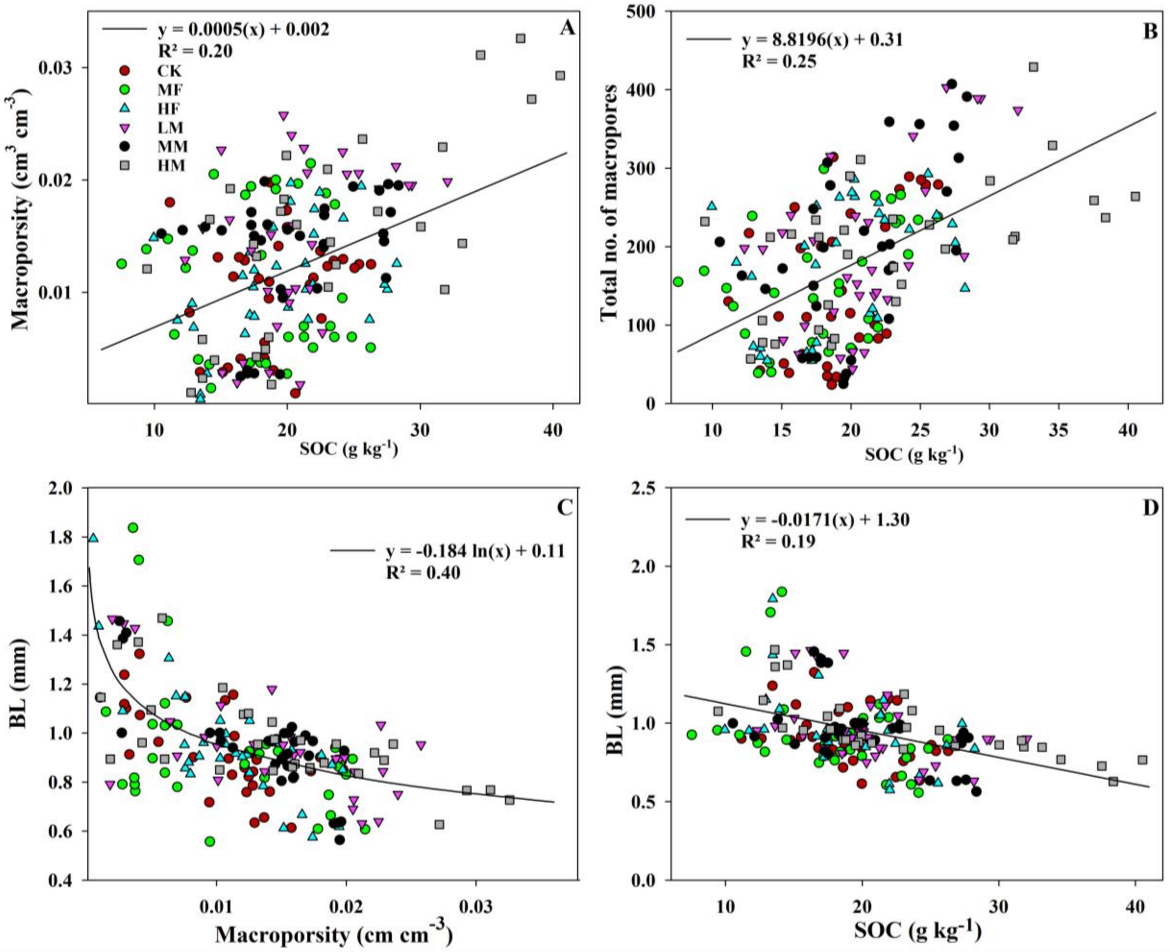

Figure 3.3. Relationship between A. SOC content and macroporosity, B. SOC content and the total number of macropores, C. branch length (BL, mm) and macroporosity, and D. SOC and BL. Nutrient applications include medium fertilizer (MF), high fertilizer (HF), low manure (LM), medium manure (MM), high manure (HM) rate applications, and control (CK) at Brookings and Beresford sites.

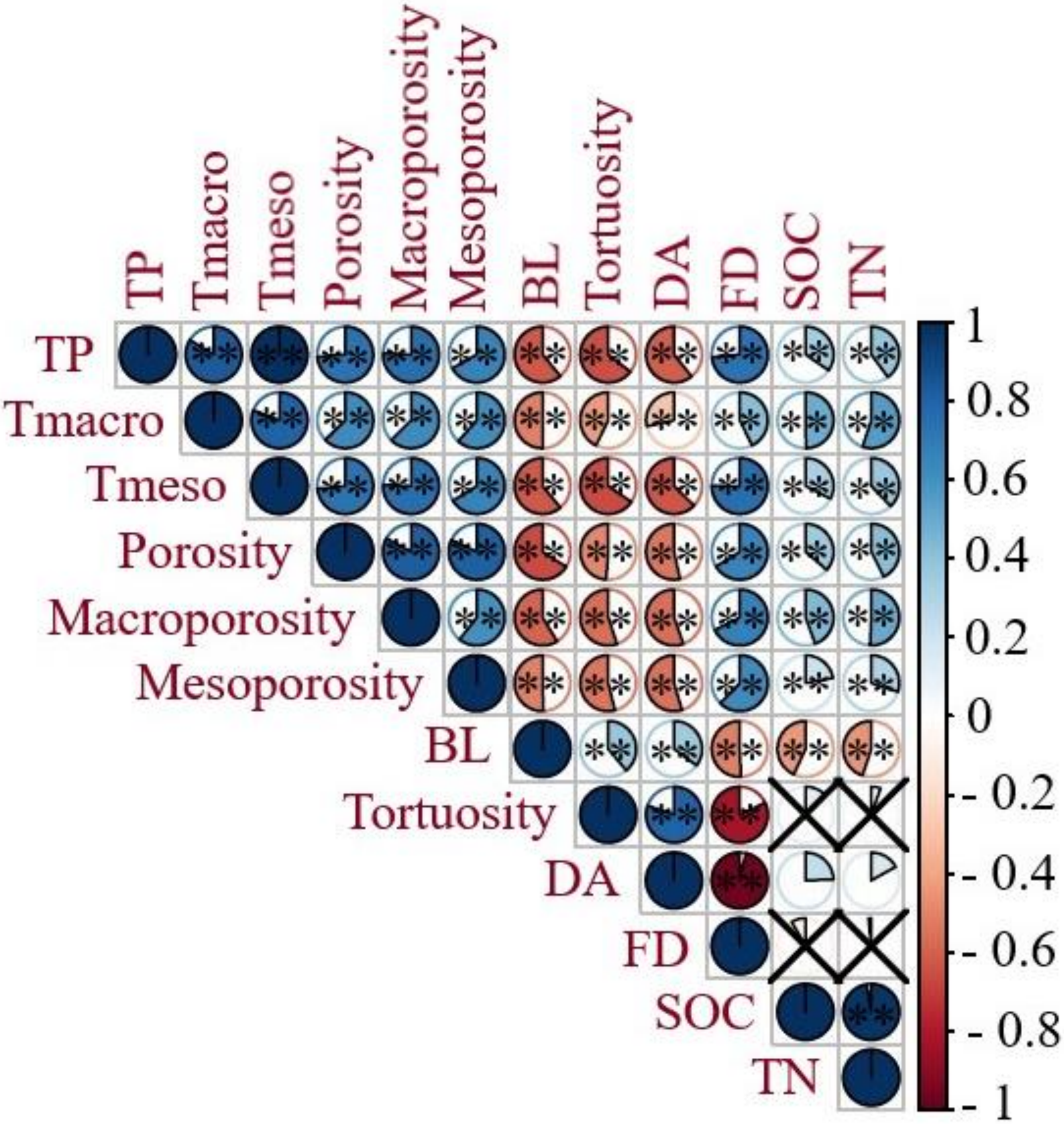

Figure 3.4. Correlation between SOC, TN content, and XCT-derived soil pore characteristics as influenced by long-term medium fertilizer (MF), high fertilizer (HF), low manure (LM), medium manure (MM), high manure (HM) rate applications, and control (CK) across 0 to 40 cm soil depth, at Brookings and Beresford sites. **Significant at the <0.01 level.

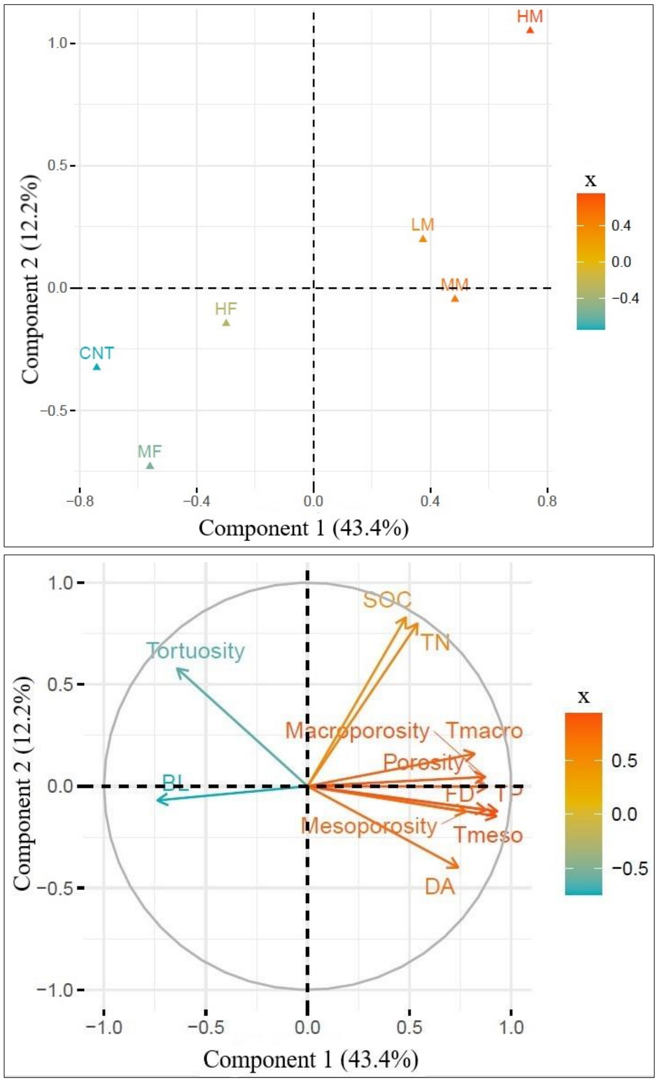

Figure 3.5. Principal component analysis (PCA) of the soil organic carbon (SOC), total nitrogen TN content, and XCT-derived soil pore characteristics (macroporosity, mesoporosity, porosity, fractal dimension – FD, degree of anisotropy – DA, total number of pores – TP, total number of macropores – Tmacro, total number of mesopores – Tmeso) as influenced by long-term medium fertilizer (MF), high fertilizer (HF), low manure (LM), medium manure (MM), high manure (HM) rate applications, and control (CK) across 0 to 40 cm soil depth, at Brookings and Beresford sites.

According to Pagenkemper et al., (2014) soil tortuosity (τ) explains the complexity of the soil pores, which was found to be negatively correlated to macroporosity, mesoporosity, and the total number of mesopores. Vogel, (1997) also reported that the τ or connectivity decreases with the increase in pore size, which explains the negative relationship observed. Zhang et al., (2017) reported a high correlation between the total number of macropores and macroporosity, which was also observed under manure and inorganic fertilizer treatment in three fields where two fields grew corn and spring wheat, and one was the control field. Previous studies have shown positive correlations between SOC content and the total no of macropores and macroporosity (Haruna et al., 2020; Xu et al., 2019). It also suggests improvement may be attributed to improved SOC content which is because of added organic matter content. This effect could explain the positive effects on the total number of macropores and macroporosity.

3.4. Conclusions

This study observed the effects of long-term (>10 years) application of different rates of manure and inorganic fertilizer on soil pore characteristics, visualized and quantified using X-ray CT and ImageJ processing. As manure binds the soil particle to form stable aggregates and increases soil moisture absorption and retention, it was observed that the MM treatment improved the TP, Tmacro, and Tmeso at 0 to 10 and 10 to 20 cm as compared to MF and HF treatments at Brookings site. While at the Beresford site, the HM treatment increased porosity and macroporosity at 0 to 10 cm as compared to other treatments, and increased porosity and macroporosity as compared to the HF treatment at 20 to 30 cm. Because of the direct organic matter addition to the soil, manure treatments also enhanced SOC content at 0 to 10 and 10 to 20 cm depths as compared to the inorganic fertilizer treatments at both sites. The SOC was positively correlated to macroporosity and Tmacro and negatively correlated to the BL. The long-term nutrient applications had little impact on the measured parameters in the subsurface (20-40 cm) depths. Overall, the high and medium rates of manure application improved XCT-derived soil pore characteristics, SOC, and TN contents as compared to the inorganic fertilizer and control treatments at 0 to 10 and 10 to 20 cm at both sites. This can stabilize the soil structure and improve the water-holding capacity of the soil. However, the long-term application of high manure rates is discouraged as this can adversely impact the environmental risk associated with above optimum nutrient application rates. Therefore, we encourage the use of medium manure application in the long term to enhance soil carbon and pore characteristics, enhancing soil health and crop production.

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CHAPTER 4

Changes in Soil Profile Organic Carbon and Hydro-Physical Properties as Impacted by Long-Term Manure and Inorganic Fertilizer Rates Under a Corn-Soybean-Spring Wheat Rotation System

Abstract

Using manure appropriately may enhance organic carbon, and hydro-physical properties while avoiding the negative impact on the environment. However, how manure impacts soils, especially at lower depths, is still not well studied. Therefore, the objective of this study was to assess the impact of different manure and inorganic fertilizer application rates on soil profile organic carbon (SOC) and hydro-physical properties under corn (*Zea mays* L.)-soybean (*Glycine max* L.)-spring wheat (*Triticum aestivum* L*)* rotation at Beresford (established, 2003) and Brookings (2008) sites in South Dakota. Treatments included: low manure (LM), medium manure (MM), high manure (HM), medium fertilizer (MF), high fertilizer (HF), and control (CK). Four replicated intact soil cores were collected from all the treatments at 0-10, 10-20, 20-30, and 30-40 cm depths. Considering treatments by depth interactions, the LM and MM decreased bulk density (ρ_b) by 6.9 to 22.1%, as compared to the CK at 0-30 cm for either site. And the HM decreased ρ_b by 16.4 to 24.7%, as compared to the HF at 30-40 cm for either site. On observing treatment as the main effect, the MM and HM increased the soil water retention (SWR) at 0, -5 kPa compared to MF, HF, and CK in Brookings, and the MM increased the SWR at -30 kPa as compared to the MF in Beresford at 0-40 cm depths.

Data suggests continuous manure application may enhance organic carbon and hydro-physical properties at lower depths. Therefore, this study concluded that long-term manure application is more beneficial when you consider the application rate and its timing. It can improve hydro-physical properties, thereby stabilizing the soil structure and improving water retention at lower depths.

4.1. Introduction

The application of manure to croplands, when used appropriately, can be a sustainable management strategy to improve productivity while enhancing SOC and other hydro-physical properties of soils. Studies showed that the incorporation of manure into the soils can reduce mineral fraction density, thereby lowering soil ρ_b (Zhou et al., 2016). The continuous application of inorganic fertilizer alone may increase the soil ρ_b (Pahalvi et al., 2021) which can adversely affect soil structure, reduce soil water retention, and thereby reduce plant available water content (Agegnehu et al., 2014). Manure application as a soil amendment has been used globally to improve hydro-physical properties (Ozlu et al., 2019). The application of manure can enhance soil aggregation, thereby promoting the formation of pores which can improve soil water retention (Usowicz & Lipiec, 2020). Yagüe et al. (2016) also revealed that manure application improved soil water infiltration regardless of the tillage practices. Manure acts as a binding agent in soil particles to improve soil aggregation (Hoover et al., 2019). Several studies reported the application of manure can enhance SOC, total nitrogen (TN) contents, and soil nutrients (Yang et al., 2014; Zhao et al., 2014). The increases in SOC due to manure application are reported to enhance porosity, soil water retention, and plant available water content (Yang et al., 2014). However, the effectiveness of manure application in increasing the SOC depends on the manure application rate (Abagandura et al., 2022), duration of continuous manure application, and manure quality (Ozlu et al., 2019).

Manure when added to the soil can also directly improve C and N fractions in the soil (Abagandura et al., 2022). This can be due to the increased organic carbon content in the manure. (Yunanto et al., 2022) reported that manure can increase the C/N ratio, which can be beneficial for soil fertility. Therefore, the composition of manure plays an important role in the amount of C and N fractions in the soil. For instance, when there is a high nitrogen content or low C/N ratio in the manure, organic matter becomes easily degraded (Gross & Glaser, 2021). Appropriate manure application and analyzing manure samples before use reduces or eliminates this issue, causing moderate manure application to be beneficial for long-term use. Inorganic fertilizer application can indirectly improve the carbon content in the soil by increasing crop dry matter production which improves the biomass inputs into the soil from roots and crop residues (Jiang et al., 2014). The labile C and N fractions form an easily decomposable sub-pool of carbon which is a sensitive indicator of the effects of management practice on the soil. Based on this, labile C and N fractions maybe early indicators of changes in soil health (Bongiorno et al., 2019). Therefore, the changes in labile C and N fractions may reveal the extent to which manure and inorganic fertilizer, tillage, and other management practices impact the soil. This also implies that SOC content is an important indicator for soil organic matter, soil fertility, and crop productivity (Nie et al., 2019).

Despite the high nutrient bioavailability of the application of inorganic fertilizer, continuous application may lead to a decline in SOC content (Jiang et al., 2014; Kizito et al., 2019). The long-term application of inorganic fertilizers can also lead to the deterioration of soil health in terms of soil physical, chemical, and biological properties (Liu et al., 2010). When compared with inorganic fertilizer application, application of manure has been found to improve the soil structure and infiltration rate in the long term (Brar et al., 2015; Gautam et al., 2022; Hou et al., 2012). Therefore, long-term application of manure can reduce the incurred cost of production by reducing or eliminating the application of inorganic fertilizer, and hence in turn protecting the environment by reducing or eliminating negative impacts of the continuous use of inorganic fertilizer. However, it is also important to know the optimal rate of manure application as a high rate of manure and inorganic fertilizer application on a long-term basis can lead to nitrogen and phosphorus pollution, surface runoff, and decreased soil aeration (Lim et al., 2015), thereby decreasing SOC and TN stock.

Depth may have a significant influence on soil hydro-physical properties and the organic carbon pool (Chakraborty et al., 2022). Therefore, the distribution of SOC across the soil depth is essential, as it can impact soil water retention and soil structure (Franzluebbers, 2002). Which can also be influenced by the application of manure and inorganic fertilizer. Although numerous studies reported the impacts of manure application on soil hydro-physical properties and organic carbon pools, yet, the majority of these studies were focused only on the surface depth (0 to 10 cm) or/and at 10 to 20 cm (Gautam et al., 2022; Gautam et al., 2020; Hou et al., 2012; Ozlu et al., 2019).

However, the effects of the continuous application of manure and inorganic fertilizer can impact soils at lower depths over time (Sukhum et al., 2021), therefore, the impacts of manure and inorganic fertilizer management on soils need to be studied at lower depths. We hypothesize that long-term manure application may enhance the organic carbon and improve physio-hydrological properties as compared to the inorganic fertilizer and control to the depth of 40 cm. Specific objectives of the study are to (i) assess the impacts of different rates of manure and inorganic fertilizer application on soil water retention (SWR), ρ_b, and plant available water (PAW) content to a depth of 40 cm, (ii) examine the effects of different rates of manure and inorganic fertilizer on SOC and TN stock, and cold and hot-water extractable C and N to a depth of 40 cm, and (iii) correlate the SOC and TN stock to PAW content across different treatments.

4.2. Materials and Methods

4.2.1. Study site and experimental design

The study was conducted at two research farms in South Dakota State University Research (Southeast research farm at Beresford and Felt research farm at Brookings). The experimental site near Beresford, SD, USA, (43˚ 02’ 33.46” N and 96˚ 53’ 55.78” W) was established in 2003 on an Egan silt loam soil (*Fine-silty, mixed, mesic Udic Haplustolls*). The plots are located on a nearly flat area with a slope of <1%, and an elevation of 390 m. The climate of this site is a humid continental climate with relatively humid summers and snowy winters with mean air temperature of 23.3°C in the summer and -5°C in the winter and in the hottest (July) and coldest (Jan.) months of the year the mean air temperature may go up to 29.5°C and -13.6°C, respectively. The mean annual precipitation is about 678 mm. Dimensions for each plot are 5 m by 20 m. The second experimental site was established in 2008 near Brookings, SD at the Felt Research Farm (44˚ 22’ 07.15” N and 96˚ 47’ 26.45” W) on a well-drained Vienna silt loam soil (*Fine-loamy, mixed, frigid Udic Haploborolls*). The plots are nearly flat with a slope of <1% and an elevation of 518 m. This site is in a humid continental climate with relatively humid summers and cold, snowy winters with mean air temperature of 22.2°C in the summer and -7.8°C and and in the hottest (July) and coldest (Jan.) months of the year the mean air temperature may go up to 27.8°C and -15.8°C, respectively. The mean annual precipitation is about 638 mm. Individual plot dimensions at these sites are 6 m by 18 m. These fields were managed under rain-fed conditions.

4.2.2. Study Treatment

Each site included six treatments, that included: three manure application rates; low manure (LM) contained a quantity of manure based on the recommended phosphorous requirement, medium manure (MM) contained a quantity of manure based on recommended nitrogen requirement, and high manure (HM) contained a quantity of manure-based on double the recommended nitrogen requirement, two inorganic fertilizer application rate; medium fertilizer (MF) contained the recommended inorganic fertilizer rate, high fertilizer (HF) contained a high fertilizer rate and a control treatment (CK) which did not receive manure nor fertilizer. Treatments were arranged in a randomized complete block design with four replicates. The cropping system was corn (*Zea mays* L.)-soybean [*Glycine max* (L.) Merr] rotation at both sites, until 2019. In 2020, spring wheat (*Triticum aestivum* L.*)* and cover crops (radish) were added to the cropping system, and it became a corn-soybean-spring wheat rotation system.

The manure and fertilizer application rates followed the state Fertilizer Recommendation guidance for 3.7 $Mg\ ha^{-1}$ spring wheat yield goal in 2020. Beef dry and dairy solid manure were used on Beresford and Brookings sites, respectively. Urea, mono ammonium phosphate, and potash fertilizer were used for the inorganic fertilizer. In 2020, the nutrient application target rate for both sites were 136 kg N ha^{-1}, 49 kg P_2O_5 ha^{-1}, and 91.5 kg K_2O ha^{-1}. The amount of manure and fertilizer applied was based on the soil test levels and target yield goal in each year applied. The manure used was analyzed by certified commercial laboratories. No manure or fertilizer was applied to the soybean. On average, dairy manure with approximately 31% and beef manure with 22% moisture for Brookings and Beresford sites, respectively, were used from 2003 to 2020. The study sites were tilled using inversion tillage about 1 to 3 days before planting, to incorporate all the treatments added and residue left on the field.

4.2.3. Soil Sampling and Sample Preparation

A total of 192 intact soil cores of 7.62 cm diameter and 7.62 cm height were collected at 0-10, 10-20, 20 -30, and 30-40 cm in July 2020, after the harvest of spring wheat (*Triticum aestivum* L.*)*. The cores were extracted from the soil manually using a core sampler vertically inserted in the soil. For each depth, plexiglass cores were inserted leaving 11.9 mm of soil at the top and bottom to minimize disturbance to the intact soil sample. Soil cores were then trimmed, using a serrated knife, sealed with plastic caps at both ends, labeled, and stored in plastic bags at 4˚C pending analysis. After SWR analysis, intact cores were ground and sieved to perform the other analysis discussed in the following sections.

4.2.4. Soil Water Retention Characteristics and Pore Size Distribution

Soil Water Retention (SWR) was measured using the intact soil cores at each depth for every treatment. After fixing the cheesecloth at the bottom, soil cores were saturated by capillarity for 24 to 48 hrs. The SWR characteristics were measured using a combination of tension tables and pressure plate extractors (Klute, 1986). The SWR characteristics were measured at five 0, -0.5, -5.0, -30.0, and -1500 kPa matric potentials (Ψ_m). Then, a subsample of the intact soil core was oven-dried at 105 °C for 48 h to determine the bulk density (ρ_b), and the rest of the soils were air-dried, ground, and sifted through a 2 mm sieve. Furthermore, the pore-size distribution (PSD) of the soil was calculated using the measured SWR data by employing the capillary rise equation (Hillel, 2014) and were grouped into; macropores (>500 μm equivalent cylindrical diameter, ECD), coarse mesopores (60–500μm ECD), fine mesopores (10–60 μm ECD), coarse micropores (0.2–10 μm ECD) and micropores (<0.2 μm ECD). Plant available water (PAW) was determined as the difference in volumetric water content retained between field capacity (− 30 kPa) and permanent wilting point (− 1500 kPa), which is also the volume of water retained by pores with ECD between 0.2 and 10 μm (coarse micropores).

4.2.4.1.van Genuchten Parameters

The van Genuchten function (Van Genuchten, 1980) was used because of its high degree of fitting to the observed SWR data, especially for undisturbed and fine-textured soils (Liu et al., 2011). The van Genuchten parameters were fitted using the RETC (RETention Curve) program (Van Genuchten et al., 1991). The function in Eq. [1] describes the SWR curve:

$$\theta(h) = \theta_r + \frac{\theta_s - \theta_r}{[1+(\alpha h)^n]^m} \quad [1]$$

where, θ (m^3 m^{-3}) is the volumetric water content, θ_r (m^3 m^{-3}) is the residual water content, θ_s (m^3 m^{-3}) is the saturated water content, α (cm^{-1}), m and n are the empirical van Genuchten parameters, respectively, and h is the soil water tension (-cm). The residual water content is defined as the water content in the range of low potentials, it is considered as the water content values at the lowest Ψ_m (−1500 kPa) (Van Genuchten, 1980). Parameters α (cm^{-1}) and n were fitted using Eq. [1]. The saturated water content was taken as the measured water content at 0 kPa. The fitted water retention parameters were used to obtain the slope of the soil water retention curve at its inflection point (Dexter, 2004) given as:

$$S = -n(\theta_s - \theta_r)\left[1 + \frac{1}{m}\right]^{-(1+m)} \quad [2]$$

4.2.5. Soil organic carbon and total nitrogen stock

Soil organic carbon and TN were determined by dry combustion method using LECO 628 CN analyzer (TruSpec; LECO Corporation, St. Joseph, MI, USA). The SOC and TN concentrations data were extracted from Sangotayo et al. (*PhD. Dissertation*).

The SOC and TN stocks were determined for each of the four depths (0-10, 10-20, 20-30, 30-40 cm) by multiplying the SOC concentration with ρ_b and soil depth (Liu et al., 2013) given as:

$$\text{SOC stocks } (\text{Mg ha}^{-1}) = \text{SOC concentration } (g\ kg^{-1}) \times \rho_b\ (Mg\ m^{-3}) \times \text{soil depth (m)} \times 10 \quad [3]$$

$$\text{TN stocks } (\text{Mg ha}^{-1}) = \text{TN concentration } (g\ kg^{-1}) \times \rho_b\ (Mg\ m^{-3}) \times \text{soil depth (m)} \times 10 \quad [4]$$

4.2.6. Water extractable carbon and nitrogen assay

Water extractable organic C and N fractions were determined using the procedure outlined by (Ghani et al., 2003). Three grams of air-dried soil sample was added into a falcon centrifuge tube. Water extractable carbon and nitrogen were obtained by adding 30 mL distilled water and putting it in an end-over-end shaker for 30 min at 140 rpm. After extraction, the suspension was centrifuged at 3000 rpm for 25 min at 4°C. The supernatant solution was filtered through 0.45 µm membrane filters and the fraction obtained was cold-water extractable organic C (CWC) and N (CWN). Another 30 mL of water was added to the same centrifuge tube and shaken vigorously for 10 sec and put into a hot-water bath at 80°C for 16 h. After extraction, the suspension was centrifuged at 3000 rpm at 25°C for 25 min and filtered. The fraction obtained is hot-water extractable organic carbon (HWC) and nitrogen (HWN). These fractions were analyzed using the TOC-L analyzer (Shimadzu Corporation, model-TNM-L-ROHS).

4.2.7. Statistical analysis

Two-way analysis of variance (ANOVA) was conducted to compare the effects of depth and treatment interactions on the soil hydro-physical parameters, carbon and nitrogen fractions, and SOC and TN stocks in the *R* modeling environment (*R* statistical Software, 2013) for each site separately. The homogeneity of the variance and normality was checked. Data were transformed when necessary, using the Box-Cox method. Significance was determined at $\alpha = 0.05$. In this study, we observed significant differences among the six treatments and the soil depths of 0 to 10, 10 to 20, 20 to 30, and 30 to 40 cm. We also observed the treatment differences for the soil parameters at different depths. The relationships between soil hydro-physical properties, SOC, and TN stock were analyzed using Pearson's correlation. The statistical significance of Pearson's correlation coefficient was determined at the $p = .05$ and $p = .01$ levels.

4.3. Results and Discussion

4.3.1 Impacts of manure and inorganic fertilizer application on SOC, TN stock, and ρ_b

The SOC and TN stocks, and ρ_b at 0 to 10, 10 to 20, 20 to 30, and 30 to 40 cm depths for Brookings and Beresford sites are presented in Figure 4.1A-F and Table 4.1. Treatments significantly impacted the ρ_b at 0 to 40 cm depths. On average, manure application decreased the ρ_b as compared to inorganic fertilizer and control at either site. Considering the depth as the main effect, the SOC and TN stocks, and ρ_b were impacted at 0 to 40 cm depth. The SOC and TN stock values were the highest and ρ_b values were the lowest at the surface (10 cm) depth as compared to the other depths at either site

(Table 4.1). Treatments by depth interactions were mainly significant for SOC and TN stock at 0 to 20 cm (Figure 4.1A-F) at Beresford site. Where the HM treatment increased the SOC and TN stocks as compared to the MF and MM at 0 to 20 cm at Beresford site. However, treatments did not always impact these parameters below 20 cm depth. Treatments by depth interactions were also significant for ρ_b at 0 to 40cm depth. The LM and MM treatments decreased the ρ_b values at 0 to 30 cm compared to the CK treatment. At 30 to 40 cm, the HM treatment decreased the ρ_b compared to the HF treatment at either site.

At the Brookings site, a significant treatment x depth effect was observed for SOC and TN stock. There was no treatment impact observed at 0 to 10 cm (Figure 4.1A). At 10 to 20 cm depth, the MM treatments increased the SOC stock by 9.8%, as compared to the MF. At 20 to 30 cm, no treatment impact was observed except that the HM had 13 to 14% lower SOC stock values when compared to the LM and CK at Brookings site. And no treatment impact was observed at 30 to 40 cm. Similarly, the HM and LM increased the TN stock more than the MF treatments at 0 to 10 cm (Figure 4.1B). No treatment impact was observed at 10 to 20 cm. At 20 to 30 cm depth, no differences were observed except that TN stock for the HF and HM was 11.8 to 26.7% lower than the MF. And the HM also had lower TN stock values compared to the HF and CK at 30 to 40 cm for Brookings study site. An almost similar trend was observed for the Beresford site. At the latter site, the HM (47.7 Mg ha^{-1}) increased SOC stock by 57.3 to 57.6% as compared to the CK and LM treatments at 0 to 10 cm. At 10 to 20 cm, the HM increased SOC stock by 20.4 to 23.4% compared to the MF and MM, and treatments did not impact SOC below 20 cm soil depth (Figure 4.1D). When observing the TN stock, the HM was 1.7 to 1.9 times higher values than the other treatments at 0 to 10 cm depth (Figure 4.1E). The TN stock for the HM was 23.3 to 37.9% higher than the CK, MF, LM, and MM treatments at the Beresford site in the 10 to 20 cm depth. There were no treatment impacts observed at lower depths (20 to 40 cm).

At Brooking site, the LM, MM, and HM treatments decreased ρ_b by 10.3 to 36.1%, as compared to the CK at 0 to 30 cm depth (Figure 4.1C). At 30 to 40 cm soil depth, the HM decreased ρ_b by 24.7% as compared to HF treatment at the Brookings site. At the Beresford site, the LM and MM (1.19 g cm^{-3} and 1.17 g cm^{-3} respectively) decreased ρ_b by 13.4 to 16.2%, as compared to the MF and CK (1.35 g cm^{-3}, and 1.36 g cm^{-3} respectively), at 0 to 10 cm depth (Figure 4.1F). Similarly, for 10 to 20 cm depth, MM and LM treatments decreased the soil ρ_b by 6.9 to 7.8% as compared to the CK treatment. At 20 to 30 cm depth, the LM and MM decreased the ρ_b by 8.3 and 8.5% as compared to CK and HF treatments. At 30 to 40 cm depth, the LM, MM, HM, and CK decreased the ρ_b by 14 to 23.5% more than the HF treatment.

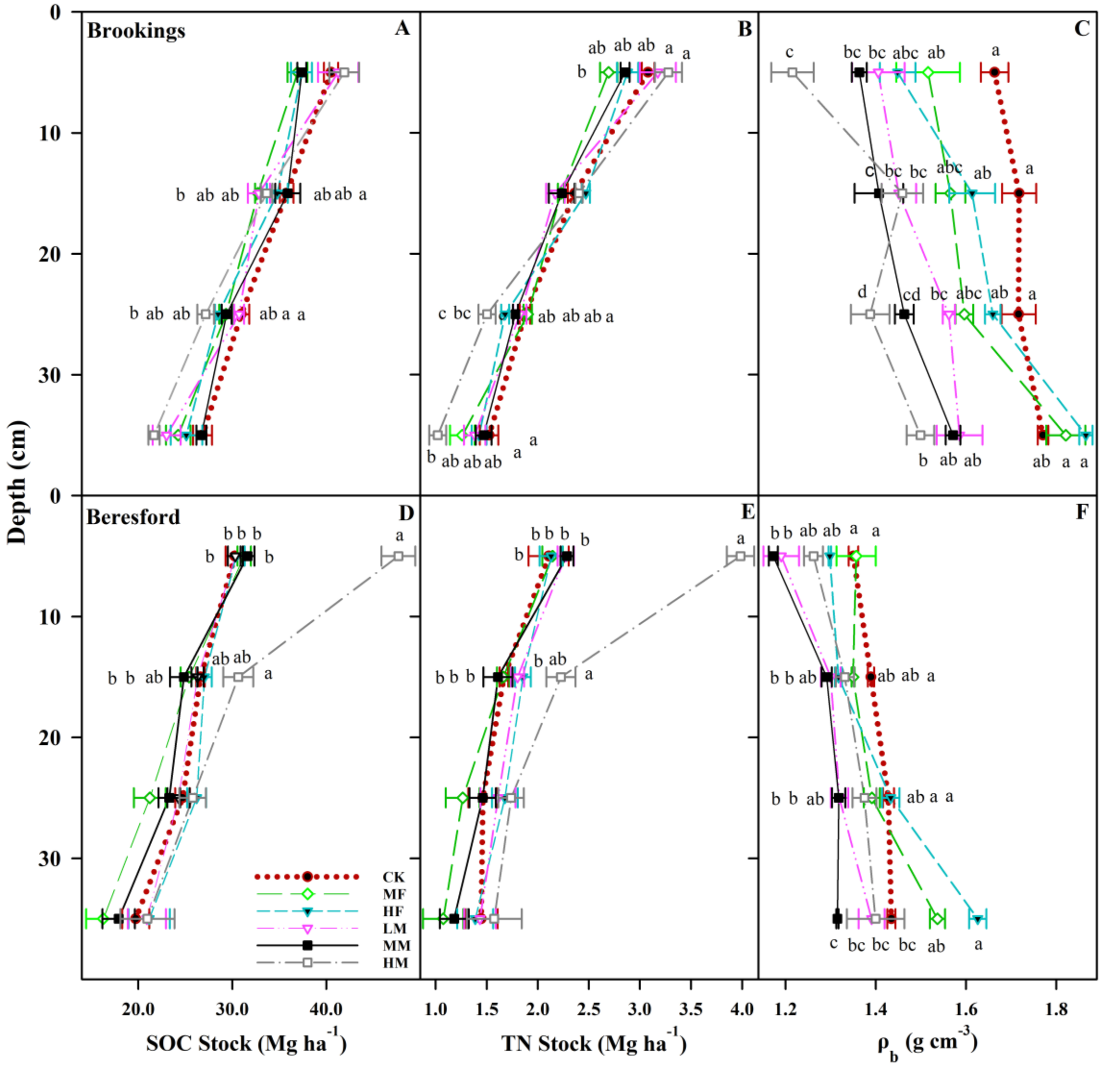

Figure 4.1. Soil organic carbon (SOC) stock, total nitrogen (TN) stock, and bulk density as impacted by medium fertilizer (MF), high fertilizer (HF), low manure (LM), medium manure (MM), high manure (HM) rate applications, and control (CK) for Brookings (A, B, C) and Beresford (D, E, F) sites in SD. Different lower-case letters indicate statistically significant different treatments at $p \leq 0.05$ level at each depth.

Manure application can increase SOC and TN stocks due to the direct incorporation of C and N fractions into the soil (Tian et al., 2015). However, the continuous addition of inorganic fertilizer alone has been proven to be unable to increase

SOC concentration in the long term (Brar et al., 2013; Triberti et al., 2008). This can explain the higher value of SOC stocks under manure (MM and HM) compared to the fertilizer treatments. It is well known that the SOC and TN stocks impact changes in ρ_b. Higher SOC can decrease the soil ρ_b (Céspedes-Payret et al., 2017). The SOC stocks not only depend on SOC concentrations but also depend on soil bulk density (Hossain et al., 2015). The decrease in soil ρ_b from manure application might be because of the addition of organic matter and its positive effects on soil aggregation, macro-pores, and macro-aggregates caused by the bonding of organic acids and polysaccharides emitted by microorganisms during the decomposition of the added organic manure (Benbi et al., 2016). Our study showed that on average, all manure application rates increased SOC stocks and reduced ρ_b at 0 to 10 and 10 to 20 cm for either site. Li et al. (2018) reported a similar reduction in ρ_b due to manure application as compared to the inorganic fertilizer. The soil ρ_b was increased with the increase in depth up to 40 cm. This might be due to the decrease in organic matter with depth. Therefore, the incorporation of manure can decrease soil ρ_b, improve soil structure, and add nutrients in the long term (Zhou et al., 2016). On the contrary, increased ρ_b has been attributed to the deterioration of the soil structure with continuous application of inorganic fertilizer (Walia et al., 2010) which can affect soil water retention. Also, the continuous application of inorganic fertilizer or high manure in the long- term can be detrimental to the environment. For instance, if there is an increase in N inputs from inorganic fertilizer or high manure application, this could in the long run lead to an increase in TN stock as compared to the SOC stock (Su et al., 2006), thereby, leading to a decrease in the C/N ratio. A decreased C/N ratio may increase microbial growth (Brust, 2019) and lower the SOC stock.

4.3.2. Impacts of manure and inorganic fertilizer application on Cold and Hot water-extractable C and N

Cold and hot water-extractable C and N data for the Beresford and Brookings sites are presented in Table 4.1 and Supplementary Table 4.1. Treatments significantly impacted the CWC at 0 to 40 cm depths. The manure application rates (LM, MM, and HM) increased CWC when compared to the CK and MF treatments at either site. On considering the depth as the main effect, all the parameters were impacted at 0 to 20 cm. The CWC, HWC, CWN, and HWN values were highest at the surface (10 cm) depth as compared to the other depths at either site (Table 4.1). However, treatment by depth interaction effects was not significant for the CWC, HWC, and CWN at either site, but was significant for HWN for Beresford site at 0 to 40 cm depths (Supplementary Table 4.1).

At Brookings site, when observing the treatment effects, the HM improved CWC as compared to CK, MF, and HF treatments. When observing the depth effect, the HWC, CWN, and HWN were 1.3 to 1.9 times higher at 0 to 10 cm as compared to 10 to 20 cm and were 1.3 to 1.8 times higher at 10 to 20 cm as compared to 20 to 40 cm at the Brookings site. At Beresford site, when observing the treatment x depth effects, the HM increased HWN by 1.6 to 2.3 times more than the CK and MF at 0 to 30 cm soil depth. The LM and HM increased HWN by 1.7 and 1.8 times more than the MF at 30 to 40 cm, respectively. On considering the treatment impact, the HM treatment improved CWC and

CWN, 1.3 to 2.0 times higher than the other treatments, at Beresford site. Lastly, when considering the depth impact, the HWC was 2.1 times higher at the 0 to 10 cm depth than the 10 to 20 cm depth, 2.7 times higher than the 20 to 30 cm depth, and 3.7 times higher than the 30-40 cm depth, respectively.

In accordance with our results, Böhme & Böhme (2006) reported that manure application increased HWC compared to inorganic fertilizer and control treatments. The positive impacts observed in this study with manure application may be attributed to the direct effect of manure on C and N fractions by enhancing microbial activities in organically amended treatments (Whalen et al., 2014) and indirect effects through providing an appropriate soil physical environment. Also, manure application can introduce external microbial populations, which also can contribute to an increase in the labile pool of C and N (Bastida et al., 2008).

Table 4.1. Soil organic carbon (SOC) and total nitrogen (TN) stock, $\rho_{b,}$ cold- and hot-water extractable carbon and nitrogen (CWC. CWN, HWC, HWN) as influenced by long term medium fertilizer (MF), high fertilizer (HF), low manure (LM), medium manure (MM), high manure (HM) rate applications, and control (CK) as a function of treatment (Trt) and soil depths.

	SOC stock (g kg^{-1})	**TN stock** (g kg^{-1})	ρ_b (g cm^{-3})	**CWC** (mg kg^{-1})	**HWC** (mg kg^{-1})	**CWN** (mg kg^{-1})	**HWN** (mg kg^{-1})
				Brookings Site			
Treatment							
CK	$33.5^{\dagger}$	$2.2^{\dagger}$	$1.72^{a\dagger}$	$44.1^{c\dagger}$	$87.4^{\dagger}$	$3.3^{\dagger}$	$5.9^{\dagger}$
MF	30.9	2.0	1.63^{ab}	43.0^{c}	83.6	3.3	5.4
HF	31.4	2.1	1.65^{a}	45.4^{bc}	86.4	3.3	6.1
LM	32.0	2.1	1.50^{bc}	51.3^{abc}	97.0	3.6	6.3
MM	32.3	2.1	1.45^{c}	54.1^{ab}	94.9	3.8	6.7
HM	31.1	2.1	1.37^{c}	55.1^{a}	105.8	4.1	7.3
Depth (cm)							
0-10	39.2^{a}	3.0^{a}	1.44^{c}	62.6^{a}	151.3^{a}	5.2^{a}	11.1^{a}
10-20	34.3^{b}	2.3^{b}	1.52^{bc}	46.5^{b}	88.6^{b}	4.1^{b}	5.8^{b}
20-30	29.3^{c}	1.8^{c}	1.56^{ab}	45.2^{bc}	67.0^{c}	2.7^{c}	4.3^{c}
30-40	24.6^{d}	1.4^{d}	1.69^{a}	41.6^{c}	63.3^{c}	2.3^{c}	3.9^{c}
				Analysis of Variance (P>F)			
Treatments	0.41	0.98	<0.01	0.01	0.56	0.41	0.62
Depth	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Trt×Depth	<0.01	<0.01	<0.01	0.98	0.68	0.37	0.48
				Beresford Site			
Treatment							
CK	26.2^{ab}	1.7^{b}	$1.40^{a\dagger}$	49.0^{c}	96.5^{b}	3.2^{b}	6.0^{ab}
MF	24.3^{b}	1.5^{b}	1.41^{a}	48.3^{c}	92.8^{b}	3.0^{b}	5.7^{b}
HF	26.2^{ab}	1.8^{b}	1.42^{a}	49.8^{c}	98.5^{b}	2.8^{b}	6.2^{ab}
LM	25.4^{ab}	1.8^{b}	1.30^{b}	61.3^{b}	119.3^{b}	3.6^{b}	7.7^{ab}
MM	24.4^{b}	1.6^{b}	1.27^{b}	56.3^{bc}	112.9^{b}	3.6^{b}	7.4^{ab}
HM	31.2^{a}	2.4^{a}	1.35^{ab}	74.2^{a}	151.1^{a}	5.7^{a}	11.0^{a}
Depth (cm)							
0-10	34.0^{a}	2.5^{a}	1.27^{b}	76.9^{a}	212.0^{a}	5.9^{a}	14.7^{a}
10-20	27.1^{b}	1.8^{b}	1.33^{bc}	50.5^{b}	100.9^{b}	3.6^{b}	6.7^{b}
20-30	24.2^{b}	1.5^{bc}	1.38^{b}	54.3^{b}	77.9^{bc}	3.1^{bc}	4.8^{bc}
30-40	$19.5.^{c}$	1.3^{c}	1.45^{a}	44.2^{b}	56.7^{c}	2.0^{c}	3.2^{c}
				Analysis of Variance (P>F)			
Treatments	0.01	<0.01	<0.01	<0.01	0.16	0.01	0.05
Depth	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Trt×Depth	<0.01	<0.01	<0.01	0.12	0.07	0.24	0.01

†Means with different letters within a column for individual sites are significantly different for different treatments or depths at $p \leq 0.05$

4.3.3 Impacts of manure and inorganic fertilizer application on soil hydro-physical parameters

Soil water retention data for Brookings and Beresford sites are presented in Figure 4.2 and Table 4.2. Treatments significantly impacted the SWR at 0, -0.5, -5, and -30 kPa for all the depths. The MM treatment increased the SWR at -30 kPa as compared to the MF at either site. Depth mainly impacted the SWR at 0, -0.5, -5, -30 kPa, and -1500 kPa at 0 to 20 cm. The SWR at 0, -0.5, -5, and -30 kPa values at these matric potentials were highest at the surface (10 cm) depth as compared to the other depths at either site (Table 4.2). Treatments by depth interactions were significant for SWR at -5, -30, and -1500 kPa in Brookings site and SWR at 0, -0.5, and -5 kPa in Beresford site, mainly at 0 to 20 cm (Figure 4.2). At Brookings site, the HM increased the SWR at -5 kPa by 26 to 70% as compared to the HF at 10 to 40 cm. Treatment did not impact the SWR at -5 kPa below 10 cm. At Beresford site, the MM increased SWR at -5 kPa by 18-22% compared to the MF at 0-10 cm and the 30-40 cm. However, varying treatment differences were observed at below 10 to 30 cm depth.

At Brookings when observing the treatment × depth effect, the MM and HM treatments had 19.4 to 68% more water retained than the CK, MF, and HF at -30 kPa for 0 to 20 cm. At 20 to 30 cm, the HM had 14 and 23% more water retained than the CK and HF, respectively, at -30 kPa. The MM and HM had 71 to 113% more water retained at -30 kPa than the CK, MF, and HF at 30 to 40 cm in Brookings site. Overall, there was no interaction effect observed for SWR at -1500 kPa except that the HM had more water retained than the fertilizers rates (MF and HF) at 10 to 20 cm depth in Brookings site.

Similarly, when observing the treatment effect, the MM and HM had 17 to 47% more water retained than the MF, HF, and CK at 0, -0.5, and -30 kPa. When considering the depth effect, it was observed that SWR at 0 and -0.5 kPa had 24 to 26% more water retained at 0 to 10 cm, than the 30 to 40 cm. However, the depth did not impact the 10 to 30 cm depth.

At the Beresford site, a significant treatment x depth interaction was observed. The MM increased SWR at -5 kPa by 18-22% when compared to the MF at 0-10 cm and 30-40 cm. However, varying treatment differences were observed at 10 to 30 cm (Figure 4.2). When treatment was considered as the main effect, the MM had 17 to 24% more water retained than the MF, HF, and CK at -30 kPa. When the depth effect is considered as the main effect, the SWR at -30 kPa for 0-20 cm was 15 to 26% higher than the 20 to 40 cm (Table 4.2).

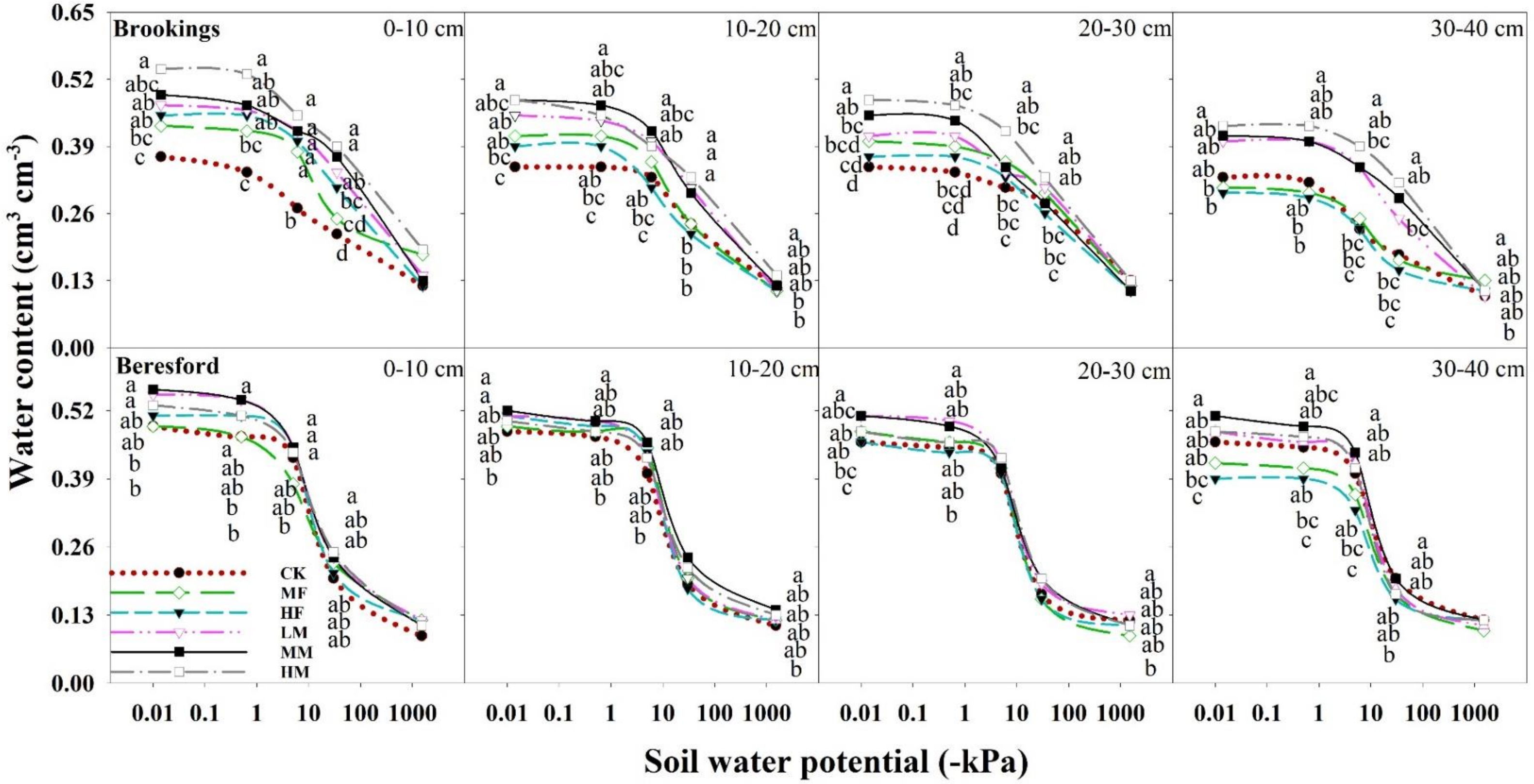

Figure 4.2. Soil water retention as influenced by long-term medium fertilizer (MF), high fertilizer (HF), low manure (LM), medium manure (MM), high manure (HM) rate applications, and control (CK) at Brookings and Beresford, SD. Different lower-case letters indicate statistically significant different treatments at *p*≤0.05 level at each depth. No alphabet indicates no significant difference.

Table 4.2. Soil water retention as influenced by long-term medium fertilizer (MF), high fertilizer (HF), low manure (LM), medium manure (MM), high manure (HM) rate applications, and control (CK) as a function of treatment (Trt) and soil depth.

	Water potential (-kPa)				
	0	**0.5**	**5**	**30**	**1500**
	--------- moisture content (cm^3 cm^{-3}) --------				
	Brookings site				
Treatments					
CK	$0.35^{c\dagger}$	$0.34^{c\dagger}$	$0.28^{c\dagger}$	$0.23^{b\dagger}$	$0.12^{\dagger}$
MF	0.39^{bc}	0.38^{bc}	0.34^{bc}	0.24^{b}	0.13
HF	0.38^{c}	0.37^{c}	0.312^{c}	0.23^{b}	0.12
LM	0.43^{ab}	0.43^{ab}	0.38^{ab}	0.30^{a}	0.12
MM	0.45^{a}	0.45^{a}	0.38^{ab}	0.31^{a}	0.12
HM	0.48^{a}	0.47^{a}	0.41^{a}	0.34^{a}	0.14
Depth (cm)					
0-10	0.46^{a}	0.44^{a}	0.39^{a}	0.31^{a}	0.15^{a}
10-20	0.43^{ab}	0.42^{a}	0.37^{a}	0.29^{b}	0.12^{b}
20-30	0.41^{bc}	0.40^{ab}	0.35^{a}	0.27^{bc}	0.12^{b}
30-40	0.361^{c}	0.36^{b}	0.30^{b}	0.23^{c}	0.11^{b}
	Analysis of Variance (P>F)				
Treatments	<0.01	<0.01	<0.01	<0.01	0.04
Depth	<0.01	<0.01	<0.01	<0.01	<0.01
Trt×Depth	0.52	0.21	0.02	<0.01	0.02
	Beresford site				
Treatments					
CK	0.48^{c}	0.46	0.41^{ab}	0.19^{bc}	0.11
MF	0.47^{bc}	0.45	0.40^{b}	0.19^{bc}	0.11
HF	0.47^{a}	0.46	0.40^{ab}	0.18^{c}	0.12
LM	0.51^{c}	0.50	0.43^{ab}	0.20^{abc}	0.12
MM	0.52^{ab}	0.51	0.44^{a}	0.22^{a}	0.12
HM	0.50^{a}	0.48	0.42^{ab}	0.21^{ab}	0.12
Depth (cm)					
0-10	0.52^{a}	0.51^{a}	0.43^{a}	0.23^{a}	0.11^{b}
10-20	0.50^{ab}	0.49^{ab}	0.43^{a}	0.21^{a}	0.13^{a}
20-30	0.48^{b}	0.47^{bc}	0.41^{ab}	0.18^{b}	0.11^{ab}
30-40	0.46^{c}	0.44^{c}	0.39^{b}	0.18^{b}	0.11^{ab}
	Analysis of Variance (P>F)				
Treatments	<0.01	<0.01	0.02	0.01	0.30
Depth	<0.01	<0.01	0.02	<0.01	0.05
Trt×Depth	0.01	0.01	0.02	0.22	0.29

[†] Means with different letters within a column for individual sites are significantly different for different treatments or depths at $p \leq 0.05$

The pore size distribution (PSD) for Brookings and Beresford sites is presented in Table 3 and Supplementary Table 2. Treatment significantly impacted coarse microporosity, microporosity, and total porosity at 0 to 40 cm. The MM treatment increased coarse microporosity and microporosity as compared to the HF at either site. Depth mainly impacted fine mesoporosity, coarse microporosity, microporosity, and total porosity at 0 to 20 cm (Table 4.3). The microporosity and total porosity values were highest at the surface (10 cm) depth as compared to the other depths at either site. Treatment by depth interaction effect was significant for coarse and fine mesoporosity at either site (Supplementary Table 4.2).

At the Brookings site, there was a significant treatment x depth effect, the HM showed 9.7 times more macroporosity than the MF and MM for 10 to 20 cm. However, treatment did not impact other depths. Similarly, the coarse-mesoporosity for the LM and MM was 1.8 to 4.6 times higher than the MF and CK at 20 to 30 cm, respectively. At 30 to 40 cm depth, the CK showed higher coarse mesoporosity than the MF, LM, MM, and HM. However, treatment did not impact the coarse-mesoporosity at 0 to 20 cm. In addition, the fine mesoporosity (ECD 10-60 μm) for the MM was lower than MF by 62% at surface depth (0 to 10 cm). Treatment did not impact fine mesoporosity below 10 cm. The coarse microporosity (ECD 2 -10 μm) for the MM was 1.3 to 4.5 times higher than the MF and CK at 0 to 10, 10 to 20, and 30 to 40 cm. However, treatment did not impact the 20 to 30 cm depth. In addition, the LM, MM, and HM increased microporosity (ECD <0.2 μm) 1.2 to 1.8 times more than the MF, HF, and CK at 0 to 20 cm. At 20 to 30 cm, the HM increased <0.2 μm pores by 14 to 23% as compared to MM, HF, and CK.

Similarly, at 30 to 40 cm, the MM and HM increased <0.2 μm pores 1.70 to 2.1 times as compared to the MF, HF, and CK. When treatment was observed as the main effect, the total porosity for MM and HM was 17 to 37% higher than the MF, HF, and CK, respectively, for this study site. Considering the depth impact, the total porosity at 0 to 10 cm was 1.1 and 1.3 times higher than 20 to 30 and 30 to 40 cm, respectively. However, depth did not impact total porosity at 0 to 10 and 10 to 20 cm.

At the Beresford site, a significant treatment x depth impact was observed. The LM increased macroporosity (ECD >500 μm) 23 times more than the HF at 30 to 40 cm. Treatment did not impact for 0 to 30 cm. Similarly, the LM, HF, and MM resulted in 2.2 to 2.4 times more coarse-mesoporosity (ECD 60-500 μm) than the CK at 0 to 10 cm. Treatment did not impact coarse mesoporosity below 10 cm. In addition, the MF showed 54% lesser fine mesoporosity (10-60 μm) as compared to the CK at 0 to 10 cm depth. At 30 to 40 cm depth, the LM, MM, and HM increased the fine mesoporosity compared to the HF at Beresford site. Treatment did not impact fine mesoporosity at 10 to 30 cm. The LM and MM increased total porosity by 12 to 14% higher than the MF and CK at surface depth. Total porosity for the LM and MM was 6% higher than CK at 10 to 20 cm depth, and the MM increased total porosity by 19 and 31% as compared to the HF at 20 to 30 cm, and 30 to 40 cm, respectively. On observing the treatment effects, the MM increased the PAW content (ECD 0.2-10 μm) and microporosity at Beresford site, as compared to HF. Considering the depth effect, the PAW and microporosity at surface depth (0 to 10 cm) were higher than 20 to 30, and 30 to 40 cm by 25 to 76%. However, there was no depth impact for microporosity at 0 to 10 and 10 to 20 cm.

Table 4.3. Pore size distribution (PSD) as influenced by long-term medium fertilizer (MF), high fertilizer (HF), low manure (LM), medium manure (MM), high manure (HM) rate applications, and control (CK) as a function of treatment (Trt) and soil depth.

	Macro-porosity	**Coarse meso-porosity**	**Fine meso-porosity**	**Course micro-porosity (PAW)**	**Micro-porosity**	**Total porosity**
	(>500 µm)	**(60-500µm)**	**(10-60 µm)**	**(2-10µm)**	**(<2µm)**	
	---------------------cm^3 cm^{-3}---------------------					
Brookings Site						
Treatments						
CK	0.012†	0.055†	0.06^{b†}	0.109^{b†}	0.229^{b†}	0.352^{c†}
MF	0.009	0.038	0.10^{a}	0.106^{b}	0.240^{b}	0.387^{bc}
HF	0.006	0.056	0.08^{ab}	0.119^{b}	0.234^{b}	0.379^{c}
LM	0.006	0.050	0.07^{ab}	0.182^{a}	0.303^{a}	0.434^{ab}
MM	0.006	0.062	0.08^{ab}	0.191^{a}	0.307^{a}	0.452^{a}
HM	0.013	0.057	0.07^{a}	0.198^{a}	0.341^{a}	0.483^{a}
Depth (cm)						
0-10	0.014	0.056	0.07^{ab}	0.167^{a}	0.314^{a}	0.458^{a}
10-20	0.009	0.048	0.10^{a}	0.152^{ab}	0.292^{b}	0.426^{ab}
20-30	0.007	0.052	0.06^{b}	0.169^{a}	0.270^{bc}	0.410^{bc}
30-40	0.006	0.056	0.08^{ab}	0.116^{b}	0.227^{c}	0.364^{c}
Analysis of Variance (P>F)						
Treatments	0.224	0.160	0.032	<0.001	<0.001	<0.001
Depth	0.073	0.660	0.001	0.006	<0.001	<0.001
Trt×Depth	0.061	<0.001	0.002	<0.001	<0.001	0.516
Beresford Site						
Treatments						
CK	0.017	0.049	0.22	0.082^{ab}	0.190^{bc}	0.475^{c}
MF	0.018	0.054	0.21	0.080^{ab}	0.190^{bc}	0.472^{bc}
HF	0.012	0.052	0.23	0.062^{b}	0.179^{c}	0.468^{a}
LM	0.014	0.067	0.23	0.082^{ab}	0.202^{abc}	0.513^{c}
MM	0.017	0.068	0.22	0.100^{a}	0.222^{a}	0.523^{ab}
HM	0.017	0.054	0.22	0.093^{ab}	0.213^{ab}	0.496^{a}
Depth (cm)						
0-10	0.019	0.074^{a}	0.20	0.118^{a}	0.227^{a}	0.524^{a}
10-20	0.014	0.052^{b}	0.23^{ab}	0.082^{b}	0.208^{a}	0.501^{ab}
20-30	0.018	0.053^{b}	0.23^{a}	0.067^{b}	0.180^{b}	0.483^{b}
30-40	0.013	0.050^{b}	0.21^{ab}	0.067^{b}	0.181^{b}	0.456^{c}
Analysis of Variance (P>F)						
Treatments	0.497	0.151	0.637	0.032	0.001	<0.001
Depth	0.085	0.003	0.031	<0.001	<0.001	<0.001
Trt×Depth	0.009	0.003	0.004	0.738	0.222	0.001

[†] Means with different letters within a column for individual sites are significantly different for different treatments and depths at $p \leq 0.05$

Data on van Genuchten water retention parameters α and *n* and the slope of the soil water retention curve at the inflection point (|S|) for both study sites are given in Table 4.4 and Supplement Table 4.3. Treatment did not impact all the parameters at 0 to 40 cm, except for α at Brookings site. The CK treatment was found to have higher α values as compared to the LM, MM, and HM at Brookings site. On considering the depth as the main effect, |S| and α parameters were impacted at 0 to 40 cm. The α values were highest at surface depth as compared to all other depths at either site. Treatment by depth interaction effect was significant for α at either site.

At the Brookings site, when considering depth as the main effect, the |S| at the surface depth was higher than the 30 to 40 cm depth. On observing the treatment x depth effect, the CK had higher α values as compared to the LM, MM, and HM at 0 to 10 cm and 30 to 40 cm. However, at 20 to 30 cm soil depth, the α values for the MM were higher than all the other treatments. At the Beresford site, when considering depth as the main effect, the parameter *n* was lower at 0 to 10 cm as compared to the corresponding lower depths (10-20, 20-30, 30- 40 cm). On observing the treatment x depth effect, the |S| for the MF was lower than the CK at 0 to 10 cm and the α for the HM was higher than the CK and HF at surface depth by 38%.

Table 4.4. Soil water retention curve at its inflection point (|S|), van Genutchen's parameter α and *n* as influenced by long-term medium fertilizer (MF), high fertilizer (HF), low manure (LM), medium manure (MM), high manure (HM) rate applications, and control (CK) as a function of treatment (Trt) and soil depths.

	|S| ($cm^3\ cm^{-3}$)	***α***	***n***
	Brookings Site		
Treatments			
CK	$0.06^{\dagger}$	$0.048^{a\dagger}$	$1.57^{\dagger}$
MF	0.08	0.026^{ab}	1.74
HF	0.08	0.020^{ab}	1.77
LM	0.08	0.010^{b}	1.62
MM	0.09	0.015^{b}	1.56
HM	0.08	0.016^{b}	1.51
Depth (cm)			
0-10	0.09^{a}	0.04^{a}	1.69
10-20	0.08^{ab}	0.02^{b}	1.60
20-30	0.07^{ab}	0.01^{b}	1.50
30-40	0.07^{b}	0.02^{ab}	1.72
	Analysis of Variance (P>F)		
Treatments	0.06	<0.01	0.14
Depth	0.02	0.02	0.10
Trt×Depth	0.20	<0.01	0.84
	Beresford Site		
Treatments			
CK	0.14	0.014	2.07
MF	0.15	0.018	2.08
HF	0.16	0.014	2.28
LM	0.16	0.016	2.07
MM	0.14	0.017	1.95
HM	0.14	0.015	2.00
Depth (cm)			
0-10	0.13^{b}	0.019^{a}	1.78^{b}
10-20	0.16^{ab}	0.014^{b}	2.12^{a}
20-30	0.17^{a}	0.015^{b}	2.24^{a}
30-40	0.15^{ab}	0.014^{b}	2.15^{a}
	Analysis of Variance (P>F)		
Treatments	0.50	0.21	0.11
Depth	<0.01	0.01	<0.01
Trt×Depth	0.01	0.01	0.33

[†] Means with different letters within a column for individual sites are significantly different for different treatments or depths at $p \leq 0.05$

Manure application can improve water holding capacity by increasing the total number of storage pores (Bhattacharyya et al., 2008). Generally, soil pore sizes are closely related to soil aggregates (Shi et al., 2016). Therefore, improvement in soil aggregation can increase macro- and meso- pores. In addition, studies have reported that long-term inorganic fertilization could degrade or have no effects on soil structure despite the increase in SOC content (Zhou et al., 2016). Also, the improvement in SWR due to manure treatments might be attributed to the action of polysaccharides and fulvic acid components of cattle manure (Prasad & Sinha, 2000). Similar results were reported in the literature (Ankenbauer & Loheide, 2017; Shi et al., 2016). Zhou et al., (2016) reported that long-term manure application increased the intra- and inter-aggregate pores and porosity in comparison with inorganic fertilization at different rates. Especially at the surface depth, the higher organic matter content leads to more water storage (Bot & Benites, 2005). Manure applications provided more organic matter to surface soil and therefore, the effect of manure application on soil porosity was found to be higher at 0 to 10 cm at both sites. Manure application can also increase the available water for plant growth (Bhattacharyya et al., 2008). However, the field capacity and wilting point may vary depending on the texture, organic matter, structure, clay content, etc. (Karahan & Öztürk, 2014). In this study, the application of inorganic fertilizer had little to no impact on soil hydro-physical properties. Long-term application of inorganic fertilizer and high manure can lead to the accumulation of NH_4^+, which is a dispersing agent of soil aggregates (Haynes & Naidu, 1998). Therefore, inorganic fertilizer application alone is not an efficient approach to improving the soil structure and water holding capacity in the soil.

According to Dexter (2004), the soil water retention curve at the inflection point (|S|) can be considered as a soil physical quality index that is reliable with observations on soil compaction, effects of soil organic matter content, and root growth. Higher values indicate the presence of structural pores. It was also proposed that $|S| > 0.035$ indicates well-structured soils from poor-structured soil ($|S| < 0.020$). For all the soil samples, irrespective of treatment and depth, the value of |S| is higher than 0.035, indicating well-structured soil at both study sites. (Benson et al., 2014) showed that soils with larger pores have larger α and soils with a wider distribution of pore sizes have lower n. In relation to water content, (Hodnett & Tomasella, 2002) reported that lower α values indicate little change in water content as potential (ψ) becomes more negative, which is usually associated with fine-textured and unstructured soils. Furthermore, higher values of α indicate a sudden change in water content, where some pores empty under very small negative heads, and this is usually associated with well-structured soils. The parameter n, on the other hand, is dimensionless and determines the steepness of the water-release curve. If the value of n is large the curve is steep, which indicates a rapid decrease in water content as ψ becomes more negative, and with a lower n value, the change in water content is slow. In this study, the parameter α varied from 0.006 to 0.127, and n varied from 1.39 to 2.50.

In general MM and HM, treatment was found to influence some hydro-physical properties at different soil depths. However, the long-term application of high manure is discouraged because apart from it being less cost-effective, the long-term application can be detrimental to the environment. For instance, Guo et al. (2018) found that after 34-year of continuous application of manure decreased aggregate stability in vertisol was observed because of accumulated exchangeable Na^+. This shows that excessive application of manure may have a negative impact on the soil structure.

4.3.4. Relationship between Soil organic carbon pool, PSD, and PAW

The correlations among CWC, CWN, HWC, HWN, SOC, and TN stock, pore size distribution, |S|, and PAW are presented in Table 4.5. The data from Beresford and Brookings sites were pooled together to observe the correlation between these parameters. Also, a positive correlation was observed between the PAW content and SOC stock [Pearson correlation coefficient (δ) = 0.42] (Figure 4.3). In addition, ANCOVA analysis taking residuals of SOC-stock as a covariate was undertaken to observe a significant treatment: covariate effect. Therefore, we fitted regression lines separately for each of the treatments to obtain the following regression equations:

CK: $PAW = 0.0024\,(SOCstock) + 0.0248\ (R^2=0.17^*)$ [5]

HF: $PAW = 0.0054\,(SOCstock) - 0.0656\ (R^2=0.29^*)$ [6]

HM: $PAW = 0.0015\,(SOCstock) + 0.0972\ (R^2=0.05)$ [7]

LM: $PAW = 0.0055\,(SOCstock) - 0.0244\ (R^2=0.40^{**})$ [8]

MF: $PAW = 0.0023\,(SOCstock) + 0.0314\ (R^2=0.10)$ [9]

MM: $PAW = 0.0063\,(SOCstock) - 0.0339\ (R^2=0.51^{**})$ [10]

The C and N fractions (CWC, CWN, HWC, and HWN) were positively correlated with SOC stock, indicating that an increase in SOC stock increased the C fractions. The maximum increase in the PAW content per unit increase in SOC stock was observed for the MM treatment. Interestingly, for HM, the change in PAW as a function of SOC stock was not significant. Macroporosity was positively correlated with the HWC and CWC, this may be because an increase in soil organic matter leads to an improvement in soil aggregation (Zhou et al., 2016). In addition, total porosity was observed to be positively correlated with HWC, CWC as well as SOC stock.

Several studies in the literature reported a positive correlation between SOC and PAW (Minasny & McBratney, 2018; Palmer et al., 2017). In this study as well, we observe a correlation between SOC stock and PAW. SOC stock may influence total porosity by its direct adsorption to soil water and its indirect effects on soil structure (Saxton & Rawls, 2006). Tisdall & Oades (1982) suggest that aggregates could protect SOC from physical and biological decay, and this impacts soil water retention by modifying the development of soil pores (Bhattacharyya et al., 2008). This explains that management practices that improve aggregate stability can improve SOC stock which can directly improve the soil water retention capacity and indirectly enhance the soil structure.

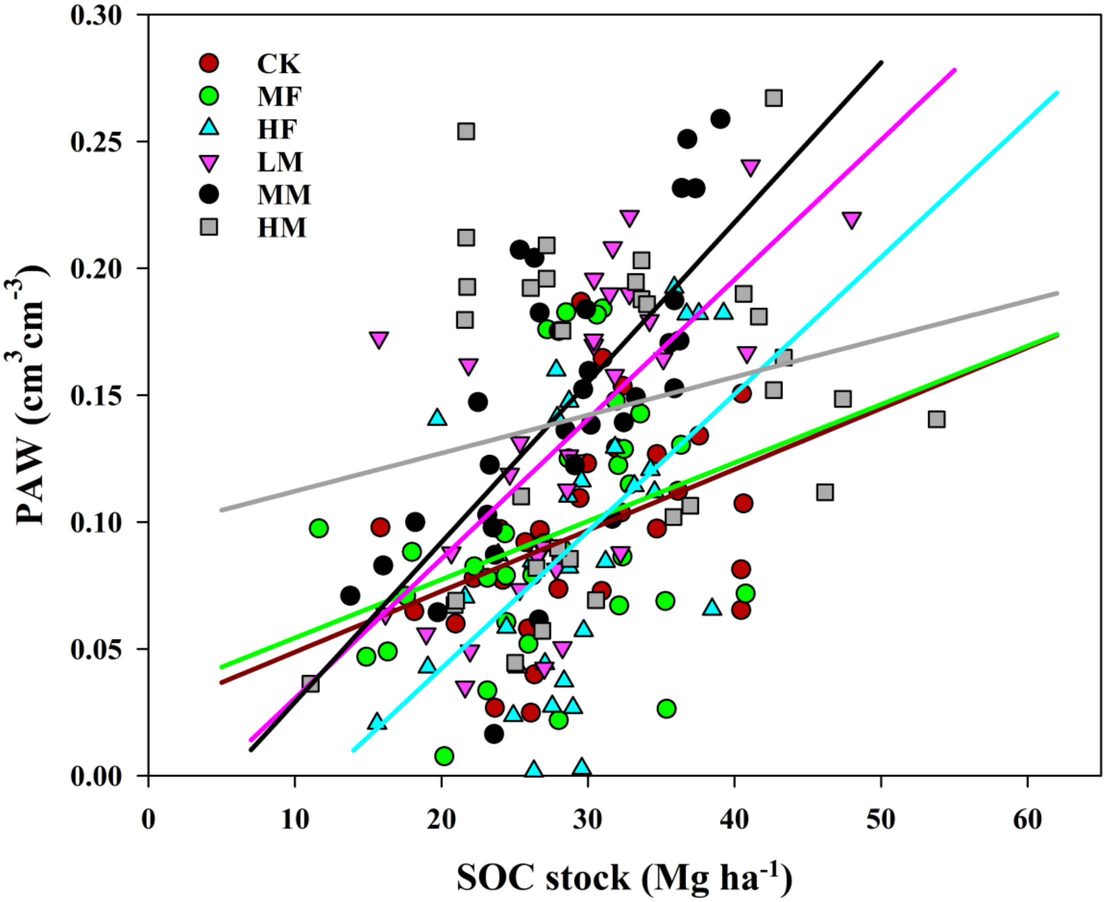

Figure 4.3. Relationship between SOC stock and plant available water (PAW). Nutrient applications include medium fertilizer (MF), high fertilizer (HF), low manure (LM), medium manure (MM), high manure (HM) rate applications, and control (CK) at Brookings and Beresford study sites. Regression equations are in-text.

Table 4.5. Correlation table of hot- and cold-water extractable carbon and nitrogen (CWC, HWC, CWN, HWN), soil organic carbon (SOC) stock, pore size distribution, soil water retention curve at its inflection point (|S|), and plant available water (PAW) overall depths at Beresford and Brookings, SD

	CWC	***CWN***	***HWC***	***HWN***	***SOCstock***	***TNstock***	***Macro-porosity***	***Coarse-mesoporosity***	***Fine mesoporosity***	***Micro-porosity***	***Total porosity***	**|S|**	***PAW***
CWC	1.00												
CWN	0.78**	1.00											
HWC	0.88**	0.84**	1.00										
HWN	0.87**	0.87**	0.99**	1.00									
SOCstock	0.64**	0.76**	0.78**	0.81**	1.00								
TNstock	0.66**	0.76**	0.80**	0.83**	0.96**	1.00							
Macro-porosity	0.26**	0.16**	0.23**	0.20**	0.12	0.17**	1.00						
Coarse mesoporosity	0.25**	0.22**	0.29**	0.28**	0.14*	0.13	-0.21**	1.00					
Fine mesoporosity	0.15*	-0.05	0.08	0.03	-0.23**	-0.13	0.29**	-0.07	1.00				
Microporosity	0.23**	0.33**	0.25**	0.29**	0.47**	0.42**	-0.15*	-0.06	-0.62**	1.00			
Total porosity	0.53**	0.36**	0.48**	0.45**	0.24**	0.32**	0.28**	0.21**	0.61**	0.17**	1.00		
|S|	0.14*	-0.06	0.05	0.01	-0.20**	-0.11	0.26**	-0.13	0.91**	-0.48**	0.61**	1.00	
PAW	0.21**	0.29**	0.22**	0.26**	0.42**	0.36**	-0.17**	-0.03	-0.60**	0.91**	0.11**	-0.48**	1.00

** Shows significant differences at 0.01

* Shows significant differences at 0.05

4.4. Conclusions

This study observed that long-term (> 10 years) manure application increased SOC stock and TN stock and decreased ρ_b as compared to inorganic fertilizer and control treatments, mainly at 0 to 20 cm. This may be attributed to the low density of manure which when added to the soil can reduce mineral fractions, thereby decreasing bulk density. The SOC stock increase is a result of direct organic matter input to the soil via manure. Manure application was also observed to increase cold- and hot-water extractable C and N as well as SWR at 0, -0.5, -5, and -30 kPa to a soil depth of 40 cm in this study. In addition, a positive correlation was observed between the SOC stock and PAW, which explains the relevance of manure application in improving the soil water holding capacity and soil structure. It is also observed that the inorganic fertilizer application improved hot-water extractable C and N, coarse and fine mesoporosity at 0-10 cm of soil depth. This might be because of the indirect effects that inorganic fertilizer has on increasing crop dry matter production thereby increasing carbon content and biomass input majorly at the surface depth. However, the long-term application of inorganic fertilizer when compared to the long-term application of manure may deteriorate the soil structure, which may negatively impact the soil hydro-physical properties, in the long run. As this study observed that the application of manure at high as well as medium rates improved the soil hydro-physical parameters, this study encourages farmers to adopt the long-term use of an appropriate rate of manure according to the nitrogen requirement rate to avoid over-application of manure, thereby enhancing organic carbon and hydro-physical properties of soil.

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CHAPTER 5

Impact of Long-Term Cattle Manure and Inorganic Fertilizer on Greenhouse Gas Emissions under a Corn-Soybean-Spring Wheat Rotation System

Abstract

Greenhouse gas (GHG) emissions are presumed to cause global warming. The application of manure has been suggested as an effective strategy to mitigate climate change. However, the effect of moderate manure application rate on GHG emissions to agricultural soils under various environmental conditions still remains unclear and need to be further studied. This study was conducted to examine the impacts of cattle manure and inorganic fertilizer on soil surface greenhouse gases (GHG) [carbon dioxide (CO_2), nitrous oxide (N_2O), and methane (CH_4)] fluxes from soils managed under corn (*Zea mays* L.)-soybean (*Glycine max* L.)- spring wheat (*Triticum aestivum)* rotation. The experiment was established on a silty loam soil, and the treatments included nitrogen-based recommended rate (medium manure, MM), recommended inorganic fertilizer rate (medium fertilizer, MF), and control (CK) replicated four times. Soil GHG fluxes were monitored at least once a week during the growing season for 2020 and 2021. Data from this study showed treatment did not significantly impact the daily average GHG emissions in 2020 and 2021. However, the daily average CO_2 emission rate was significantly higher on July 26, 2020 and July 23, 2021. The cummulative soil surface N_2O, and CH_4 fluxes were increased by the MF treatment compared to the MM and CK in 2020. In general, the MF treatment resulted in higher cummulative GHG

emissions compared to the MM and CK in both years. Also the global warming potential rate increased in 2020 by inorganic fertilizer rate as compared to the MM and CK. Data from this study showed that inorganic fertilizer application in crops can be harmful to the environment by emitting higher GHG emissions, therefore, sustainable management practices need to be explored to mitigate GHG emissions and reduce or eliminate the negative environmental impacts.

5.1. Introduction

Greenhouse gas (GHG) emissions are presumed to cause global warming, and agriculture contributes around 10-12% of global anthropogenic GHG emissions (Smith et al., 2014). The three main greenhouse gases emitted from agricultural soil are N_2O, CO_2, and CH_4. These gases have been recognized as major contributors to global warming (Ren et al., 2017). Therefore, incorporating sustainable management practices such as the application of inorganic fertilizer and manure may mitigate GHG emissions (Niggli et al., 2009; Zhang et al., 2013).

Soil N_2O is formed from microbial processes through denitrification and nitrification under dry and wet conditions (Smith & Conen, 2004). Inorganic fertilizers, primarily nitrogen-based fertilizers, contribute to emissions of nitrous oxide (N_2O) from agricultural soils (Hui et al., 2017). Soil N_2O produced from manure application, can be produced directly by nitrification–denitrification processes or indirectly when N is lost through NH_3 volatilization or nitrate leaching and then converted to N_2O (Sun et al., 2014). Stalenga & Kawalec, (2008) reported that the application of inorganic fertilizer resulted in almost double the soil N_2O emissions than that of the application of animal manure. Soil CO_2 emission in agricultural soils is generated from soil microbial respiration (Ding et al., 2007). One management practice that increases soil CO_2 emissions is soil tillage, and, therefore, increases soil carbon losses (Rakotovao et al., 2017). Implying that soil carbon accumulation in agricultural soil is a strategy to reduce CO_2 emissions (Dossou-Yovo et al., 2016). Recently, it was found that manure application can potentially sequestrate more C in the soils and thus convert the soils to a net CO_2 sink (Gattinger et al., 2012). Increasing soil organic carbon can be a mitigation strategy for reducing CO_2 emissions (Smith, 2004). Soil CH_4 is formed when organic matter is decomposed and CO_2 is reduced under highly anaerobic conditions (Gao et al., 2019). Therefore, indicating an increased emission in well-aerated agricultural soil. Manure is a significant source of methane (CH_4) emissions. Although, the effect of inorganic fertilizer use on CH_4 emissions is still poorly understood and requires further research (Smith et al., 2014). Soil CO_2 and CH_4 are produced from manure degradation (Sun et al., 2014). These findings highlight the need for sustainable agricultural practices that can help reduce GHG emissions from the agriculture sector. Therefore, it is important to consider the impacts of manure and inorganic fertilizer on GHG emission fluxes when making decisions about agricultural practices.

GHG emission fluxes are greatly influenced by climatic conditions (Bowden et al., 1998). Research has shown that an increased CO_2 emission could lead to an increase in N_2O emissions (Dijkstra et al., 2012; Van Groenigen et al., 2011). An increase in temperature can both increase and decrease N_2O emissions. This is because an increase in temperature can reduce soil moisture, which may slightly reduce N_2O flux; but heat can also encourage N mineralization (Rustad et al., 2001) and the transformation rate from N into N_2O (Bai et al., 2013), thereby increasing N_2O emissions. The impact of GHG emissions may also be dependent on the emissions rate of other greenhouse gases. For instance, Wang et al. (2018) reported that under increased temperature and CO_2, higher CH_4 emissions were reported than under increased temperature without increased CO_2 emission. Also, the changes in precipitation intensity have been shown to greatly impact soil CO_2, CH_4, and N_2O fluxes (Martins et al., 2017). For example, Yan et al. (2018) reported that increased precipitation significantly increased CO_2 and N_2O emissions, but Knorr et al. (2008) reported that increased precipitation suppressed soil CO_2 emissions due to weak soil aeration and enhanced CO_2 dissolution. This explains that GHG emissions can be affected by different factors, including climate and management strategy. Therefore, it is important to observe these differences, to better understand the effect of manure and inorganic fertilizer on GHG emissions.

A sustainable management strategy such as manure and inorganic fertilizer application on GHG emissions can be an alternative method to mitigate GHG emissions (Lin et al., 2011). However, there are inconsistent results about the overall effects of fertilization rate and type, climate, and soil conditions on GHG emissions (Mbonimpa et al., 2015). The impact of manure and inorganic fertilizer on GHG fluxes needs to be studied, to understand the variation of GHG emission under different climatic conditions. As this will be of great assistance in mitigating GHG emissions. Therefore, the objective of this study was to assess differences in GHG fluxes when medium rates of manure and inorganic fertilizers along with the control (no manure or inorganic fertilizer) were applied under corn-soybean-spring wheat rotation systems.

5.2. Materials and Methods

5.2.1. Experimental site and experimental design

The experimental site was located at the South Dakota State University Felt Research Farm (44° 22′ 07.15″ N and 96° 47′ 26.45″ W) in Brookings, South Dakota on well-drained silty loam Vienna soil (Fine-loamy, mixed, frigid Udic Haploborolls). The experimental plots were established in 2008 in a corn (*Zea mays* L.)-soybean (*Glycine max* L.) rotation system, until 2019. In 2020, spring wheat (*Triticum aestivum)* followed by cover crops were added to this cropping system. Converting it to a corn-soybean-spring wheat rotation system. The dimensions for Felt Research Farm plots are 6 m by 18 m. The plots are nearly flat with a slope of <1% and elevation of 518 m, and it was managed under a reduced tillage system. The experimental areas were characterized by a continental climate having relatively humid summers, and cold and snowy winters.

5.2.2. Study treatments

The experimental site included six different treatments in total, where there were three manure application rates; low manure (LM) contained a quantity of manure based on the recommended phosphorous requirement, medium manure (MM) contained a quantity of manure-based on recommended nitrogen requirement, high manure (HM) contained a quantity of manure-based on double the recommended nitrogen requirement, two inorganic fertilizer application rate; medium fertilizer (MF) contained the suggested inorganic fertilizer rate, high fertilizer (HF) contained a high fertilizer rate, and control (CK) which received no manure and no fertilizer. The amount of manure and inorganic fertilizer treatments were calculated using South Dakota Department of Agriculture and Natural Resources (DANR) tool and considering crop nutrients needed according to crop yield goal, soil nutrient contents, and manure nutrient contents for each treatment. The manure and fertilizer treatments were applied manually and incorporated by disking at 20 cm depth one to three days before planting. Treatments were arranged in a randomized complete block design with four replicates.

5.2.3. Sampling and analysis of greenhouse gas fluxes

Soil surface GHG fluxes were monitored from July through November 2020 and March through October 2021 where gas samples were taken at least once a week depending on the weather conditions. Static closed chamber technique was used for measuring GHG fluxes (Parkin & Venterea, 2010), where Polyvinyl chloride (PVC) static chambers (25 cm diameter × 15 cm height) were installed in the plots with medium manure (MM), medium fertilizer (MF), and control (CK) treatment to monitor soil surface GHG fluxes. The chamber was installed to a depth of 5 cm between crop with minimum soil disturbance and were removed only during field operations. In addition to soil surface GHG flux monitoring, during each sampling time, soil moisture was measured volumetrically using a HH2 moisture sensor (Delta-T-Devices, Cambridge, England), and soil temperature was measured using a thermometer (Taylor 14769 Digital

LCD folding thermometer) at 0-5 cm soil depth, respectively. Gas samples were collected at 0-, 20-and 40- min intervals using a 10-ml syringe. It was collected between 8:00 am and noon to minimize the effect of diurnal variations on GHG fluxes. These samples were taken via a chamber septum and transferred to a 10-ml argon-filled sterilized vial sealed with a gas-tight septum. Gas concentrations of CO_2, CH_4, and N_2O were measured within 2 to 3 days of sampling using a Gas Chromatograph (GC-2014; Shimadzu, Columbia, MD, USA) using a lepton capture detector each at 260 °C for N_2O, and a flame ionization detector for CO_2 and CH_4. The daily flux of gases was estimated from the concentration in the chamber headspace over 40 min collection period. Daily flux (F, mass of g gas ha^{-1} day^{-1}) was computed as:

$$F = \left(\frac{\Delta \mathrm{g}}{\Delta \mathrm{t}}\right)\left(\frac{\mathrm{V}}{A}\right)k$$

where $\Delta g/\Delta t$ is the rate of gas change (CH_4, CO_2 or N_2O) concentration inside the chamber (mg CH_4-C, CO_2-C or mg N_2O-N m^{-2} min^{-1}); V is the chamber volume (m^3); A is the surface area circumscribed by the chamber (m^2) and k is the time conversion factor (1440 min day^{-1}) (Ussiri & Lal, 2009). Calibration was routinely performed using dilutions of a certified gas standard mix (Scott Specialty Gases, Plumsteadville, PA, USA). Daily gas flux was calculated from the concentration vs. time data using linear regression or the algorithm of Hutchinson and Mosier (1981) when the concentration vs. time data were curvilinear. Cumulative flux for each site was calculated using linear interpolation between individual GHG flux observations and summing over the total observation days. Gas fluxes were calculated from the change in the chamber gas concentration during the 40-minute period where static chambers are closed. The linearity of gas diffusion into the headspace over this closure period (40 min) had previously been determined, and each flux could be calculated from a single determination at the end of closure by considering the chamber volume and soil surface (Hutchinson & Mosier, 1981). A positive daily flux (*F)* shows a net emission of gas from the soil to the atmosphere, and a negative *F* value indicates a net transfer of gas from atmosphere into the soil. The global warming potential (GWP) in CO_2-equivalent for CH_4 and N_2O were calculated using the following equations (Griggs & Noguer, 2002):

$GWP_{(N2O)} = N_2O \times 298$

$GWP_{(CH4)} = CH_4 \times 25$

Based on a 100-year time frame, the GWP of CH_4 and N_2O are, respectively, 25 and 298 times higher than that of CO_2 (IPCC, 2007). The changes in SOC during the growing seasons were not measured so the $GWP_{(CO2)}$ was not determined (Robertson & Grace, 2004). Although the concentrations of CH_4 and N_2O are lower in the atmosphere than the CO_2, their GWPs are sufficiently high that small changes have an inconsistent effect on radiative forcing. The net GWP was also calculated using the following equation given by (Six et al., 2004) as:

$\text{Net GWP} = GWP_{(N2O)} + GWP_{(CH4)}$

5.2.4. Statistical analysis

Statistical analysis on the effects of manure and inorganic fertilizer applications on GHG fluxes and GWP were conducted based on the Proc GLIMMIX procedure in

SAS (2014) using the DandA.sas macro (Saxton 2013). Least square means were separated using Fisher's protected least significant differences (LSD) test at $p \leq 0.05$ significance level. The data were tested for normality using Shapiro-Wilk and for homogeneity of variance using levene's test and were log-transformed when necessary.

5.3. Results and Discussion

5.3.1. Weather, soil moisture, soil temperature

The mean precipitation, maximum and minimum air temperature for 2020 and 2021 growing seasons are given in Figure 5.1. Mean annual air temperature in 2020 and 2021 was −15.8 °C in the winter and 27.8 °C in the summer, respectively. The mean annual precipitation for 2020 and 2021 was approximately 672 mm. Soil moisture content (m^3 m^{-3}) was associated with air temperature and precipitation. A decline in air temperature and precipitation for 2020 and 2021 was observed with a decline in soil moisture content under all treatments. An increased soil moisture was observed with increased precipitation in 2020 and 2021. Soil temperature was positively associated with precipitation and air temperature. Treatment did not significantly impact the soil temperature and soil moisture in 2020 and 2021 (Figure 5.1).

According to Piao et al. (2009), changes in soil moisture depends on the stability of precipitation (Piao et al., 2009). Therefore, this explains why soil moisture was observed to be increased when precipitation consistent increased under all the treatments. Apart from the weather condition, soil moisture can be impacted by agricultural management practice (Mishra, 2020) such as manure and inorganic fertilizer application. In this study, treatment did not significantly impact the soil moisture. However, soil moisture was higher under manure treatment compared to inorganic fertilizer. In relation to green house gas emissions fluxes, improved soil moisture content may increase minerialization (Cassman & Munns, 1980), and increased minerialization is a strategy in mitigating greenhouse gas emissions (Alturki, 2022). However, soil moisture and temperature are two main factors that affects minerialization (Cassman & Munns, 1980). This may also explain the why variation in greenhouse gas fluxes needs to be studied futher, as various factors like management practices and climatic factors influences the greenhouse gas emission rate.

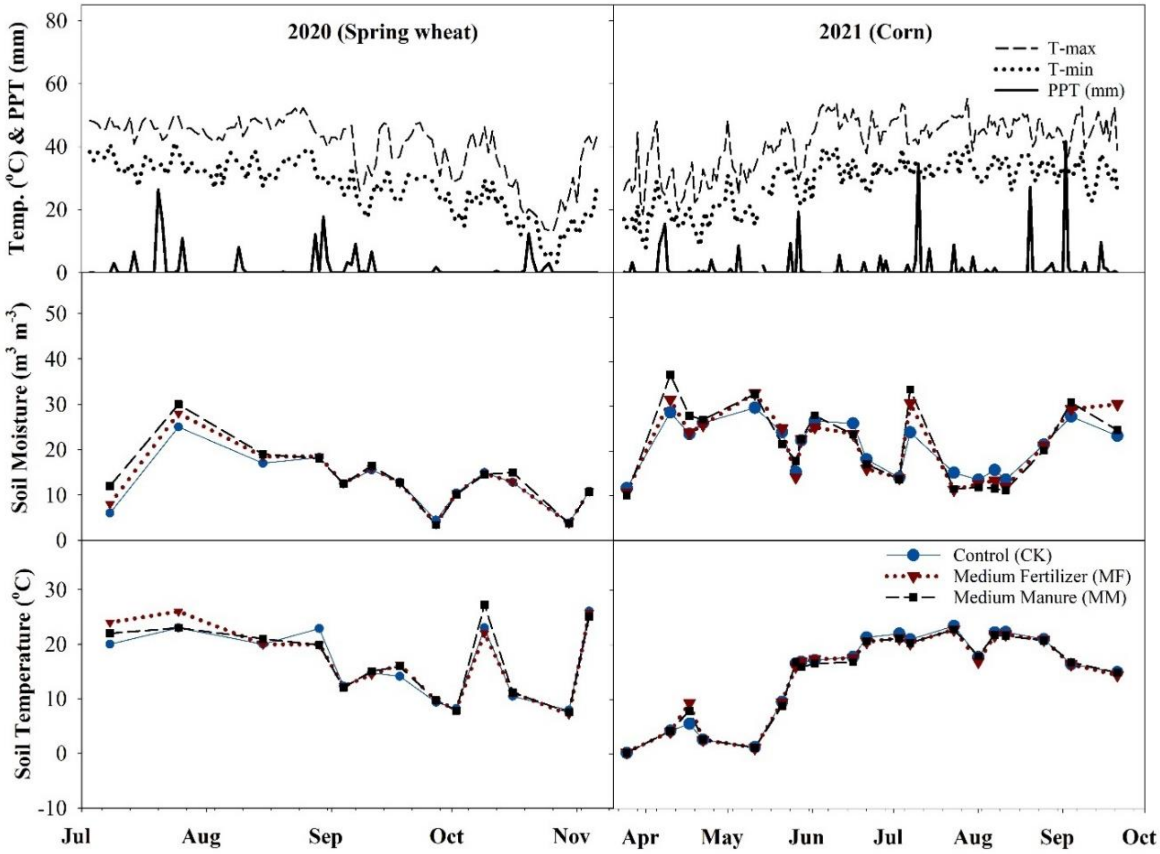

Figure 5.1. Soil moisture and soil temperature as influenced by long-term manure and inorganic fertilizer management under corn-soybean-spring wheat rotation. Weather data from mesonet website.

5.3.2. Daily average of CO_2, N_2O and CH_4 fluxes

Daily average soil surface CO_2 fluxes were not significantly impacted by treatments (Figure 5.2). However, higher CO_2 fluxes were observed in wet and warm periods of the year. The highest peak was observed in July and decreased until November. The largest treatment difference was observed in soil CO_2 fluxes between the MF (48.2 kg ha^{-1} day^{-1}) and CK (23.4 kg ha^{-1} day^{-1}) treatments, on July 26, 2020. While the least difference was observed on November 4th, 2020, between the MF and MM treatments. The peak of CO_2 fluxes for the MF was 2.1 times higher than the CK. The CO_2 fluxes were significantly higher on July 26, 2020, as compared to other dates in 2020 and on July 23, 2021, as compared to other dates in 2021. Comparing the CO_2 peak in 2020 to that in 2021, the MF treatment was 2.3 times higher on July 26, 2020, than on July 23, 2021.

Daily average soil surface N_2O fluxes did not differ under the MF and MM treatment through the month of July 2020. Higher fluxes were observed under warm and wet periods of the year. Soil N_2O fluxes peaked in July and started decreasing until November. The largest treatment difference was observed in soil N_2O fluxes between the MF (18.6 g ha^{-1} day^{-1}) and CK (2.7 kg ha^{-1} day^{-1}) treatments, on June 2, 2021. The peak of N_2O fluxes for the MF was 6.9 times higher than the CK. While a minimum difference was observed on April 17, 2021, between the MF and MM treatments. Comparing the

N_2O peak in 2020 to that in 2021, the MF treatment was 1.9 times higher on July 26, 2020, than on June 2, 2021.

Daily average soil surface CH_4 fluxes under both manure and inorganic fertilizer application varied with the climatic conditions. During the warm and wet periods of both years, alternating episodes of release and uptake of CH_4 emission was observed. Higher changes were observed under the MF treatment in 2020 as compared to other treatments. Data showed that the measured CH_4 fluxes varied over the two years, both positively as a GHG source and negatively as a sink, however, no significant differences in CH_4 fluxes due to the treatments were observed in 2020 and 2021 (Figure 5.2). Inorganic fertilizer application significantly increased CH_4 fluxes compared to manure treatments only in July 2020.

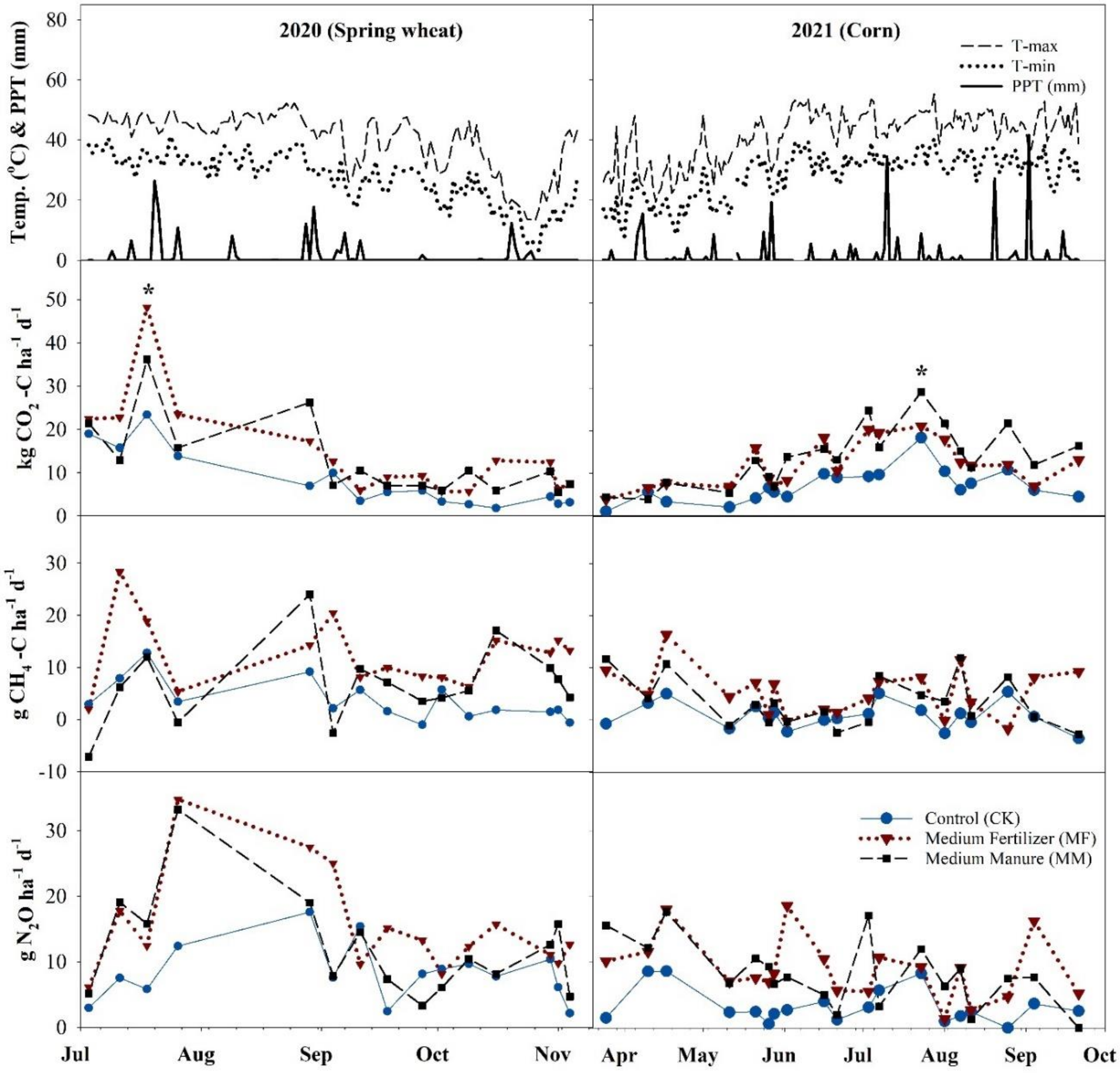

Figure 5.2. Daily average greenhouse gas (GHGs) fluxes as influenced by long-term manure and inorganic fertilizer management under corn-soybean-spring wheat rotation.

According to Wagle & Kakani, (2014), variations in CO_2 fluxes during the growing season depends on temperature and moisture (Wagle & Kakani, 2014). In this study, higher fluxes were observed in warmer and wetter periods in the year. This may be attributed to higher microbial activities and increased soil temperature (Smith et al., 2008). In this study, the MF treatment did not significantly differ from the MM. However, N_2O emissions were slightly higher under the MF treatment which was similar to the finding of Wei et al., (2020). This may be attributed to application of inorganic fertilizer (Wei et al., 2020). According to Shimizu et al. (2013), CH_4 emission is not associated to air temperature and precipitation but was associated to soil moisture (Shimizu et al., 2013). This may explain why the complexity of CH_4 emissions can be seen with the lack of significance and little to no difference when observing the averages between treatments.

5.3.3. Cumulative CO_2, N_2O and CH_4 fluxes

Cumulative CO_2, N_2O, and CH_4 fluxes for the two years are presented in Table 5.1. Data showed that the treatments did not impact CO_2 fluxes throughout the sampling period in 2020 but significantly impacted CO_2 fluxes in 2021 ($p < .05$; Table 5.1). Cumulative CO_2 fluxes were higher under the MF and MM treatment compared to the CK in 2021. Treatment significantly impacted the N_2O fluxes in both years ($p < .05$; Table 5.1). Cumulative N_2O fluxes were higher under the MF treatment than the MM and CK treatment in 2020. In 2021, the cumulative N_2O fluxes were higher under the MF and MM treatment compared to the CK. Treatment significantly impacted the CH_4 fluxes in 2020, but did not impact CH_4 fluxes in 2021 ($p < .05$; Table 5.1). Cumulative CH_4 fluxes were higher under the MF treatment than the MM and CK treatment in 2020. In general, inorganic fertilizer application significantly increased N_2O and CH_4 fluxes compared to manure application in 2020 (Table 5.1).

Table 5.1. Cumulative greenhouse gas (GHG) fluxes as influenced by long-term manure and inorganic fertilizer management under corn-soybean-spring wheat rotation.

Treatments	**CO_2**		**N_2O**		**CH_4**	
	kg ha^{-1} d^{-1}		**g ha^{-1} d^{-1}**		**g ha^{-1} d^{-1}**	
	2020	**2021**	**2020**	**2021**	**2020**	**2021**
	(Spring wheat)	**(Corn)**	**(Spring wheat)**	**(Corn)**	**(Spring wheat)**	**(Corn)**
	Annual emissions					
MM	$189.47^{a\dagger}$	$229.84^{a\dagger}$	$182.60^{b\dagger}$	$169.59^{a\dagger}$	$100.51^{b\dagger}$	$103.85^{a\dagger}$
MF	221.30^{a}	261.89^{a}	230.99^{a}	157.92^{a}	186.15^{a}	65.17^{a}
CK	121.98^{a}	135.08^{b}	124.58^{b}	62.91^{b}	55.02^{b}	17.13^{a}
	Analysis of variance					
Treatment	0.1547	0.0167	0.0003	0.9657	0.0103	0.1908

† Means within the same column followed by different superscript letters are significantly different at $p \le .05$.

In this study, treatmment did not significantly impact cumulative CO_2 in 2020, and the CK treatment was observed to be significantly lower in 2021 compared to manure and inorganic fertilizer. Similar report were found in (Ding et al., 2007). Also, the cumulative GHG emissions were lower in 2020 under spring wheat rotation + cover crops compared to corn rotation period. This may attributed to the fact that GHG emissions were not totally captured during the growing season in 2020 and was captured in 2021. In 2020, the MF treatment increased cumulative CH_4 emissions compared to the MM and the CK. This may be explained by the fact that manure treatment over a long period of time improves soil porosity and structure which may decrease cumulative CH_4 emissions compared to inorganic fertilizer treatment (Hui et al., 2017). In 2021, treatment did not impact the cumulative CH_4 emissions under corn rotation, this is similar to (Jumadi et al., 2008) finding. They explained that corn field could be a sink for CH_4 gas.

Higher cumulative N_2O emissions were observed under inorganic fertilizer treatment compared to manure treatment in 2020, which was similar to Chirinda et al. (2021) and may be due to the direct nitrogen supply and the effect that inorganic fertilizers have on soil organic matter.

5.3.4. Global warming potential

The global warming potential for N_2O, CH_4, and net global warming potential under the medium applications of manure and inorganic fertilizer rates in 2020 and 2021 are presented in Table 5.2. The MF treatment produced higher $GWP_{(N2O)}$ compared to the MM and CK in 2020. The MF and MM treatments produced significantly higher $GWP_{(N2O)}$ in 2021, compared to the CK (Table 5.2). When observing the $GWP_{(CH4)}$, the MF treatment produced a higher $GWP_{(CH4)}$ compared to the CK in both years. Treatment was found to have significantly impacted $GWP_{(CH4)}$ in both years. In 2020, the MF treatment produced higher $GWP_{(CH4)}$ compared to the MM and CK treatments. In 2021, the MF and MM treatments produced higher $GWP_{(CH4)}$ compared to the CK treatment (Table 5.2).

Table 5.2. The global warming potential for N_2O, CH_4, and net global warming potential as influenced by long-term manure and inorganic fertilizer management under corn-soybean-spring wheat rotation in 2020 and 2021.

Treatments	**GWP$_{(N2O)}$**		**GWP$_{(CH4)}$**		**Net GWP**	
	(kg CO_2-eq ha^{-1} yr^{-1})					
	2020	**2021**	**2020**	**2021**	**2020**	**2021**
	(Spring wheat)	**(Corn)**	**(Spring wheat)**	**(Corn)**	**(Spring wheat)**	**(Corn)**
MM	$3200.98^{b\dagger}$	$2457.66^{a\dagger}$	$167.51^{ab\dagger}$	$85.74^{a\dagger}$	$3368.49^{b\dagger}$	$2543.41^{a\dagger}$
MF	4589.18^{a}	2659.82^{a}	333.58^{a}	136.65^{a}	4922.76^{a}	2796.47^{a}
CK	2901.61^{b}	981.56^{b}	111.69^{b}	22.54^{b}	3013.30^{b}	1004.10^{b}
	Analysis of variance					
Treatment	0.0153	0.0001	0.0761	0.0117	0.0053	0.0022

†Means within the same column followed by different superscript letters are significantly different at $p \leq .05$.

The values of the net GWP for the two years of this study (Table 5.2) were positive for all treatments, indicating that all treatments were GHG sources. The contribution of CH_4 in the net GWP was lower than N_2O. The net GWP for 2020 and 2021 showed that treatments significantly impacted the net GWP (Table 5.2). Where the MF treatment produced higher net GWP compared to the MM and CK treatments in 2020. In 2021, the MF and MM treatments produced higher net GWP compared to the CK treatment (Table 5.2). GWP$_{(N2O)}$ & GWP$_{(CH4)}$ were averagely higher for the MF treatment compared to the MM and CK in both years. This may be because manure enhances carbon sequestration and reduces N_2O which is suggested in reducing the GWP (Kitamura et al., 2021; Mukumbuta & Hatano, 2020). Similar report was found in Owen et al. (2015), where net GWP were lower under the MM treatment compared to the MF.

5.4. Conclusions

A study was conducted in South Dakota to observe the influences of manure and inorganic fertilizers on GHG emissions; methane (CH_4), carbon dioxide (CO_2), and nitrous oxide (N_2O). Results from this study showed the manure and inorganic fertilizer applications did not show significant impacts on the daily average GHG emission. However, the daily average CO_2 emission rate was significantly higher on July 26, 2020 and July 23, 2021. The cummulative soil surface N_2O, and CH_4 fluxes were increased by the MF treatment compared to the MM and CK in 2020. In general, the MF treatment resulted in higher cummulative GHG emissions compared to the MM and CK in both years. Also the global warming potential rate increased in 2020 by inorganic fertilizer rate as compared to the MM and CK. Data from this study showed that inorganic fertilizer application in crops can be harmful to the environment by emitting higher GHG emissions, therefore, sustainable management practices needs to be explored to mitigate GHG emmission and reduce or eliminate the negative environmental impacts.

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CHAPTER 6

CONCLUSION

Soil hydro-physical properties, X-ray computed tomography-derived soil pore characteristics, and greenhouse gas emissions from the soils managed under low manure (LM) contained a quantity of manure rate based on the recommended phosphorous requirement, medium manure (MM) contained a quantity of manure rate based on recommended nitrogen requirement, and high manure (HM) contained a quantity of manure-based on double the recommended nitrogen requirement, two inorganic fertilizer application rate; medium fertilizer (MF) contained the suggested inorganic fertilizer rate, high fertilizer (HF) contained a high fertilizer rate and a control treatment (CK) which did not receive manure and fertilizer, were studied from 2020 through 2023 at two study sites. These study sites are at (i) Felt Research Farm (44˚ 22’ 07.15” N and 96˚ 47’ 26.45” W) near Brookings, South Dakota on a well-drained Vienna soil, and (ii) Southeast Research Farm in Clay County, near Beresford, (43˚ 02’ 33.46” N and 96˚ 53’ 55.78” W) South Dakota, USA. The experiment was a randomized complete block design with four replications. Soils at Brookings and Beresford sites were dominated by Fine-loamy, mixed, frigid Udic Haploborolls and Fine-silty, mixed, mesic Udic Haplustolls, respectively.

The following conclusions were determined from the experimental studies:

***Study 1** – X-ray Computed Tomography-Derived Soil Pore Characteristics*

1. Impact of manure and inorganic fertilizer application on X-ray computed tomography-derived soil pore characteristics were mainly observed at 0 to 20 cm of soil depth.
2. Manure application enhanced soil organic carbon (SOC) content at both studies sites as compared to inorganic fertilizer and control treatments.

***Study 2** – Soil Hydro-physical Properties*

1. Manure application reduced the soil bulk density, increased SOC stock and hydro-physical properties as compared to the inorganic fertilizer rates for either site.
2. Medium manure treatment (MM) impacted the SOC stock and hydro-physical properties for 0-20 cm depth.
3. Soil organic carbon (SOC) stock was positively correlated to plant available water (PAW) content.

***Study 3** – Greenhouse Gas Emissions*

1. Cumulative carbon dioxide soil surface N_2O, and CH_4 fluxes were increased by the MF treatment compared to the MM and CK in 2020. In general, the MF treatment resulted in higher cummulative GHG emissions compared to the MM and CK in both years.
2. The global warming potential rate increased in 2020 by the MF as compared to the MM and CK.

3. Treatment did not significantly impact the daily GHG emission fluxes.

SUMMARY

A study was conducted to investigate the long-term (over 10 years) impacts of different rates of manure and inorganic fertilizer application on soil hydro-physical properties, X-ray computed tomography soil pore characteristics, and surface GHG. This study showed that the MM and HM, in general, reduced the bulk density and enhanced the soil water retention and total porosity at the 0-10 and 10-20 cm depths. The positive effects of the MM and HM on soil hydro-physical properties suggest that the MM and HM can improve the water flow in the soils and can reduce the risks of water erosion. Visualization using X-ray CT and ImageJ processing confirmed that manure treatments improved soil pore characteristics, SOC, and TN contents at shallower depths, stabilizing soil structure and improving water-holding capacity. The long-term nutrient applications had little impact on the measured parameters in the subsurface (20-40 *cm*) depths. The MM may need a longer time to manifest a noticeable change in the measured GHG fluxes. The GHG fluxes are also controlled by soil water content and soil temperature.

In summary, the study emphasizes the intricate relationship between fertilizer types, application rates, and their long-term effects on GHG emissions and soil properties. It underscores the importance of sustainable management practices for mitigating environmental impacts while promoting soil health and crop production.

APPENDICES

Supplementary Table 3.1. Cold water extractable carbon (CWC), hot water extractable carbon (HWC), cold water extractable nitrogen (CWN), and hot water extractable nitrogen (HWN) as influenced by long-term medium fertilizer (MF), high fertilizer (HF), low manure (LM), medium manure (MM), high manure (HM) rate applications, and control (CK) across 0 – 40 cm soil depth.

	CWC **(mg kg^{-1})**				**HWC** (mg kg^{-1})				**CWN** (mg kg^{-1})				**HWN** (mg kg^{-1})			
	0-10	10–20	20-30	30-40	0-10	10–20	20-30	30-40	0-10	10–20	20-30	30-40	0-10	10–20	20-30	30-40
	-----------*Depth(cm)*-----------				------------*Depth(cm)*----------				-----------*Depth(cm)*------------				------------*Depth(cm)*------------			
Brookings Site																
Treatments																
CK	$57.2^{cd\dagger}$	$39.4^{d\dagger}$	$41.5^{c\dagger}$	$38.5^{bc\dagger}$	$144.1^{bc\dagger}$	$81.7^{d\dagger}$	$64.4^{b\dagger}$	$59.4^{b\dagger}$	$4.6^{a\dagger}$	$3.5^{bc\dagger}$	$2.9^{\dagger}$	$2.1^{b\dagger}$	$10.3^{cd\dagger}$	$5.5^{bc\dagger}$	$4.3^{ab\dagger}$	$3.6^{b\dagger}$
MF	54.4^{d}	38.1^{d}	41.7^{c}	38.0^{c}	129.7^{c}	75.3^{d}	68.6^{ab}	60.8^{b}	5.0^{b}	3.3^{c}	2.8	2.1^{b}	9.0^{d}	4.8^{c}	4.3^{ab}	3.7^{b}
HF	52.8^{d}	45.8^{c}	43.3^{bc}	39.8^{bc}	140.8^{bc}	84.0^{cd}	59.2^{c}	61.6^{ab}	4.8^{b}	3.9^{abc}	2.3	2.1^{b}	10.6^{bc}	6.4^{ab}	3.8^{b}	3.9^{b}
LM	63.9^{bc}	51.8^{ab}	49.9^{a}	39.7^{bc}	155.1^{b}	96.2^{ab}	71.5^{a}	65.2^{ab}	4.6^{a}	4.8^{ab}	2.8	2.2^{ab}	11.4^{bc}	5.6^{abc}	4.2^{ab}	3.8^{b}
MM	70.6^{ab}	49.9^{b}	48.4^{a}	47.6^{a}	149.3^{b}	91.3^{bc}	71.1^{a}	68.0^{a}	5.5^{a}	4.1^{abc}	2.9	2.9^{a}	11.9^{b}	5.7^{abc}	4.5^{ab}	4.8^{a}
HM	76.5^{a}	54.2^{a}	46.5^{ab}	45.8^{a}	188.6^{a}	103.0^{a}	67.1^{ab}	64.4^{ab}	6.6^{ab}	4.9^{a}	2.6	2.1^{ab}	13.9^{a}	7.0^{a}	4.5^{a}	3.8^{b}
Analysis of variance (p > F)																
	<0.01	<0.01	<0.01	0.02	<0.01	<0.01	<0.01	0.01	0.01	0.01	0.09	0.03	<0.01	0.02	0.05	<0.01
Beresford Site																
Treatments																
CK	62.0	43.5^{b}	52.9^{b}	37.6^{b}	172.9^{b}	88.3^{b}	72.2^{b}	52.6^{b}	3.8^{a}	4.6^{a}	2.7^{b}	1.5^{c}	11.2^{b}	5.8^{b}	4.3^{b}	2.9^{ab}
MF	71.1	37.5^{b}	43.7^{b}	40.8^{b}	190.5^{ab}	71.3^{b}	63.0^{b}	46.4^{b}	5.2^{a}	2.4^{a}	2.4^{b}	1.8^{bc}	12.3^{b}	4.5^{b}	3.5^{b}	2.3^{c}
HF	66.8	43.8^{b}	48.8^{b}	39.8^{b}	173.0^{b}	92.9^{b}	79.1^{b}	49.6^{a}	4.2^{a}	2.6^{a}	2.6^{b}	1.7^{bc}	11.4^{b}	5.9^{b}	4.7^{ab}	2.6^{bc}
LM	84.4	54.6^{ab}	50.4^{b}	55.8^{a}	228.2^{ab}	104.8^{b}	78.2^{ab}	66.0^{a}	6.0^{a}	3.4^{a}	2.8^{b}	2.4^{ab}	15.4^{ab}	6.6^{b}	5.0^{ab}	3.8^{ab}
MM	81.9	49.2^{b}	51.9^{b}	42.3^{ab}	220.1^{ab}	98.7^{b}	72.1^{b}	60.9^{ab}	6.5^{a}	3.1^{a}	2.9^{b}	2.0^{bc}	15.3^{ab}	6.6^{b}	4.5^{b}	3.2^{abc}
HM	95.0	74.5^{a}	78.3^{a}	48.9^{ab}	287.4^{a}	149.6^{a}	102.6^{a}	64.7^{a}	9.5^{a}	5.6^{a}	4.9^{a}	2.7^{a}	22.6^{a}	10.5^{a}	6.7^{a}	4.2^{a}
Analysis of variance (p > F)																
	0.07	<0.01	<0.01	0.01	0.03	<0.01	0.01	0.04	0.08	0.06	<0.01	<0.01	0.01	<0.01	0.01	0.01

†Means with different letters within the same column for individual sites are significantly different for different treatments or depths at $p < 0.05$.

Supplementary Table 3.2. Macroporosity, coarse mesoporosity, and fine mesoporosity as influenced by long-term medium fertilizer (MF), high fertilizer (HF), low manure (LM), medium manure (MM), high manure (HM) rate applications, and control (CK) across 0 – 40 cm soil depth.

	Macroporosity (>500 µm)				**Coarse mesoporosity (60 – 500 µm)**				**Fine mesoporosity (10 – 60 µm)**			
	0-10	10-20	20-30	30-40	0-10	10-20	20-30	30-40	0-10	10-20	20-30	30-40
	-----------*Depth(cm)*-----------				-----------*Depth(cm)*-----------				-----------*Depth(cm)*-----------			
	Brookings site											
Treatments												
CK	0.027^{a}[†]	0.005^{ab}[†]	0.011^{z}[†]	0.007^{a}[†]	0.075^{a}[†]	0.017^{b}[†]	0.036^{c}[†]	0.091^{a}[†]	0.05^{b}[†]	0.09^{a}[†]	0.03^{c}[†]	0.06^{a}[†]
MF	0.011^{a}	0.003^{b}	0.012^{a}	0.011^{a}	0.037^{a}	0.043^{ab}	0.021^{c}	0.053^{b}	0.13^{a}	0.13^{a}	0.06^{ab}	0.08^{a}
HF	0.008^{a}	0.005^{ab}	0.004^{a}	0.008^{a}	0.048^{a}	0.078^{a}	0.044^{bc}	0.056^{ab}	0.09^{ab}	0.09^{a}	0.07^{a}	0.08^{a}
LM	0.011^{a}	0.009^{ab}	0.004^{a}	0.002^{a}	0.039^{a}	0.047^{ab}	0.066^{b}	0.046^{b}	0.08^{ab}	0.09^{a}	0.03^{bc}	0.10^{a}
MM	0.013^{a}	0.003^{b}	0.004^{a}	0.004^{a}	0.055^{a}	0.046^{ab}	0.096^{a}	0.049^{b}	0.05^{b}	0.13^{a}	0.07^{a}	0.06^{a}
HM	0.012^{a}	0.029^{a}	0.009^{a}	0.003^{a}	0.081^{a}	0.057^{ab}	0.048^{bc}	0.041^{b}	0.05^{ab}	0.07^{a}	0.07^{a}	0.07^{a}
	Analysis of Variance (P>F)											
	0.44	0.03	0.54	0.08	0.16	0.01	<0.01	0.01	0.02	0.06	<0.01	0.41
	Beresford site											
Treatments												
CK	0.026^{a}	0.012^{a}	0.017^{a}	0.012^{ab}	0.040^{b}	0.063^{a}	0.047^{a}	0.047^{a}	0.23^{a}	0.21^{a}	0.23^{a}	0.21^{ab}
MF	0.026^{a}	0.013^{a}	0.020^{a}	0.014^{ab}	0.089^{a}	0.030^{a}	0.051^{a}	0.048^{a}	0.15^{b}	0.25^{a}	0.25^{a}	0.19^{ab}
HF	0.008^{a}	0.015^{a}	0.022^{a}	0.001^{b}	0.057^{ab}	0.053^{a}	0.038^{a}	0.059^{a}	0.24^{a}	0.26^{a}	0.24^{a}	0.16^{b}
LM	0.015^{a}	0.014^{a}	0.005^{a}	0.023^{a}	0.087^{a}	0.067^{a}	0.077^{a}	0.037^{a}	0.21^{ab}	0.23^{a}	0.24^{a}	0.24^{a}
MM	0.018^{a}	0.012^{a}	0.019^{a}	0.020^{ab}	0.097^{a}	0.047^{a}	0.078^{a}	0.049^{a}	0.21^{ab}	0.21^{a}	0.21^{a}	0.23^{a}
HM	0.020^{a}	0.017^{a}	0.024^{a}	0.007^{ab}	0.070^{ab}	0.054^{a}	0.030^{a}	0.060^{a}	0.19^{ab}	0.20^{a}	0.23^{a}	0.23^{a}
	Analysis of Variance (P>F)											
	0.11	0.94	0.10	0.02	<0.01	0.20	0.13	0.22	0.01	0.04	0.78	0.01

[†] Means with different letters within a column for individual sites are significantly different for different treatments or depths at $p \leq 0.05$

Supplementary Table 3.3. Coarse microporosity (plant available water, PAW), microporosity, and total porosity as influenced by long-term medium fertilizer (MF), high fertilizer (HF), low manure (LM), medium manure (MM), high manure (HM) rate applications, and control (CK) across 0 – 40 cm soil depth.

	Coarse mesoporosity (PAW; 0.2 - 10 µm)				**Microporosity (>0.2 µm)**				**Total porosity**			
	0-10	10-20	20-30	30-40	0-10	10-20	20-30	30-40	0-10	10-20	20-30	30-40
	-----------Depth(cm)-----------				*-----------Depth(cm)-----------*				*-----------Depth(cm)-----------*			
					Brookings site							
Treatments												
CK	0.10^{c}†	0.12^{b}†	0.14^{a}†	0.07^{bc}†	0.22^{d}†	0.24^{b}†	0.28^{bc}†	0.17^{b}†	0.10^{c}†	0.12^{b}†	0.14^{a}†	0.07^{bc}†
MF	0.13^{bc}	0.13^{b}	0.18^{a}	0.04^{c}	0.31^{cd}	0.23^{b}	0.30^{ab}	0.17^{b}	0.13^{bc}	0.13^{b}	0.18^{a}	0.04^{c}
HF	0.18^{abc}	0.11^{b}	0.14^{a}	0.04^{c}	0.31^{bc}	0.21^{b}	0.26^{c}	0.15^{b}	0.18^{abc}	0.11^{b}	0.14^{a}	0.04^{c}
LM	0.21^{ab}	0.19^{a}	0.19^{a}	0.15^{ab}	0.35^{ab}	0.31^{a}	0.31^{ab}	0.25^{ab}	0.21^{ab}	0.19^{a}	0.19^{a}	0.15^{ab}
MM	0.24^{a}	0.17^{a}	0.17^{a}	0.18^{a}	0.37^{a}	0.29^{a}	0.28^{bc}	0.29^{a}	0.24^{a}	0.17^{a}	0.17^{a}	0.18^{a}
HM	0.20^{abc}	0.19^{a}	0.19^{a}	0.21^{a}	0.39^{a}	0.33^{a}	0.32^{a}	0.32^{a}	0.20^{abc}	0.19^{a}	0.19^{a}	0.21^{a}
					Analysis of Variance (P>F)							
	<0.01	<0.01	0.03	<0.01	<0.01	<0.01	<0.01	0.01	<0.01	<0.01	0.03	<0.01
					Beresford site							
Treatments												
CK	0.11^{a}	0.08^{a}	0.05^{a}	0.08^{ab}	0.19^{b}	0.19^{ab}	0.17^{a}	0.19^{ab}	0.49^{b}	0.48^{b}	0.46^{abc}	0.46^{ab}
MF	0.11^{a}	0.10^{a}	0.07^{a}	0.07^{ab}	0.23^{ab}	0.20^{ab}	0.16^{a}	0.17^{ab}	0.49^{b}	0.49^{ab}	0.45^{bc}	0.42^{bc}
HF	0.10^{a}	0.06^{a}	0.05^{a}	0.04^{b}	0.21^{ab}	0.18^{ab}	0.16^{a}	0.16^{b}	0.52^{ab}	0.51^{ab}	0.43^{c}	0.39^{c}
LM	0.12^{a}	0.08^{a}	0.05^{a}	0.07^{ab}	0.24^{ab}	0.20^{ab}	0.19^{a}	0.18^{ab}	0.55^{a}	0.51^{a}	0.49^{ab}	0.48^{ab}
MM	0.13^{a}	0.09^{a}	0.09^{a}	0.08^{a}	0.24^{ab}	0.24^{a}	0.20^{a}	0.20^{a}	0.56^{a}	0.51^{a}	0.51^{a}	0.51^{a}
HM	0.14^{a}	0.10^{a}	0.09^{a}	0.05^{ab}	0.25^{a}	0.22^{ab}	0.20^{a}	0.17^{ab}	0.53^{ab}	0.50^{ab}	0.48^{ab}	0.48^{ab}
					Analysis of Variance (P>F)							
	0.21	0.51	0.15	0.03	0.03	0.02	0.09	0.03	0.21	0.51	0.15	0.03

†Means with different letters within a column for individual sites are significantly different for different treatments and depths at $p \leq 0.05$.

Supplementary Table 3.4. Soil water retention curve at its inflection point (|S|), van Genutchen's parameter α and *n* as influenced by long-term medium fertilizer (MF), high fertilizer (HF), low manure (LM), medium manure (MM), high manure (HM) rate applications, and control (CK) across 0 – 40 cm soil depth.

	|S| cm^3 cm^{-3}				**α**				***n***			
	0-10	10–20	20-30	30-40	0-10	10–20	20-30	30-40	0-10	10–20	20-30	30-40
	-----------*Depth(cm)*-----------				------------*Depth(cm)*----------				-----------*Depth(cm)*------------			
					Brookings Site							
Treatments												
CK	$0.05^{\dagger}$	$0.08^{ab\dagger}$	$0.06^{ab\dagger}$	$0.05^{\dagger}$	$0.127^{a\dagger}$	$0.007^{c\dagger}$	$0.011^{b\dagger}$	$0.049^{a\dagger}$	$1.41^{\dagger}$	$1.81^{a\dagger}$	$1.54^{\dagger}$	$1.50^{b\dagger}$
MF	0.08	0.09^{ab}	0.07^{ab}	0.07	0.051^{ab}	0.012^{c}	0.010^{b}	0.031^{ab}	1.85	1.66^{ab}	1.59	1.84^{b}
HF	0.11	0.07^{b}	0.06^{ab}	0.07	0.008^{b}	0.029^{a}	0.013^{b}	0.032^{ab}	1.81	1.49^{c}	1.50	2.26^{ab}
LM	0.11	0.09^{ab}	0.06^{ab}	0.08	0.006^{b}	0.010^{c}	0.013^{b}	0.012^{b}	1.88	1.55^{bc}	1.45	1.57^{a}
MM	0.10	0.10^{a}	0.07^{b}	0.07	0.008^{b}	0.010^{c}	0.030^{a}	0.011^{b}	1.65	1.66^{ab}	1.39	1.52^{ab}
HM	0.08	0.07^{b}	0.09^{a}	0.09	0.026^{b}	0.021^{b}	0.010^{b}	0.006^{b}	1.51	1.40^{c}	1.54	1.59^{b}
					Analysis of variance (p > F)							
	0.13	0.01	0.06	0.74	0.01	<0.01	<0.01	<0.01	0.28	<0.01	0.30	0.21
					Beresford Site							
Treatments												
CK	0.15^{a}	0.14	0.17	0.14	0.013^{b}	0.015^{a}	0.014	0.013^{ab}	1.93^{ab}	1.97	2.35	2.05
MF	0.10^{b}	0.18	0.18	0.13	0.029^{ab}	0.011^{b}	0.016	0.014^{ab}	1.63^{b}	2.34	2.27	2.08
HF	0.15^{a}	0.19	0.18	0.12	0.013^{b}	0.014^{ab}	0.014	0.016^{a}	2.01^{a}	2.37	2.50	2.22
LM	0.14^{ab}	0.15	0.17	0.16	0.019^{ab}	0.015^{a}	0.016	0.013^{b}	1.76^{ab}	2.02	2.30	2.20
MM	0.14^{ab}	0.15	0.14	0.15	0.021^{ab}	0.013^{ab}	0.020	0.014^{ab}	1.70^{ab}	2.12	1.93	2.03
HM	0.12^{ab}	0.13	0.15	0.17	0.018^{a}	0.015^{a}	0.012	0.014^{ab}	1.65^{b}	1.92	2.07	2.34
					Analysis of variance (p > F)							
	0.01	0.07	0.54	0.12	<0.01	0.01	0.37	0.06	0.01	0.31	0.28	0.40

†Means with different letters within a column for individual sites are significantly different for different treatments and depths at $p \leq 0.05$.

VITAE

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