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Long-Term Impacts of Manure and Inorganic Fertilization on Soil Physical, Chemical and Biological Properties

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LONG-TERM IMPACTS OF MANURE AND INORGANIC FERTILIZATION ON SOIL PHYSICAL, CHEMICAL AND BIOLOGICAL PROPERTIES

BY

ASMITA GAUTAM

A thesis submitted in partial fulfillment of the requirement for the

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THESIS ACCEPTANCE PAGE

Asmita Gautam

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

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(Asmita Gautam)

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ABBREVIATIONS

AMF	Arbuscular mycorrhizal fungi
C	Carbon
CO_2	Carbon dioxide
CWC	Cold water extractable carbon
CWN	Cold water extractable nitrogen
fPOM	Fine particulate organic matter
HWC	Hot water extractable carbon
HWN	Hot water extractable nitrogen
MBC	Microbial biomass carbon
MBN	Microbial biomass nitrogen
MWD	Mean weight diameter
N	Nitrogen
NH_3	Nitrate
NH_4	Ammonium
OM	Organic matter
P	Phosphorus
PLFA	Phospho-lipid fatty acid
POM	Particulate organic matter
SMAF	Soil management assessment framework
SOC	Soil organic carbon
SOM	Soil organic matter
SQ	Soil quality
SQI	Soil quality index
TN	Total nitrogen
WSA	Water-stable aggregate

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ABSTRACT

LONG-TERM IMPACTS OF MANURE AND INORGANIC FERTILIZATION ON SOIL PHYSICAL, CHEMICAL AND BIOLOGICAL PROPERTIES

ASMITA GAUTAM

2019

The intensive use of mineral fertilizers to achieve high crop yield has led to soil degradation and poor soil health. Thus, manure application as an alternative to mineral fertilizers can be an effective fertilization strategy to improve soil health and biodiversity. This study aims to assess the impacts of long-term manure and mineral fertilizers on soil physical, chemical and biological properties. The experimental site was initiated in 2003 near Beresford, South Dakota on Egan soil under a randomized complete block design with four replications and six treatments. The study treatments included: three manure rates [low manure (LM), manure application based on the phosphorous requirement; medium manure (MM), manure application based on nitrogen requirement; high manure (HM), two times prescribed nitrogen rate], two chemical fertilizer rates [medium fertilizer (MF), recommended inorganic fertilizer rate; high rate of the fertilizer (HF)], and control (CK, without any manure or fertilizer application). Data from this study showed that bulk density under HM was 19 and 9% lower compared to the CK in 2018 and 2019, respectively. Data from 2019 showed that manure application significantly increased soil wet aggregate stability (0-10 cm) compared with the CK. Particulate organic matter (POM) and soil organic matter (SOM) were increased with manure application compared with the CK. However, inorganic fertilizer application did not impact organic matter components. The HM treatment significantly increased urease, β-glucosidase, and alkaline phosphatase enzyme activities, and soil microbial community PLFA biomass for the 0-10 cm depth as compared to those with CK treatment in 2018. Similar trend was observed for 2019. However, both fertilizer rates (MF and HF) did not show any differences in microbial community for either depth. Carbon and nitrogen fractions were significantly increased with HM treatment but remained unaffected with mineral fertilization. Cold water nitrogen (CWN) was increased under MF treatment as compared to the CK for 0-10 cm soil depth, whereas, both MF and HF increased CWN by 121 and 86%, respectively, for 10-20 cm depth in 2018. Soil quality index (SQI) was higher for the HM treatment as compared to the CK and fertilizer treatments in 0-10 cm and 10-20 cm soil depths, which indicate that manure application improves the soil quality. However, fertilizer treatments did not impact SQI. This study concluded that application of manure for long-term enhances the soil physical, chemical and biological properties as compared to the inorganic fertilizers, however, further study needed which can monitor the environmental impacts that include water quality and greenhouse gas emissions associated with the manure application.

CHAPTER 1

INTRODUCTION

Agriculture intensification with the heavy use of chemicals has negative impact on soils and the environment, and serves as threat to sustainability (Tilman et al., 2011). The overuse of fertilizers not only decrease the fertilizer use efficiency, but also led to degradation of environment through nutrients runoff and biodiversity loss (Li et al., 2015; Zhang et al., 2012). Mineral fertilizer application decreases the soil pH (Cai et al., 2015) which could alter the nutrient availability. Since mineral fertilizer fastens the mineralization of soil organic carbon (SOC), therefore, continuous use of these fertilizers can deplete SOC (Ju et al., 2009). Mineral fertilization can also negatively impact the microbial diversity by altering the bacterial and fungal population (Zhang et al., 2012). Replacement of mineral fertilizer with manure can be an alternative of mineral fertilizer application which can enhance soil quality (Jiang et al., 2018; Martínez et al., 2017). Higher mineral fertilizer application can be expensive and also can increase the nitrogen (N) loss when compared to manure application (Martínez et al., 2017).

Manure application helps to increase soil quality and productivity by its favourable effect on soil properties (Martínez et al., 2017; Ozlu and Kumar, 2018). Several studies on manure application reported soil organic matter (SOM) restoration and maintaining the soil quality (Benbi et al., 2018; Bending et al., 2004; Ye et al., 2019). SOM restoration has beneficial effects on soil structure and aggregate stability, preventing soil degradation (Lal et al., 2016), and sustaining the productivity of agroecosystem (Ding et al., 2012). The increase in SOM can enhance aggregate stability, improve soil structure (Are et al., 2018), increase nutrient cycling, microbial diversity, microbial biomass, and enzyme activities (Lupwayi et al., 2019; Weitao et al., 2018). Long-term manure application increases enzyme activities, and the legacy effect of manure was observed even 29 years after manure application (Lupwayi et al., 2019). There are other various benefits of manure application including improvement in soil physical, chemical (Cai et al., 2019) and biological properties (Lupwayi et al., 2019). Manure application enhances soil structure leading to higher porosity, water holding capacity and water infiltration, as well as maintaining the soil pH (Ozlu and Kumar, 2018). Therefore, manure application is being promoted as an alternative to the heavy chemical fertilization to enhance agricultural sustainability (Gai et al., 2019), and to restore SOC and N pools and improve soil aggregation (Choudhary et al., 2018).

Manure application also results in accumulations of SOC, which contributes to the formation of soil macro-aggregates (Annabi et al., 2011; Are et al., 2018; Zou et al., 2018). Manure amendments aggregate soil particles together to form well aggregated soils as compared to the fertilizer application (Ozlu and Kumar, 2018). Whereas, the decrease in aggregate stability with increase in rate of N fertilizer application was also reported (Brtnický et al., 2017). Manure application helps to maintain the soil pH, whereas, mineral fertilizer decreases the soil pH (Cai et al., 2015). Increase in SOM through manure application results in the decrease in soil compaction and decrease in bulk density (Guo et al., 2016). Some studies have also shown that increase in SOM improves soil aggregations and lowers the bulk density and the degree of compaction (Leroy et al., 2008). Increase in SOC can contribute to increase microbial biomass and can change community structure (Peacock et al., 2001). Manure application increases microbial biomass carbon (MBC), microbial biomass nitrogen (MBN) and enzyme activities involved in C, P, and N cycling (Li et al., 2015). Abbasi and Khizar (2012) reported decrease in MBC with the addition of urea, whereas, use of poultry manure as soil amendments resulted increase in MBC.

However, improper nutrient management practices are one of the reasons for further degradation in soil through decline in SOC (Ju et al., 2009). Animal manure contains substantial but variable quantities of macro and micronutrients those needed for the plant growth. The N:P ratio of manure is generally lower than that of crop uptake (Eghball, 2002). Therefore, it is difficult to meet all the nutrient demand on a recommended level. The N-based manure management often oversupplies the crop-soil system with P, which can contribute to eutrophication of water bodies through nitrate leaching and accumulation in water bodies (Sileshi et al., 2019). Higher long-term manure application could also have a loose structure due to monovalent cations like sodium ion (Na^+) present in animal manure, which acts as dispersing agent to break the structure (Bronick and Lal, 2005). Therefore, the long-term study the effect of different rates of manure and fertilizers on important soil physical, chemical and microbial properties is important.

Study Objectives

Differences in the rate of application over a long-term could have different impact on the performance of different soil physical, chemical and microbial properties. Therefore, purpose of this study was to understand the influences of different rates of manure and fertilizer application on soil health. The study was divided into two separate objectives, and those are listed below as:

Study 1. To assess long-term impact of manure application and fertilization on soil aggregate stability, soil organic carbon, and nitrogen in different aggregate fractions.

Study 2. To study long-term impact of manure and mineral fertilizer application on soil microbial properties and overall soil health.

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CHAPTER 2

LITERATURE REVIEW

Agricultural management practices can have diverse impact on soil health (Bai et al., 2018). Therefore, understanding the impacts of different management practices on soils is important (Lal, 2015). The management of agricultural systems affects soil quality and structure through fertilization, tillage, cover crops, crop rotation and other practices (Garcia-Franco et al., 2015; Madari et al., 2005). Among these different management systems, fertilizer management practices such as manure and inorganic fertilizers are commonly studied (Cai et al., 2019; Geisseler and Scow, 2014). Manures impacts soils by influencing especially the soil organic carbon (Ye et al., 2019). The application of manure and chemical fertilizers can improve soil properties and provide various additional benefits to enhance the soil quality (Choudhary et al., 2018). The present review will focus on investigating the impacts of manure and inorganic fertilizer application on soil health.

2.1 Manure application in agroecosystems

Manure application, when managed properly, improves soil fertility and crop yield through enhancing the soil organic matter (SOM) and other soil properties (Benbi et al., 2018; Patel et al., 2015). There are various benefits of manure application that include: soil physical, chemical (Cai et al., 2019) and biological (Lupwayi et al., 2019) properties. The increase in SOM can lead to higher aggregate stability, improved structure (Are et al., 2018), increase in nutrient cycling, microbial diversity, microbial biomass, and enzyme activities (Lupwayi et al., 2019; Weitao et al., 2018). Manure application enhances soil porosity, water holding capacity, and water infiltration (Ozlu and Kumar, 2018). Manure application also improves soil fertility supplying macro and micronutrients (Cai et al., 2019; Ozlu et al., 2019). However, excess manure application can have detrimental effects on the environment (Parchomenko and Borsky, 2018; Sharpley et al., 1994). The N:P ratio of manure is generally lower than that of crop uptake (Eghball, 2002). Therefore, it is difficult to meet all the nutrient demand on the recommended level. The N-based manure management often oversupplies the crop-soil system with P, which can be lost into the environment and contribute to eutrophication of water bodies through nitrate leaching and accumulation in water bodies (Sileshi et al., 2019). Heavy metal accumulations is a major drawback of excess manure application. The higher concentration of monovalent cations (Na^+ and K^+) in the animal manure can break down the soil colloids resulting the breakdown of aggregates (Guo et al., 2019). The optimum rate of manure application is important for a sustainable agroecosystem, and there is a need to apply manure rates based on phosphorus. Therefore, it can be summarized that application of manure should be at recommended rates and nutrient based according to analysis of soil and manure under consideration of yield, management practices and environmental risks.

2.2 Mineral fertilizer application in agroecosystems

Inorganic fertilization application is common nutrient management practice for enhancing soil fertility and crop yield (Geisseler and Scow, 2014). However, the overuse of fertilizers can led to the degradation of environment through nutrient runoff and biodiversity loss (Li et al., 2015; Zhang et al., 2012). Chemical fertilizer application could lead to SOC losses due to increase SOC mineralization and reduce aggregate stability (Le Guillou et al., 2011). The decrease in aggregate stability with the increase in rate of N fertilizer application was reported by Brtnický et al. (2017). The replacement of mineral fertilizer with manure can manage deposition of animal waste, while also provide an opportunity to improve soil quality (Jiang et al., 2018; Ozlu and Kumar, 2018). Therefore, previous studies suggest that understanding the response of different application rate to different soil properties is important.

2.3 Manure and fertilizer impacts on soil properties

2.3.1 Aggregate stability and soil structure

Soil water-stable aggregate (WSA) stability has been widely used as an important indicator to evaluate soil health. It is also an indicator to detect the response of soils to agronomic management and environmental change (Wang et al., 2016). Soil aggregates are the main components of soil structures, and their characteristics create the physical environment enabling or disabling connections for C stabilization or loss (Kravchenko and Guber, 2017). A well-aggregated soil favours optimum condition for crop growth by maintaining good aeration through well-balanced soil pore-size distribution containing air and water. Application of organic manure, in general, increases SOC (Ozlu and Kumar, 2018), hence, increases the stability of soil aggregates. Soil aggregates stabilize SOC against rapid mineralization, by making it inaccessible to microorganisms through several mechanisms like physical entrapment of C (within macro- and micro- aggregates), chemical protection (through adsorption), biological stabilization (by recalcitrance transformation and condensation reactions within aggregates) (Lal, 2004). These macro aggregates generally have more C than the micro aggregates, and macro aggregate-associated C have less mean residence time compared with the micro aggregate-associated C.

The improvement of soil structure through addition of organic manure can lead to a high degree of aggregation and a large portion of macro-aggregates, which remain stable when wetted (Tisdall and Oades, 1982). Soils with good structure, generally provides suitable soil physical properties including a high water-holding capacity, moderate saturated hydraulic conductivity, and sufficient aeration for plant establishment and growth (Bronick and Lal, 2005). Reduced aggregate stability may decrease rate of water infiltration and crop production and increase slaking and crusting, and runoff erosion. Applying organic manure can also improve water storage, restore C, sources of biodiversity, and prevent soil degradation (Lal et al., 2016). The SOM serves as a major binding agent of mineral particles into aggregates while on the other hand soil aggregates protect SOM from rapid decomposition by microorganisms and act as a storage for C and other key important soil nutrients (Are et al., 2018). SOM further stimulates the activities of the soil biota and maintain physiochemical conditions of the soil such as cation exchange capacity (CEC) and pH. The negative correlation ($r=-0.16$) between increased N fertilizer application rates and WSA in study conducted by Are et al. (2018) indicates that SOC mineralization is enhanced by higher N fertilizer application and leads to lower SOC stabilization. However, manure application resulted on increased WSA stability corresponding to higher SOC content (Are et al., 2018). Zou et al. (2018) reported that crop rotation and manure amendment increased macroaggregate (>250 μm) proportion and geometric mean diameter and decreased the proportion of microaggregates and silt-clay sized fractions (<250 μm) compared to monoculture crop and conventional fertilizer management. Therefore, previous studies suggest that understanding the response of different application rates of manure and fertilizers to different aggregate stability and aggregate associated carbon is important.

2.3.2 Soil organic matter (SOM) components

The SOM impacts physical, chemical and biological properties of soil, and is a key indicator of soil health (Riley et al., 2008). The SOM restoration has beneficial effects on aggregate stability, soil properties and crop production (Karami et al., 2012). Several studies found the strong correlation among soil aggregate stability, soil structure and SOM contents (Darwish et al., 1995; Haynes and Naidu, 1998). Data on SOM quantity and quality are therefore important for agricultural sustainability. Continuous cultivation without organic inputs caused significant losses of SOM (Mando et al., 2005).

Soil organic carbon plays a crucial role in maintaining agriculture productivity by enhancing soil physical chemical and biological properties (Stockmann et al., 2013). Soil is the largest carbon reservoir in the terrestrial ecosystems (Lal, 2004). SOC sequestration is important for mitigating the effects of greenhouse gases and possibility of agricultural soils to store surplus atmospheric carbon (Lal, 2013). The physical protection of SOC is affected by aggregate sizes, those play an important role in equilibrium of carbon pool (Zheng et al., 2017). Manure and fertilization can be recognized as important agricultural measures to restore the SOC pool to an optimum level (Lal et al., 2016).

2.3.3 Enzyme activities

Soil microorganisms play a major role in soil nutrient cycling through biochemical processes by decomposing organic compounds. They are sensitive to management practices (Bending et al., 2004), and are also a good indicator of soil health (Bünemann et al., 2018). However, less is understood about enzyme activities and microbial community under the different levels of long-term manure and inorganic fertilizer application rates. Soil enzymes, each with a specific biochemical action, mediate biochemical processes and considered as the indicators of the ability of soils to perform biochemical functions and reactions (Nannipieri et al., 2018). Manure effects on soil enzyme activities have been reported in many studies, and results usually show enhanced enzyme activities with the manure application (Benbi et al., 2018; Li et al., 2015; Lupwayi et al., 2019).

β-Glucosidase is an important enzyme in carbon cycle which is produced mainly by saprotrophic microorganisms such as bacteria and fungi. This enzymes is also present in root exudates and in the gut of soil fauna (Lammirato et al., 2010), which breaks β-D-glucosidic linkages in glucose-substituted molecules or disaccharide such as cellobiose. β-Glucosidase activity has been found to be sensitive to soil management and is an early indication of changes in organic matter status and its turnover (Mariscal-Sancho et al., 2018; Stege et al., 2010). Researchers found higher enzyme activities in organic management as compared to the conventional management (Benbi et al., 2018). β-glucosidase activity was found to be enhanced by more than 200% in the organic amended soil as compared to the non-amended soil (Medina et al., 2004). Manure application increases soil organic C, and hence glucosidase activity was enhanced with the increase in total organic C (Ma et al., 2010).

Urease activity is often used to represent organic N mineralization (Nannipieri et al., 2018). It acts as a catalyst for hydrolysis of urea and urea-associated compound into CO_2 and NH_3 (Das and Varma, 2010). It originates from microorganisms, and presence of urea and alternative N sources enhance the urease activity, whereas, presence of NH_4^+

in the cell of microorganisms depress the urease enzyme production (Geisseler et al., 2010). The increase in urease activity under manure application shows the close relationship of this enzyme with the soil organic matter and N cycling. However, the decrease in activity of urease in soils with long-term nitrogen fertilization was a result of the absorption of mineral N by soil microorganisms because of higher accumulation of ammonia as metabolites (Konig et al., 1966). A meta-analysis observed that there is no any impact of mineral fertilizer on urease activity (Geisseler and Scow, 2014).

Phosphatases enzymes (acid and alkaline) play a major role in P cycling for release of bioavailable inorganic phosphorus (P) from organic form of P in soil (Nannipieri et al., 2011). Phosphatases in soil are regulated by a combination of factors such as chemical and physical soil properties and organic P mineralization processes (Nannipieri et al., 2011). Addition of nutrients irrespective of the forms affects soil chemical and biological processes over time. Long-term manure application increases enzyme activities, and legacy effect of manure was observed even 29 years after manure application (Lupwayi et al., 2019). Therefore, understanding the response of different rate of manure and fertilization to enzyme activities is important.

2.3.4 Microbial properties

Soil microorganisms play an important role in soil biological processes. Microbial diversity is one of the most important microbial parameters in the soil (Li et al., 2015; Zhong and Cai, 2007). They are the critical factors that determine soil organic matter decomposition, and nutrient cycling. Several studies have reported that fertilizer management affects microbial diversity (Böhme et al., 2005; Zhang et al., 2012). Several studies have documented the effects of nutrient management on microbial community composition using PLFA analysis (Böhme et al., 2005; Lupwayi et al., 2018; Weitao et al., 2018). Manure application enhanced PLFA and enzyme activities, whereas, nitrogen fertilizer had no effect (Lupwayi et al., 2018). Manure application to the soil can increase microbial biomass and led to changes in community structure (Peacock et al., 2001). Inorganic fertilizer effects on soil microorganisms and enzyme activities are variable. They can have positive effect directly because of nutrients being added to the soil (Lupwayi et al., 2012) as well as indirect positive effect because of increased root exudates by crops or crop biomass which adds organic C (Geisseler and Scow, 2014). In contrast, inorganic fertilization can have direct negative effect due to acidification, leading to changes in soil microbial community composition (Peacock et al., 2001).

2.3.5 Carbon and nitrogen pools

Cold (CWC) and hot (HWC) water extractable organic carbon are the most important active pools of soil organic matter (Tobiašová et al., 2016) and play an important role in global C cycling (Bu et al., 2011). The CWC concentration is smaller than the other labile forms of C but it is the primary source of energy for soil microorganisms, and control nutrients turnover as well as plays important role in determining soil physical, chemical and biological properties (Gong et al., 2009).

Microbial biomass of carbon (MBC) is a living active component of SOC, and is an important attribute of soil quality. The MBC serves as a sensitive indicator of changes and future trends in organic C (da Silva et al., 2012). Manure application increased MBC, MBN and enzyme activities those involved in C, P, and N cycling (Li et al., 2015). A decrease in MBC with the addition of urea, whereas, an increase in MBC with the addition of poultry manure was reported by Abbasi and Khizar (2012). A meta-analysis study conducted by Treseder (2008) reported that N-fertilization results in 9% decrease in the microbial biomass. Another meta-analysis by Geisseler and Scow (2014) reported that 15.1% increase in MBC due to long-term mineral fertilization. Therefore, understanding the responses of carbon and nitrogen fractions to different rates of manure and fertilization is important to investigate.

2.3.6 Soil quality index (SQI)

Soil quality is defined as capacity of soil to function (Karlen et al., 1997), whereas, soil health treats soil as a living biological entity that affects plant health, animal, human and ecosystem (Doran and Zeiss, 2000). The Food and Agriculture Organization of the United Nations (FAO) describes soil health as the "capacity of soil to function as a living system, with ecosystem and land use boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and promote plant and animal health". The complexity associated with SQ has led to the development and testing of several approaches and tools (Andrews et al., 2004; Bai et al., 2018; Mukherjee and Lal, 2014). Among the several tools, soil management assessment framework (SMAF) is more accepted tool because is an accurate, sensitive and dynamic tool for the assessment of soil changes induced by different uses and management (Andrews et al., 2004; da Luz et al., 2019).

The SMAF is a tool that could determine the soil functions and can be used for sustainable soil management (Andrews et al., 2004). Soil functions are associated with soil attributes (physical, chemical, biological and ecological), contributes to ecosystem (environment) individually and through interaction (Lal, 2016). The SMAF is a tool for assessing the impact of management practices on soil functions associated with management goals of crop productivity, waste recycling, or environmental protection (Andrews et al., 2004). Specific soil properties, or indicators, are transformed via scoring algorithms into unitless scores (0 to 1) that reflect the level of function of that indicator with 1 representing the highest potential. The nonlinear scoring algorithms take one of three general shapes more is-better, less-is-better, or midpoint optimum (Andrews et al., 2004).

The use of SMAF to evaluate the effect of different uses and management practice has been reported by Cherubin et al. (2016); da Luz et al. (2019); Lal (2016). There are various research carried out that has connected soil quality indicators to different management practices along with manure and fertilizer application (Jokela et al., 2009; Wienhold, 2005). Although several research studies have been carried out to determine the effect manure application and fertilization on soil quality indicator, the translation of the effect to SQI values is still unclear.

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CHAPTER 3

MANURE AND INORGANIC FERTILIZATION IMPACTS ON SOIL AGGREGATE STABILITY, ORGANIC CARBON AND NITROGEN IN DIFFERENT AGGREGATE FRACTIONS

ABSTRACT

Manure application can enhance soil fertility and crop yield, however, knowledge of optimum application rates of manure is needed to prevent negative impact on soils and the environment. The aim of this study was to compare the long-term effect of manure and inorganic fertilizer application at different rates on soil aggregate stability, aggregate associated carbon and nitrogen, SOM and other physical properties. The experimental site was initiated in 2003 near Beresford on Egan soil under a randomized complete block design with six treatments replicated four times. The six treatments were three manure treatments; low manure (LM; application based on the phosphorous requirement), medium manure (MM; application based on nitrogen requirement), High manure (HM; two times prescribed nitrogen rate); two chemical fertilizer treatments; Medium fertilizer (MF; suggested inorganic fertilizer rate), high rate of the fertilizer (HF), and control (CK no manure nor fertilizer). Soil samples were collected in late spring 2018 and 2019. Bulk density was lowered in HM treatment by 18.9 and 9.20 % in 2018 and 2019, respectively as compared to CK. Manure application significantly increased soil wet aggregate stability (0-10 cm) compared with the CK and fertilizer application. Particulate organic matter (POM) and soil organic matter (SOM) were increased in higher manure application compared with the CK. However, no significant differences were observed in inorganic fertilizer application in comparison with CK. Findings from this study suggest

that manure application can improve soil aggregate stability, aggregate associated carbon and nitrogen, SOM and other physical properties.

3.1 Introduction

Soil organic matter (SOM) is regarded as a key indicator of soil quality (Riley et al., 2008). The SOM has beneficial effects on soil aggregate stability, hydrological properties and crop production (Karami et al., 2012). Sustainable land use and soil management practices are being adopted to add and stabilize soil organic carbon (SOC) (Gao et al., 2019). Addition of organic manure helps to increase SOC, soil quality and productivity by its favourable effects on soil properties (Ozlu and Kumar, 2018).

Several studies have found the strong correlation among soil aggregate stability, soil structure and SOM content (Darwish et al., 1995; Haynes and Naidu, 1998). Soil aggregates stabilize SOC against rapid mineralization, by making it inaccessible to microorganisms through several mechanisms; physical entrapment of C (within macro- and micro- aggregates), chemical protection (through adsorption), and biological stabilization (by recalcitrance transformation and condensation reactions within aggregates) (Lal, 2004). Some studies have also shown that increase in SOM improves soil aggregation and lowers the bulk density and the degree of compaction (Leroy et al., 2008). In contrast, SOM losses deteriorate soil quality and crop productivity, which can be challenging to restore to the original SOM content (Ding et al., 2011). Declines in SOM content can increase soil compaction which has negative impact on root growth through increased penetration resistance and bulk density (Celik et al., 2010). Bulk density is related with soil compaction, which can also alters the air-soil and water interactions and affects microbiological activity (Martınez and Zinck, 2004).

Additionally, SOM retains water and helps soil particles to bind and resist against soil compaction (Celik et al., 2010). An adequate amount of SOM can stabilize the soil structures, making the soil more resistant to degradation (Riley et al., 2008).

Many studies have shown the benefits of SOM restoration and maintaining the soil quality (Benbi et al., 2018; Bending et al., 2004; Ye et al., 2019). Sustainable agriculture practices such as conservation agriculture, effective rotation practices (Fuentes et al., 2012), manure and residue incorporation (Ye et al., 2019), cover crops in crop rotation (Nouri et al., 2019) are beneficial in enhancing the SOC, and other soil properties. Fertilization practices can maintain SOM at optimum level (Gong et al., 2009). However, improper nutrient management practices are one of the reasons for further degradation in soil through decline in SOM or SOC. Continuous use of chemical fertilizer can deplete SOC since chemical fertilizer fastens the mineralization of SOC (Ju et al., 2009). Therefore, manure application is being promoted as an alternative to the heavy chemical fertilization to enhance agricultural sustainability (Gai et al., 2019), and to restore SOC and nitrogen (N) pools and improve soil aggregation (Choudhary et al., 2018). Some studies shave shown that inorganic fertilization decreases SOC, while others have shown no negative effects, and it depends on source of fertilizer, rate, climate, and crop rotations (Geisseler and Scow, 2014). Therefore, further long-term fertilizer management research to restore SOC, aggregate stability, and maintain soil quality is needed.

Different rates of fertilizer application and their source have different effects on soil quality. Animal manure contains substantial but variable quantities of macro and micronutrients those needed for the plant growth. The N:P ratio of manure is generally lower than that of crop uptake (Eghball, 2002). Therefore, it is difficult to meet all the nutrient demand on the recommended level. The N-based manure management often oversupplies the crop-soil system with P, which can be lost into the environment and contribute to eutrophication of water bodies through nitrate leaching and accumulation in water bodies (Sileshi et al., 2019). Whereas, P-based manure management is usually unable to supply the crop N requirement. Miller et al. (2011) observed no difference on concentrations and loads of N fractions in runoff for the P- and N-based applications, whereas, Fan et al. (2017) reported higher nitrate level in fertilizer applied treatment than the manure application. Therefore, the long-term study based on recommended rates of nutrients is important.

Although many studies have shown that manure application increases soil physical stability through aggregation and overall soil health (Cai et al., 2019; Ozlu et al., 2019). There is a need for further investigation to study the long-term impact of different rates of manure application on SOC, soil structure and aggregates associated properties. Thus, this study was based on the hypothesis that long-term manure application based on different nutrient recommendation can enhance soil physical properties such as aggregate stability, SOC and organic matter components. The objective of this study was to examine the effects of long-term manure application and inorganic fertilizer application on soil physical properties such as aggregate stability, SOC and organic matter components.

3.2 Materials and methods

3.2.1 Site description and sampling

The experimental site was initiated in 2003 (16-yr) near Beresford (43º 02’ 33.46” N and 96º 53’ 55.78” W) at the Southeast Research Farm of the South Dakota State University in Clay County on Egan silty loam soil (Fine-silty, mixed, mesic Udic Haplustolls). The study treatments included: three manure rates [low manure (LM), manure application based on the phosphorous requirement; medium manure (MM), manure application based on nitrogen requirement; high manure (HM), two times prescribed nitrogen rate], two chemical fertilizer rates [medium fertilizer (MF), recommended inorganic fertilizer rate; high rate of the fertilizer (HF)], and control (CK, without any manure or fertilizer application). The experimental design was randomized complete block design with four replications. There were total 24 plots; each plot was 4.6 m (wide) by 20 m (length).

The amount of manure and mineral fertilizer treatments were calculated using South Dakota Department of Environmental and Natural Resources (DENR) tool and considering crop nutrients needed according to crop yield goal (190 bu ac^{-1} yield goal for corn and 60 bu ac^{-1} for soybean). The treatment details used in this study since 2003 can be found in Ozlu and Kumar (2018). The nutrient contents of beef manure used in 2018 for this study are mentioned in Table S1. The manure was applied using manual application and incorporated by disk at 6-cm deep within 1 to 3 d before planting in spring. A similar calculation process was determined to calculate amount of inorganic fertilizer applications for corn (*Zea mays* L.); however, no nutrient recommendation of fertilizer for soybean (*Glycine max* L.) was used. Soil samples were collected in June

2018 and May 2019 from 0-10 cm and 10-20 cm soil depths. The aggregate associated C and N analysis and organic matter components analysis were carried out only in 2018. All other parameters were analyzed on both years.

3.2.2 Soil structure and water stable aggregates size distribution

Soil samples were extracted from 0-10 cm using a hand shovel from each plot in mid-June 2019. Soil samples were then gently sieved through an 8 mm sieve to remove any undesirable plant residues and rocks. Soil samples were air-dried, and then stored for analysis. The procedure developed by Kemper and Rosenau (1986) was followed to determine water stable aggregates (WSA) size distribution with some modifications. One hundred grams of soil sample was used for wet sieving for five minutes in deionized water at room temperature by lowering and then raising the sieves with a stroke length of 13 mm and a frequency of 90 strokes per minute, using a custom-made sieving machine that can fit 20 cm (7.9 in) diameter sieves. Seven aggregate-size fractions were collected. Aggregates that passed through all sieves including the 0.053 mm (0.002 in) sieve were categorized as <0.053 mm. The other six fractions were 0.053 to 0.25, 0.25 to 0.5, 0.5 to 1, 1 to 2, 2 to 4, and 4 to 8 mm. Each soil sample was first misted and then submerged in water in the top sieve for at least five minutes before wet sieving began to slake off air-dried soil. Following wet sieving, soil samples were immediately poured into tubs and oven dried at 65°C (150°F) until all water was completely evaporated, and dry weight was recorded for each size fraction. In addition, WSA dry weights were adjusted to soil moisture corrections from air-dried subsamples of WSA. The data were analyzed to compute WSA (Kemper and Rosenau, 1986), and the mean weight diameter (Youker and McGuinness, 1956).

The mean weight diameter was calculated as follow:

$$\mathrm{MWD} = \Sigma_{i=1}^{n} X_i W_i$$

where, MWD is the mean weight diameter of water-stable aggregates, X_i is the mean diameter of each size fraction (mm) and W_i is the proportion of total sample mass in the corresponding size fraction. Aggregate size fractions with the range >2 mm, 2- 0.25mm, 0.25 -0.053mm and <0.053 mm were classified as large macro-aggregates (LMA), small macro-aggregates (SMA), micro aggregates (MI), and silt and clay (SC) as reported by Zou et al. (2018).

3.2.3 Determination of C and N

The aggregate fractions obtained from each sieve were grounded into fine powder with the help of mortar and pestle. Then, the grounded samples were analyzed with a LECO CN analyzer (LECO Corporation, St. Joseph, Michigan) to determine WSA–associated C and total N (TN) concentrations by dry combustion. Further, soil samples were tested for the presence of inorganic C (Nelson and Sommers, 1996) to remove carbonates in the samples having a pH >7.0. There was no inorganic C detected, therefore, measured total C were considered as organic C. Hence, the aggregate associated SOC and TN were determined. Similarly, SOC and TN for 0-10 cm, 10-20 cm, 20-30 cm and 30-40 cm soil depths were determined with a LECO CN analyzer (LECO Corporation, St. Joseph, Michigan). The concentrations of SOC and TN were expressed in g kg^{-1} dry soil.

3.2.4 Soil organic matter components

Soil organic matter (SOM) was determined by loss on ignition (Cabardella et al., 2001). Approximately 30 g of soil was dispersed in 90 ml 0.5 M sodium hexametaphosphate solution for 24 h, mixed for 5 min with a mechanical stirrer, poured, and rinsed though a set of nested sieves of mesh sizes 0.5 and 0.053 mm to separate samples for coarse particulate organic matter (coarse POM) and fine particulate matter (fine POM). The separated sieved materials were transferred to aluminum weighing pans (Cabardella et al., 2001). Each POM mass was determined using the loss on ignition method. Total POM is determined as the sum of coarse POM and fine POM. Sand (%) is determined from the remaining amount present in coarse separated samples.

3.2.5 Soil bulk density

Bulk density (BD) samples were taken at depths of 0 to 10, 10-20, 20-30 and 30 to 40 cm depths. Soil samples from each individual depth was then oven dried at 105°C for 24 h and weighed. Soil BD, (g cm^{-3}) was calculated as the dried soil mass divided by the soil core volume (Blake and Hartge, 1986).

3.2.6 Statistical analysis

One-way analysis of variance (ANOVA) with Duncan multiple comparison tests for mean comparison was conducted to compare the effects of different treatments within the year and the soil depth on soil physical parameters using the R-studio. The level of significance was determined at $\alpha= 0.05$.

3.3 Results

3.3.1 Soil structure and water aggregate size distribution

Wet aggregate stability (%) data under different treatments are shown in Table 3.1. Aggregate stability generally increases with the increase in amount of manure applied. In 2018, WSA was increased by 18, 22, and 46% in LM, MM and HM treatments, respectively, as compared to the CK (Table 3.1). In 2018, MWD was increased in LM, MM, and HM treatments by 50, 60, and 77%, respectively, compared to the CK (Table 3.1). Large macro-aggregates (LMA) were significantly increased in LM, MM, and HM treatments by 68, 74, and 127%, respectively, compared to the CK (Table 3.1). Small macro aggregates (SMA) were increased in MM and HM treatments by 3.4 and 16%, respectively, compared to the CK. Micro aggregates (MI) were significantly lower only in HM treatment by 33% when compared to the CK. Manure application lowered SC by 33.5 and 80% in MM and HM treatments, respectively, as compared to the CK treatment. Fertilizer application rates did not impact LMA, SMA, MI, and SC (Table 3.1).

In 2019, wet aggregate stability (%) data under different treatments are were similar as observed in 2018 (Table 3.1). Aggregate stability generally increased as the rate of manure application rate increased. Wet stable aggregates (WSA) was increased by 21, 28, and 44% in LM, MM and HM treatments, respectively, as compared to the CK (Table 3.1). Mean weight diameter (MWD) was increased in LM, MM, and HM treatments by 40, 51, and 72%, respectively, compared to the CK (Table 3.1). Large macro-aggregates (LMA) was significantly increased in LM, MM, and HM treatments by 62, 62, and 95%, respectively, compared to the CK (Table 3.1). The SMA was increased in MM and HM treatments by 13, and 20%, respectively, compared to the CK. The MI was 40% lower in HM treatment as compared to the CK. Manure application lowered SC by 50, 57 and 85% in LM, MM, and HM treatments as compared to the CK treatment. Fertilizer application rates did not impact LMA, SMA, MI, and SC (Table 3.1).

3.3.2 Carbon and nitrogen

Aggregate associated SOC under different size distribution of different treatments are shown in Table 3.2. Manure application has significantly increased aggregate associated SOC in most of the size fractions as compared to the CK. Aggregate associated SOC was increased in 8-4 mm, 4-2 mm, 2-1 mm, 1-0.5 mm, 0.5-0.25 mm and 0.25-0.053 mm size fractions in HM treatment by 62.7, 58.4, 58.7, 71.9, 47.7, and 54.1%, respectively as compared to the CK (Table 3.2). Similarly, aggregate associated SOC was increased in 8-4 mm, 4-2 mm, 2-1 mm, 1-0.5 mm, 0.5-0.25 mm and 0.25-0.053 mm size fractions in MM treatment by 18.2, 15.6, 13.1, 27.7, 17.3, and 20 %, respectively, as compared to the CK (Table 3.2). Low manure treatment (LM) had higher aggregate associated SOC in 4-2 mm, 2-1 mm, 1-0.5 mm, and 0.25-0.053 mm size aggregate fractions by 18.5, 13.1, 22.1, and 15.5 %, respectively as compared to the CK (Table 3.2). However, fertilizer application rates did not impact aggregate associated SOC.

Aggregate associated N under different size distribution of different treatments are shown in Table 3.3. Manure application significantly increased aggregate associated N in most of the size fractions as compared to the CK. Aggregate associated N was increased in 8-4 mm, 4-2 mm, 2-1 mm, 1-0.5 mm, 0.5-0.25 mm and 0.25-0.053 mm size fractions in HM treatment by 66.5, 63.5, 61.8, 67.1, 48.5, and 53.2%, respectively, as compared to the CK (Table 3.3). Similarly, aggregate associated N was increased in 4-2 mm, 2-1 mm, 1-0.5 mm, 0.5-0.25 mm and 0.25-0.053 mm size fractions in MM treatment by 11.7, 21.5, 15.3, and 14.9%, respectively, as compared to the CK (Table 3.3). Low manure (LM) treatment had higher aggregate associated N in 4-2 mm, and 1-0.5 mm size aggregate fractions by 17.4, 14.3, and 18.4% as compared to the CK (Table 3.3). However, fertilizer application rate did not impact aggregate associated N.

The SOC (g kg^{-1}) data under different treatments are shown in Table 3.4. SOC was significantly higher in HM treatment by 51.7, 9.27, 11.7, and 33.3% in 0-10 cm, 10-20 cm, 20-30 cm, and 30-40 cm soil depths, respectively, as compared to the CK. Similarly, MM treatment has higher SOC by 3.47 and 44.9% in 10-20 cm, 30-40 cm soil depths, respectively, as compared to the CK. The SOC was higher in LM and HF at 10-20 cm soil depth by 4.24 and 3.47%, respectively, as compared to the CK. The TN (g kg^{-1}) data under different treatments are shown in Table 3.4, which is significantly higher in HM treatment by 48.3, 8.84, 8.92, and 15.7% in 0-10 cm, 10-20 cm, 20-30 cm, and 30-40 cm soil depths, respectively as compared to the CK. Similarly, MM treatment has higher TOC by 4.08 % in 10-20 cm soil depth, respectively, as compared to the CK. At 10-20 cm soil depth, TOC was higher in LM and HF by 4.42 and 3.40 %, respectively, as compared to the CK.

3.3.3 Organic matter components

Different soil organic matter components based on different treatments are shown in Table 5. Manure application significantly increased coarse POM and fine POM as well as total POM in HM treatment by 1.86, 1.14, and 1.21 times as compared to the CK (Table 3.5). However, fertilizer application rate did not impact soil organic matter components.

3.3.4 Soil bulk density

Bulk density (g cm^{-3}) data under different treatments are shown in Table 3.6. Higher manure application significantly decreased the bulk density as compared to the

CK for the 0-10 cm soil depth. Bulk density was lowered in HM treatment by 14.7 and 9.20 % in 2018 and 2019, respectively, as compared to the CK (Table 3.6). However, fertilizer application rate did not impact BD in any soil depths

3.4 Discussion

3.4.1 Soil structure and water aggregate size distribution

Soil structure and WSA are among the most important physical indicators of soil quality due to its influences on soil biological, chemical and physical properties. The WSA formation, stabilization and degradation are some of the most complex processes that occur in the soil (Are et al., 2018). The stability of soil structure directly reflects the effects of land uses and crop management on nutrient soil fertility, aggregation or degradation (Kemper and Rosenau, 1986) and overall soil quality. In this study, irrespective of treatments, small macro aggregates (SMA) were found to be the highest size fractions among the water stable aggregates, which is consistent with other research findings (e.g., Kumari et al., 2011). Aggregate stability, in general, increased with the increase in amount of manure applied. Similar findings were reported by various researchers (e.g., Annabi et al., 2011; Are et al., 2018; Zou et al., 2018). The WSA and MWD values were higher in HM treatment as compared to the CK, and the increase was more than that in LM and MM treatments. This phenomenon could be explained by a higher SOM pool in higher manure as compared to the lower manure rates (Table 3.4). Manure can raise the organic matter of the soil which contributes to the formation of soil macro aggregates. Manure amendments can aggregate soil particles together to make more aggregated than the soils without the manure amendment. In the soil with higher

WSA, MI and SC fractions are lower, which is consistent with other research findings (e.g., Kumari et al., 2011). However, some researchers have found long-term manure application showing detrimental effects on soil structure due to the accumulation of Na^+ which can led to salinity (Guo et al., 2019).

Fertilization and manure application can improve soil fertility and crop production, often link with increase in SOC (Are et al., 2018). Our study also shows that SOC was generally increased with the increase in rate of manure application and higher chemical fertilizer, although the values were not significant in all depths. We did not observed differences in aggregate stability (WSA, MWD and other aggregate values) between different rates of chemical fertilizer application treatments. Reduction in soil pH could be a reason to repeal the positive effect of SOC in WSA stability. Our result is similar with a study where researchers reported a negative correlation ($r = -0.16$) between increased N rates and WSA stability, regardless of increased higher SOC content (Are et al., 2018). Higher aggregate stability in the manure application indicates the potentiality of manure application to maintain soil structure and decelerate soil degradation.

3.4.2 Carbon and nitrogen

Aggregate associated SOC and total nitrogen concentration were higher in macro aggregates than the micro aggregates irrespective of the treatments. Our research also supports this finding. The TN and SOC showed similar pattern in all aggregate size fractions, which is consistent with other research findings (e.g., Kumari et al., 2011). The higher concentrations of SOC and TN in macro aggregates observed in our study compared to the micro aggregates also reported by Zou et al. (2018).

Soil organic carbon (SOC) and TN play vital role in soil functions that produce a wide range of services to ecosystem. Organic matter in manure come form stable organic compounds on decomposition (Abdelhafez et al., 2018) and can the reason for maintaining the higher level of SOC in long-term manure application. In our study, the SOC was significantly higher in HM treatment as compared to the LM and MM. Liang et al. (2012) showed that 15-year farmyard manure increased SOC by 56, 46, and 14% for 0- to 10-cm, 10- to 20-cm, and 20- to 30-cm depths, respectively, compared with the control (without any application). In all treatments, numerically higher values of SOC were found at the surface compared to the deeper layers, indicating the slower translocation of SOC and TN through the soil profile. Similar findings were reported by Sithole et al. (2019). Increase in SOC and TN contents in HM treatment can be explained by directly higher addition of manure and crop residues (Li et al., 2015). Inorganic fertilizer effects on SOC and TN are variable. They can have positive effect directly because of nutrients being added to the soil (Lupwayi et al., 2012) as well as indirect positive effect because of increased root exudates by crops or crop biomass which adds organic C (Geisseler and Scow, 2014). In contrast, inorganic fertilization can have direct negative effect due to acidification, leading to lower SOC and TN and microbial activity (Peacock et al., 2001).

3.4.3 Soil organic matter components

The SOM controls soil physical, chemical and biological properties, and is a key factor in soil quality (Riley et al., 2008). SOM restoration has beneficial effects on soil structure and aggregate stability, preventing soil degradation (Lal et al., 2016), and sustaining the productivity of agroecosystem (Ding et al., 2012). Several studies have found the strong correlation among soil aggregate stability, soil structure and SOM content (Darwish et al., 1995; Haynes and Naidu, 1998). Data on SOM quantity and quality are therefore important for agricultural sustainability. Continuous cultivation without organic inputs caused significant losses of SOM (Mando et al., 2005). This study also observed lower SOM in treatments with no manure application as compared to HM treatment. The SOM was mainly stored in the size-fraction between 0.053 and 2 mm (particulate organic matter, POM). The HM treatment increased POM concentrations as compared to the CK. This study observed that SOM and POM were affected in HM treatment. Some studies found grain yield to be positively correlated with the total POM but not correlated with total SOM (Mando et al., 2005), which indicates the greater importance of POM in productivity.

3.4.4 Soil bulk density

Bulk density is used to characterize the soil compaction which influences the structural functions and characteristics of soils (Celik et al., 2010). In this study, HM decreased the soil bulk density as compared to the CK and HF on the 0-10 cm soil depth in both years. Soil compaction alters the air-soil and water interactions and hence impacts the microbiological activity (Martınez and Zinck, 2004). SOM addition has organic components which loosens the soil and lowers the bulk density (Bronick and Lal, 2005). The increase in organic matter content results in greater total porosity and lowers soil bulk density (Guo et al., 2016). Some studies have also shown that increase in SOM improves soil aggregations and lowers the bulk density and the degree of compaction (Leroy et al., 2008). Decline in SOM content can increase soil compaction which has negative impact on root growth through increased penetration resistance and bulk density

(Celik et al., 2010). SOM retains soil moisture and helps soil particles to bind and resist against soil compaction (Celik et al., 2010). An adequate amount of SOM can stabilize the soil structure which makes the soil more resistant to degradation (Riley et al., 2008).

3.5 Conclusions

A study was conducted in South Dakota to investigate the impacts of long-term manure and inorganic fertilizer application on soil organic carbon and select soil properties. The following conclusions were drawn from this study, and those are mentioned below as:

- Manure application, in general, increased the aggregate stability, and aggregate associated SOC and TN as compared to the CK in all the aggregate size fractions. Further, higher manure application increased the SOC and TN as compared to the CK.
- Higher manure application increased the SOM, coarse POM and fine POM, whereas, fertilizer application did not influence these parameters.
- Higher manure application decreased soil bulk density as compared to the CK and HF for 0-10 cm soil depth in both years.

Findings from this study show that manure addition, when used in optimum amount, can positively influences the aggregate stability. However, there are some negative soils and environmental effects of the high manure and fertilizer application, those were not the scope of this study, and this needs to be studied in the future.

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Table 3.1 Response of wet stable aggregates (WSA), mean weight diameter (MWD, large macroaggregates (LMA>2 mm), small macroaggregates (SMA, 2-0.25 mm), micro aggregates (MI, 0.25-0.053) and sand clay (SC,<0.053 mm) as influenced by manure (low, LM, based on P requirement; medium, MM, based on N requirement; and high, HM, double rate of manure based on N), and fertilizer (medium, MF, recommended rate; and high, HF, higher dose) application, and the control (CK) treatments (TRT) at 0-10 cm soil depth in 2019.

TRT	**WSA**	**MWD**	**LMA**	**SMA**	**MI**	**SC**
	%	Mm	%			
			2018			
CK	$56.9^{c\dagger}$	1.691^{b}	15.4^{b}	41.5^{b}	18.1^{ab}	25.1^{ab}
MF	49.1^{c}	1.63^{b}	15.4^{b}	33.7^{c}	18.4^{ab}	32.6^{a}
HF	51.9^{c}	1.566^{b}	16.0^{b}	35.9^{c}	22.4^{a}	25.7^{ab}
LM	67.1^{b}	2.532^{a}	25.8^{a}	41.3^{b}	14.6^{bc}	18.3^{bc}
MM	69.7^{b}	2.709^{a}	26.8^{a}	42.9^{a}	13.6^{bc}	16.7^{c}
HM	82.9^{a}	2.992^{a}	34.9^{a}	48.0^{a}	12.1^{c}	4.94^{d}
			Analysis of Variance (*P*>*F*)			
Trt	<0.0001	<0.0001	0.0009	0.0002	0.0108	<0.0001
			2019			
CK	$59.6^{c\dagger}$	1.218^{c}	18.5^{b}	41.4^{c}	18.3^{a}	22.1^{a}
MF	59.5^{c}	1.114^{c}	17.1^{b}	42.4^{bc}	18.7^{a}	21.8^{a}
HF	59.6^{c}	1.144^{c}	17.4^{b}	42.2^{bc}	19.6^{a}	20.8^{a}
LM	72.4^{b}	1.709^{b}	29.9^{a}	42.5^{bc}	16.5^{ab}	11.1^{b}
MM	76.5^{b}	1.837^{ab}	29.9^{a}	46.6^{ab}	13.9^{ab}	9.61^{b}
HM	85.6^{a}	2.094^{a}	36.1^{a}	49.5^{a}	11.0^{b}	3.32^{b}
			Analysis of Variance (*P*>*F*)			
Trt	<0.0001	<0.0001	<0.0001	0.0157	0.0492	0.0001

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

Table 3.2 Response of aggregate associated carbon on different size fractions (8-4 mm, 4-2 mm, 2-1 mm, 1-0.5 mm, 0.5-0.25 mm and 0.25-0.053 mm as influenced by manure (low, LM, based on P requirement; medium, MM, based on N requirement; and high, HM, double rate of manure based on N), and fertilizer (medium, MF, recommended rate; and high, HF, higher dose) application, and the control (CK) treatments (TRT) at 0-10 cm soil depth in 2019.

TRT	**Aggregate associated carbon on different size fraction**					
	8-4 mm	**4-2 mm**	**2-1 mm**	**1-0.5 mm**	**0.5-0.25 mm**	**0.25-0.053 mm**
	g SOC kg^{-1}					
CK	$23.6^{c\dagger}$	24.3^{c}	25.2^{c}	23.1^{c}	24.3^{cd}	22.0^{d}
MF	24.9^{c}	24.4^{c}	24.6^{c}	23.8^{c}	23.5^{d}	22.7^{d}
HF	24.9^{c}	24.5^{c}	25.2^{c}	24.5^{c}	24.8^{cd}	23.5^{cd}
LM	26.6^{bc}	28.8^{b}	28.5^{b}	28.2^{b}	27.1^{bc}	25.4^{bc}
MM	27.9^{b}	28.1^{b}	28.5^{b}	29.5^{b}	28.5^{b}	26.4^{b}
HM	38.4^{a}	38.5^{a}	40.0^{a}	39.7^{a}	35.9^{a}	33.9^{a}
	Analysis of Variance (*P*>*F*)					
Trt	<0.0001	<0.0001	<0.0001	<0.0001	<0.001	<0.001

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

Table 3.3 Response of aggregate associated nitrogen on different size fractions (8-4 mm, 4-2 mm, 2-1 mm, 1-0.5 mm, 0.5-0.25 mm and 0.25-0.053 mm as influenced by manure (low, LM, based on P requirement; medium, MM, based on N requirement; and high, HM, double rate of manure based on N), and fertilizer (medium, MF, recommended rate; and high, HF, higher dose) application, and the control (CK) treatments (TRT) at 0-10 cm soil depth in 2019.

	Aggregate associated nitrogen on different size fractions					
TRT	**8-4 mm**	**4-2 mm**	**2-1 mm**	**1-0.5 mm**	**0.5-0.25 mm**	**0.25-0.053 mm**
	g TN kg^{-1}					
CK	2.29^{b}[†]	2.30^{c}	2.38^{cd}	2.28^{c}	2.35^{cd}	2.22^{cd}
MF	2.27^{b}	2.24^{c}	2.28^{d}	2.23^{c}	2.18^{d}	2.14^{d}
HF	2.39^{b}	2.32^{c}	2.32^{d}	2.33^{c}	2.39^{cd}	2.33^{bcd}
LM	2.48^{b}	2.70^{b}	2.72^{b}	2.70^{b}	2.62^{bc}	2.47^{bc}
MM	2.49^{b}	2.57^{b}	2.61^{bc}	2.77^{b}	2.71^{b}	2.55^{b}
HM	3.78^{a}	3.76^{a}	3.85^{a}	3.81^{a}	3.49^{a}	3.40^{a}
	Analysis of Variance ($P>F$)					
Trt	<0.0001	<0.0001	<0.0001	<0.0001	<0.001	<0.001

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

Table 3.4 Response of soil organic carbon (SOC) and total nitrogen (TN) as influenced by manure (low, LM, based on P requirement; medium, MM, based on N requirement; and high, HM, double rate of manure based on N), and fertilizer (medium, MF, recommended rate; and high, HF, higher dose) application, and the control (CK) treatments (TRT) at 0-10 cm,10-20 cm, 20-30 cm and 30-40 cm soil depths in 2018.

TRT	**SOC** (g kg^{-1})			
	0-10 cm	**10-20 cm**	**20-30 cm**	**30-40 cm**
CK	29.2^{bc†}	25.9^{c}	22.2^{bc}	15.6^{b}
MF	28.1^{c}	25.5^{c}	21.2^{c}	18.9^{ab}
HF	30.2^{bc}	26.8^{b}	24.2^{ab}	20.4^{a}
LM	33.5^{bc}	27.0^{b}	23.8^{ab}	19.7^{ab}
MM	34.9^{b}	26.8^{b}	24.1^{ab}	22.6^{a}
HM	44.3^{a}	28.3^{a}	24.8^{a}	20.8^{a}
	Analysis of Variance (P>F)			
Trt	<0.001	<0.001	0.019	0.036
	TN (g kg^{-1})			
CK	3.31^{bc†}	2.94^{c}	2.69^{bc}	2.35^{b}
MF	3.10^{c}	2.94^{c}	2.60^{c}	2.27^{b}
HF	3.36^{bc}	3.04^{b}	2.76^{ab}	2.47^{b}
LM	3.73^{bc}	3.07^{b}	2.82^{ab}	2.49^{ab}
MM	3.92^{b}	3.06^{b}	2.82^{ab}	2.45^{b}
HM	4.91^{a}	3.20^{a}	2.93^{a}	2.72^{a}
	Analysis of Variance (P>F)			
Trt	0.001	<0.001	0.008	0.025

[†] Mean values within the same column followed by different small letters for SOC and TN are significantly different for each depth at p<0.05 for treatment.

Table 3.5 Response of coarse particulate organic matter (coarse POM), fine POM, total POM, and soil organic matter (SOM) as influenced by manure (low, LM, based on P requirement; medium, MM, based on N requirement; and high, HM, double rate of manure based on N), and fertilizer (medium, MF, recommended rate; and high, HF, higher dose) application, and the control (CK) treatments (TRT) at 0-10 cm soil depth in 2019.

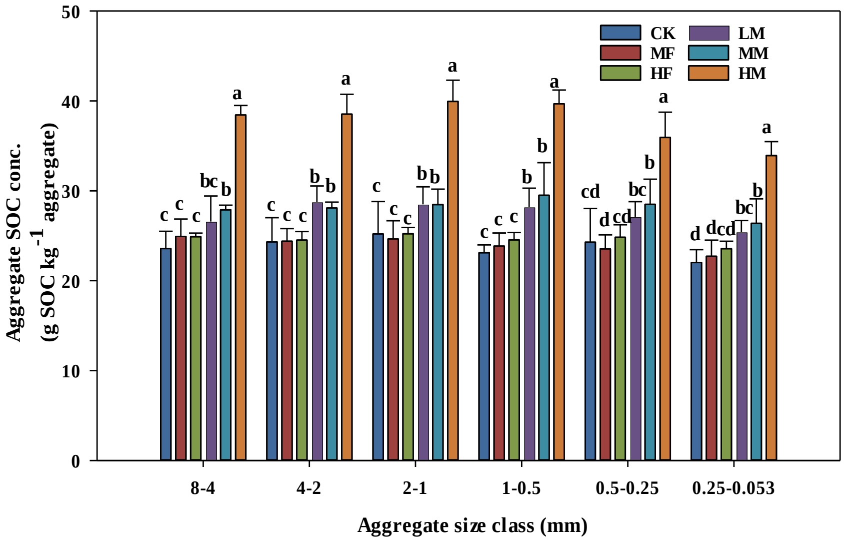
TRT	**SOM**	**Coarse POM**	**Fine POM**	**Total POM**	**Sand**
		mg g^{-1}			%
CK	$65.2^{b†}$	0.816^{b}	6.867^{b}	7.68^{b}	12.1^{b}
MF	65.6^{b}	0.927^{b}	6.394^{b}	7.32^{b}	13.4^{b}
HF	70.0^{b}	1.145^{b}	8.484^{b}	9.63^{b}	11.9^{b}
LM	69.9^{b}	0.742^{b}	7.820^{b}	8.56^{b}	12.2^{b}
MM	70.8^{b}	1.132^{b}	8.795^{b}	9.93^{b}	13.9^{ab}
HM	86.9^{a}	2.337^{a}	14.67^{a}	17.0^{a}	16.8^{a}
		Analysis of Variance (*P*>*F*)			
Trt	0.0001	<0.0001	<0.0001	<0.0001	0.010

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

Table 3.6 Response of bulk density (g cm^{-3}) as influenced by manure (low, LM, based on P requirement; medium, MM, based on N requirement; and high, HM, double rate of manure based on N), and fertilizer (medium, MF, recommended rate; and high, HF, higher dose) application, and the control (CK) treatments (TRT) at 0-10 cm, 10-20 cm, 20-30 cm and 30-40 cm soil depths in 2018 and 2019.

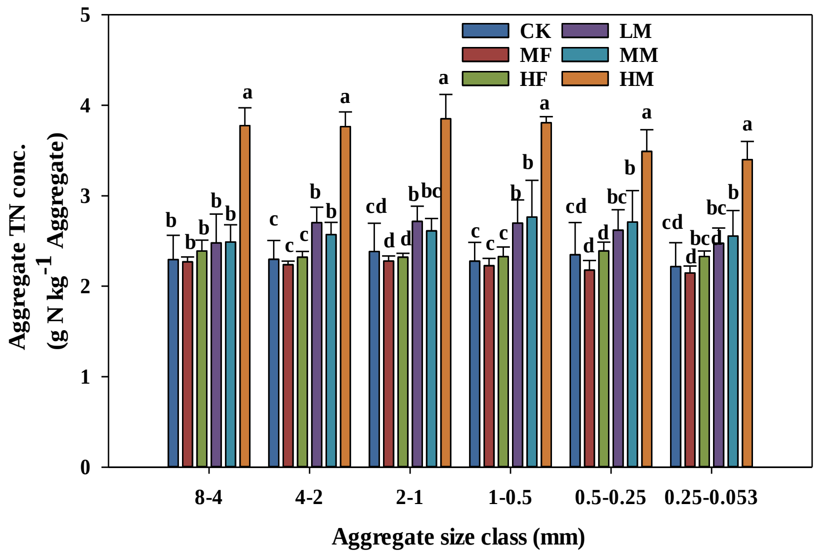
TRT	**Depths**			
	0-10 cm	**10-20 cm**	**20-30 cm**	**30-40 cm**
		2018		
CK	1.32^{a}	1.42^{a}	1.32^{a}	1.38^{a}
MF	1.22^{ab}	1.42^{a}	1.34^{a}	1.29^{a}
HF	1.30^{ab}	1.38^{a}	1.28^{a}	1.35^{a}
LM	1.18^{bc}	1.38^{a}	1.32^{a}	1.32^{a}
MM	1.22^{ab}	1.37^{a}	1.33^{a}	1.30^{a}
HM	1.07^{c}	1.37^{a}	1.31^{a}	1.30^{a}
	Analysis of Variance (P>F)			
Trt	0.045	0.553	0.771	0.239
		2019		
CK	1.63^{a}	1.72^{a}	1.66^{a}	1.63^{c}
MF	1.59^{a}	1.74^{a}	1.70^{a}	1.75^{a}
HF	1.64^{a}	1.71^{a}	1.66^{a}	1.74^{ab}
LM	1.53^{ab}	1.74^{a}	1.66^{a}	1.70^{abc}
MM	1.64^{a}	1.75^{a}	1.68^{a}	1.72^{ab}
HM	1.48^{b}	1.65^{a}	1.71^{a}	1.63^{c}
	Analysis of Variance (P>F)			
Trt	0.028	0.462	0.361	0.012

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



†Different small letters are significantly different at $p<0.05$ for treatment.

Figure 3.1 Aggregate associated soil organic carbon (SOC, g kg^{-1}) as influenced by manure (low, LM, based on P requirement; medium, MM, based on N requirement; and high, HM, double rate of manure based on N), and fertilizer (medium, MF, recommended rate; and high, HF, higher dose) application, and the control (CK) in different size aggregate fraction (8-4 mm, 4-2 mm, 2-1 mm, 1-0.5 mm, 0.5-0.25 mm and 0.25-0.053 mm).



†Different small letters are significantly different at $p<0.05$ for treatment.

Figure 3.1 Aggregate associated total nitrogen (TN g kg^{-1}) as influenced by manure (low, LM, based on P requirement; medium, MM, based on N requirement; and high, HM, double rate of manure based on N), and fertilizer (medium, MF, recommended rate; and high, HF, higher dose) application, and the control (CK) in different size aggregate fraction (8-4 mm, 4-2 mm, 2-1 mm, 1-0.5 mm, 0.5-0.25 mm and 0.25-0.053 mm).

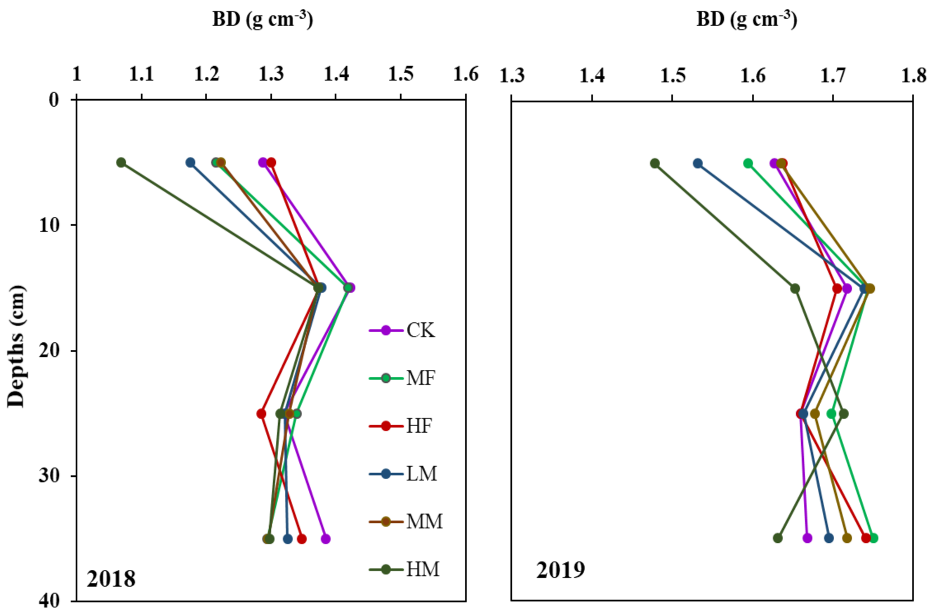


Figure 3.2 Bulk density (BD, g cm-3) as influenced by manure (low, LM, based on P requirement; medium, MM, based on N requirement; and high, HM, double rate of manure based on N), and fertilizer (medium, MF, recommended rate; and high, HF, higher dose) application, and the control (CK) in 2018 and 2019.

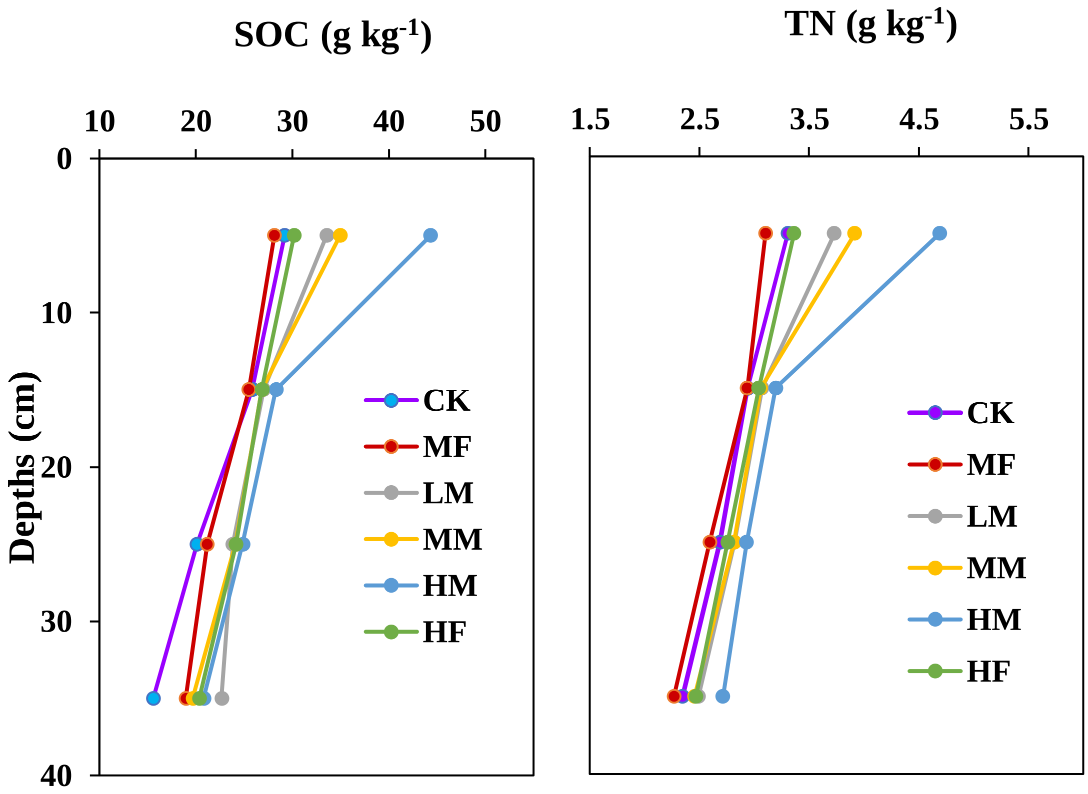


Figure 3.3 Soil organic carbon (SOC, g kg^{-1}) and total nitrogen (TN, g kg^{-1}) as influenced by manure (low, LM, based on P requirement; medium, MM, based on N requirement; and high, HM, double rate of manure based on N), and fertilizer (medium, MF, recommended rate; and high, HF, higher dose) application, and the control (CK) in 2018.

CHAPTER 4

LONG-TERM IMPACT OF MANURE AND INORGANIC FERTILIZATION APPLICATION ON SOIL MICROBIAL PROPERTIES AND OVERALL SOIL HEALTH

ABSTRACT

The intensive use of mineral fertilizers to achieve high crop yield has led to soil degradation and poor soil health. Thus, organic manure application as an alternative to mineral fertilizers can be a feasible fertilization strategy to sustain soil health and biodiversity and mitigate soil degradation. This study aims to assess the impacts of long-term manure and mineral fertilizers on key soil biochemical and biological indicators. The study was conducted on a 16-year long-term experimental site with six different manure and fertilizer treatments that included no amendments (CK), recommended mineral fertilizer (MF), double the amount of MF (HF), manure application based on the phosphorus requirement (LM), manure application based on the nitrogen (N) requirement (MM), and double the rate of MM treatment (HM). Data showed that higher rates of organic manure application (HM) significantly increased urease, β-glucosidase, and alkaline phosphatase enzyme activities, and soil microbial community PLFA biomass compared to the CK for 0-10 cm soil depth in 2018. Similar trend was observed for 2019. However, both fertilizer rates (MF and HF) did not show any differences in microbial community when compared with the CK for either depth. Carbon and nitrogen fractions were significantly increased by HM treatment but remained unaffected by mineral fertilization. Cold water nitrogen (CWN) was increased under MF treatment as compared to the CK for 0-10 cm soil depth, whereas, both MF and HF increased CWN by 121 and

86% respectively, for 10-20 cm depth in 2018. This study demonstrated that a long-term manure application strategy based on different nutrients requirement could be beneficial in enhancing soil biochemical and microbial parameters.

Keywords**:** beef manure, inorganic fertilizer, soil enzymes, PLFA.

4.1 Introduction

Inorganic fertilization and manure application are common nutrient management practices for enhancing soil fertility and crop yield. However, the overuse of fertilizers can led to the degradation of environment through nutrient runoff and biodiversity loss (Li et al., 2015; Zhang et al., 2012). This increases the concern towards sustainability of agricultural management practices. Sustainability in agricultural production can be secured by maintaining soil health. Therefore, it is essential in protecting and sustaining long-term soil productivity from destructive and unbalanced management practices such as intensive tillage and excessive application of chemicals that lead to soil and water quality degradation. Additionally, the replacement of mineral fertilizer with manure can manage deposition of animal waste while also provide an opportunity to improve soil quality (Jiang et al., 2018; Ozlu and Kumar, 2018).

Manure application increases soil carbon stock, improves aggregate stability, and maintains pH (Ozlu and Kumar, 2018), reduces nutrient loss and supports similar or higher crop production than the mineral fertilizer (Jiang et al., 2018). In contrast, mineral fertilizer application decreases the soil pH (Cai et al., 2015) which could alter the nutrient availability. Higher application of manure can create a negative environmental effect such as phosphorus and nitrate leaching. Further, higher manure application in long-term could also have a loose soil structure due to the presence of monovalent cations (e.g., Na^+) in animal manure which acts as dispersing agent to break the soil structure (Bronick and Lal, 2005). Similarly, higher mineral fertilizer application can be expensive and increase the more N loss when compared to manure application (Martínez et al., 2017).

Different rates of fertilizer application and their source have different effects on soil health. The nitrogen (N), phosphorus (P) and potassium (K) are the major macronutrients required by the crop to optimize the production. The amount of manure and fertilizer application is generally done by considering crop nutrients needed according to crop yield goal, and nutrient contents of soil and manure. Animal manure contains substantial but variable quantities of macro and micronutrients those needed for the plant growth. The N:P ratio of manure is generally lower than that of crop uptake (Eghball, 2002). Therefore, it is difficult to meet all the nutrient demand on a recommended level. The N-based manure management often oversupplies the crop-soil system with P, which can be lost into the environment and contribute to eutrophication of water bodies through nitrate leaching and accumulation in water bodies (Sileshi et al., 2019). Further, the P-based manure management is usually unable to supply the crop N requirement. Miller et al. (2011) did not observe any difference on concentrations and loads of N fractions in runoff between the P- and N-based applications. However, Fan et al. (2017) mentioned higher nitrate level in fertilizer applied treatment than the manure application.

Manure application increases soil physical stability through aggregation and enhances water holding capacity, and soil health (Cai et al., 2019; Ozlu et al., 2019).

However, the impact of the different rates of application of manure on microbial properties is still elusive. Thus, this study was based on the hypothesis that long-term manure application based on different nutrient recommendation can enhance soil biochemical properties and alter soil microbial community structure differently by increasing diversity. Soil microorganisms play an important role in soil biogeochemical processes (Sekaran et al., 2019). They are the critical factors that determine soil organic matter decomposition and nutrient cycling. Microbial diversity is one of the most important soil quality parameters in the soil (Li et al., 2015; Zhong and Cai, 2007). Several studies have reported that fertilizer management affects microbial diversity (e.g., Böhme et al., 2005; Zhang et al., 2012). However, research that focuses on assessing the impacts on manure and fertilizer impacts on detailed microbial analysis that include enzymatic analysis, microbial community structure at surface (0-10 cm) and subsurface (10-20 cm) depths under two different crop stand on the corn soybean crop rotation is limited. Thus, specific objective of this study is to determine how the long-term contrasting manure and inorganic fertilizer regimes impact soil biochemical properties including enzyme activities and microbial community.

4.2 Materials and methods

4.2.1 Site description and sampling

The experimental site was initiated in 2003 (16-yr) near Beresford (43º 02’ 33.46” N and 96º 53’ 55.78” W) at the Southeast Research Farm of the South Dakota State University in Clay County on silty loam Egan soil (Fine-silty, mixed, mesic Udic Haplustolls). The study included three manure application rates; low (LM, based on phosphorus requirement), medium (MM, based on nitrogen requirement), and high (HM, double rate of MM), and two fertilizer application rates; medium (MF; only nitrogen addition), high (HF; double the amount of MF), and control (CK, no amendments). The experimental design was randomized complete block design with four replications. There were total 24 plots, and each plot was 4.6 m wide by 20 m long.

The amount of manure and mineral fertilizer treatments were calculated using South Dakota Department of Environmental and Natural Resources (DENR) tool and considering crop nutrients needed according to the crop yield goal (190 bu ac^{1} yield goal for corn and 60 bu ac^{-1} for soybean). The details of this study site is described in previous papers (e.g., Ozlu and Kumar, 2018; Ozlu et al., 2019). The nutrient contents of beef manure used in 2018 for this study are mentioned in Tables S1. The manure was applied using manual application and incorporated by disk at 6-cm deep within 1 to 3 d before planting in spring. A similar calculation process was determined to calculate amount of inorganic fertilizer rates for corn (*Zea mays* L.); however, no nutrient recommendation of fertilizer was used for soybean (*Glycine max* L.). Soil samples were collected in June 2018 and May 2019 from 0-10 cm and 10-20 cm soil depths. These samples were kept fresh and stored in a refrigerator at 4^{o}C pending analysis. The carbon and nitrogen fractions, and metagenomics analysis were carried out only for 2018, whereas, all other parameters were analyzed for both (2018 and 2019) years.

4.2.2 Soil C and N fractions

Content of water- extractable organic carbon and nitrogen fractions were carried out by schematic procedure as described by Ghani et al. (2003). A 3 g of soil was poured with 30 mL of water (1:10; soil-to-solution ratio) and then, kept for shaking on vortex and rotatory shaker for 10 sec. and 30 min. at 40 rpm, respectively for extraction. The suspension obtained was centrifuged, and then filtration was carried out by syringe filter. The filtrate obtained was cold-water extractable organic carbon (CWC) and nitrogen (CWN). Further 30 mL of water was added to the remaining residue and kept for shaking on vortex and rotatory shaker for 10 sec. and 30 min. at 40 rpm, respectively. The suspension was left in hot-water bath at 80°C for 12-15 h. The suspension was centrifuged, and then filtration was carried out by syringe filter. The filtrate obtained was the hot water extractable organic carbon (HWC) and nitrogen (HWN). The cold and hot water carbon and nitrogen fractions were determined using the TOC-L analyzer (Shimadzu Corporation, model-TNM-L-ROHS).

4.2.3 Microbial biomass C and N

Microbial biomass carbon (MBC) and nitrogen (MBN) in soil were determined using the chloroform fumigation direct extraction method as described in Anderson and Domsch (1978); Gregorich et al. (1990). A total of 8 g soil was placed into a 50-mL glass beaker for fumigation and non-fumigation analysis. Soil samples specified as fumigated were kept in a desiccator clouded with alcohol-free chloroform for 24 h, evacuated, and extracted with 40-mL 0.5M K_2SO_4. Non-fumigated soil samples extraction was carried out with 40 mL 0.5M K_2SO_4. The suspensions obtained from both were analyzed for dissolved C and N. The MBC and MBN were calculated by the difference between C and N in the fumigated and non-fumigated samples, and with a correction factor of 0.45 for MBC (Beck et al., 1997).

4.2.4 Microbial community structure

The phospholipid fatty acid analysis (PLFA) was used to access microbial community structure in the soil samples for both the depths in either year. Soil samples were analyzed at Ward Laboratories, Inc. (Lincoln, NE). These samples were analyzed according to the method of Clapperton et al. (2005); Hamel et al. (2006). Briefly, total soil lipids were extracted by shaking approximately 2.0 g of soil in 9.5 mL dichloromethane: methanol: citrate buffer (1:2:0.8 v/v). Extracted samples were analyzed using an Agilent 7890A GC equipped with a CP-7693 auto-sampler and a flame ionization detector (FID). Individual fatty acids have been used as signatures for different functional groups of microorganisms (Bardgett et al., 1999; Bossio et al., 1998; Grayston et al., 2001; Pankhurst et al., 2002; Yao et al., 2000). Amounts were derived from the relative area under specific peaks, as compared to the 19:0 peak value (Internal standard), which was calibrated according to a standard curve made from a range of concentrations of the 19:0 FAME standard dissolved in hexane. The sum of all PLFAs and each PLFAs are expressed as C mass (ng PLFA-C g^{-1} soil).

4.2.5 Enzyme assays

Soil β-glucosidase (EC 3.2.1.21) enzyme activity was assayed by the method of Eivazi and Tabatabai (1988), using the substrate 50 mM para-nitrophenyl-β-D-glucopyranoside (pNPG). The β-glucosidase enzyme activity is expressed as μmol *p*-nitrophenol (pNP) released g^{-1} soil h^{-1}. Urease (EC 3.5.1.5) enzyme activity was assayed by the method of Kandeler and Gerber (1988). Briefly, a 5.0 g of soil was incubated with 2.5 mL of urea solution and 20 mL of borate buffer at 37°C and the urease enzyme activity was reported as μmol N-NH_4^+ g^{-1} soil h^{-1}. Alkaline (E.C.3.1.3.1) phosphatase enzyme activity was determined as described by Eivazi and Tabatabai (1977) and Tabatabai and Bremner (1970), and the activity was reported as µg pNP g^{-1} soil h^{-1}.

4.2.6 Soil quality index (SQI)

The soil management assessment framework (SMAF) is a tool for assessing the impact of management practices on soil functions associated with management goals of crop productivity, waste recycling, or environmental protection (Andrews et al., 2004). Specific soil properties, or indicators, are transformed via scoring algorithms into unit less scores (0 to 1) that reflect the level of function of that indicator, with 1 representing the highest potential. The nonlinear scoring algorithms take one of three general shapes more is-better, less-is-better, or midpoint optimum (Andrews et al., 2004). The SMAF users are directed to select 4 to 8 indicators representing physical, chemical and biological properties from the set of 13 for which algorithm have be published (Andrews et al., 2004). We used seven indicators that include: pH, EC, bulk density, beta glucosidase activity, wet aggregate stability, MBC, and SOC for 0-10 cm soil depth, and six indicators except wet aggregate stability for the 10-20 cm soil depth. We calculated SMAF scores for each parameter using scoring algorithms in an Excel spreadsheet and combined the scores to obtain soil quality index (SQI) for each treatment. The SMAF scores was calculated for the 0-10 cm and 10-20 cm soil depths.

4.2.7 Statistical analysis

One-way analysis of variance (ANOVA) with Duncan multiple comparison tests for mean comparison was conducted to compare the effects of different treatments within the year and the soil depth on soil biological parameters using the R-studio. The level of significance was determined at $\alpha = 0.05$ (McLean, 1982).

4.3 Results

4.3.1 Soil C, N fractions, and microbial biomass

Data on CWC, HWC, CWN, HWN, MBC and MBN as influenced by different treatments at 0-10 and 10-20 cm depths are presented in Table 4.3. The HM treatment increased the CWC, HWC, CWN, HWN, MBC, and MBN by 46, 102, 228, 91, 101 and 123%, respectively, as compared to the CK for the 0-10 cm depth. However, no significant differences were observed between inorganic fertilizer treatments and the CK except in CWN, which was increased by 1.3 times in MF treatment. The HM treatment increased the HWC, CWN, HWN, MBC, and MBN by 58, 123, 62, 46, and 68%, respectively, for the 10-20 cm depth (Table 4.3). However, no significant differences were observed between inorganic fertilizer treatments and the CK except in CWN, which was increased, by 86 and 121 % in MF and HF, respectively.

4.3.2 Soil enzyme activity

Data on soil enzymatic activities at 0-10 and 10-20 cm soil depths for 2018 and 2019 are presented in Fig. 4.1, 4.2, and 4.3. For 0-10 cm soil depth, manure application under HM treatment significantly increased the soil β-glucosidase activity by 44 and 64% compared to the CK in 2018 and 2019, respectively (Fig. 4.1). For 0-10 cm soil depth in 2018 and 2019, the HM treatment significantly increased the urease enzyme activity by 54 and 100% times than the CK, respectively (Fig 4.2). Alkaline phosphatase activity was also increased by 1.2 and 2.29 times with HM treatment than the CK in 2018 and 2019, respectively (Fig 4.3). However, there was no significant increase observed from inorganic fertilizer application (MF and HF) and CK for soil β-glucosidase and urease enzyme activity in either year. However, alkaline phosphatase enzyme activity was increased by 78% under HF as compared to the CK in 2019.

For 10-20 cm depth, soil enzyme activity values were lower than the 0-10 cm soil depth. In 2018, β-glucosidase enzyme activity was significantly higher with MM, HM, and HF treatments (4.79, 4.86 and 4.75 µmol PNPg^{-1} soil h^{-1}, respectively) than the CK (3.74 µmol PNPg^{-1} soil h^{-1}) treatment (Fig. 4.1). Furthermore, in 2019, HM treatment significantly increased the β-glucosidase enzyme activity by 38 and 25% than the CK and HF treatments, respectively, at 10-20 cm depth. However, no significant difference was observed for urease activity at 10-20 cm soil depth in 2018, whereas, in 2019, the HM treatment increased the urease activity by 1.08 times higher than the CK treatment (Fig 4.2). Alkaline phosphatase activity in 2018 for 10-20 cm soil depth was increased in all fertilizer treatments irrespective of the source of fertilizer when compared to the CK (Fig 4.3), however, the MM and HM treatments increased the alkaline phosphatase activity by 29 and 43%, respectively, as compared to the inorganic fertilizer (MF) treatment. In 2019, no differences were observed among the treatments for alkaline phosphatase activity.

4.3.3 Soil microbial community structure

The PLFA for 0-10 and 10-20 cm depths was significantly influenced by manure treatments in 2018 and 2019 (Table 4.1 and 4.2). The PLFA biomass at 0-10 and 10-20 cm depths was higher with the HM compared with the CK. In 2018, the HM increased the total PLFA, total bacterial, actinomycetes, Gram-negative bacteria, Gram-positive bacteria, total fungi, arbuscular mycorrhizal fungi (AMF), and saprophyte PLFA biomass by 70, 84, 65, 108, 72, 118, 92, and 1.36% than the CK treatment. However, no significant differences were observed between inorganic fertilizer and CK treatments. Similarly, in 2019, application of HM and MM treatments significantly increased the total PLFA, total bacterial, actinomycetes, Gram-negative bacteria, Gram-positive bacteria, total fungi, arbuscular mycorrhizal fungi (AMF), and saprophyte PLFA biomass than the CK (Table 4.1). Similar to 2018, there were no significant differences between inorganic fertilizer and CK treatments in 2019. The PLFA parameters for the 10-20 cm soil depth were lower than those for the 0-10 cm. Furthermore, at 10-20 cm depth, HM treatment significantly increased PLFA biomass in 2018. Total PLFA, total bacterial, actinomycetes, Gram-negative bacteria, Gram-positive bacteria, total fungi, arbuscular mycorrhizal fungi (AMF), and saprophyte PLFA biomass were significantly higher by 106, 113, 109, 200, 88, 361, 326, and 381% with HM treatment than the CK. However, no significant difference was observed in 2019 for the 10-20 cm depth.

4.3.4 Soil quality index (SQI)

The data of SQI under different treatments in 2018 for 0-10 and 10-20 cm depths are shown in Table 4.4. The SQI values in 0-10 cm soil depth was higher in LM, MM, and HM treatments by 7.95, 6.75 and 9.80 %, respectively, compared to the HF (Table 4.4). In 10-20 cm soil depth, the SQI values were higher in LM, MM, and HM treatments by 10.1, 8.73 and 16.3%, respectively, compared to the CK (Table 4.4). However, fertilizer application rate did not impact SQI at either depth.

4.4 Discussion

In the present study, we observed that application of manure significantly increased the CWC and HWC than the inorganic fertilizer and CK treatments for 0-10 cm soil depth. The trend was similar for the 10-20 cm depth. The water-soluble C and N fractions showed a decreasing trend with the increasing depth in all the treatments (Table 4.3). Different rates of inorganic fertilizer did not show any increase in the concentration of CWC and HWC when compared with the CK for both the soil depths. Water-extractable organic N, though representing only a small portion, showed the highest increase with the manure addition compared to the inorganic fertilizers and CK. Benbi et al. (2015) reported that long-term (11-year) addition of organic manure through farmyard manure and rice straw improved water extractable organic C fractions. Earlier studies have also shown that manure application practices play a vital role in determining the labile fractions of C and N in soils (Gong et al., 2009a). The response of SOC fractions to management indicates that added organic matter, aboveground biomass, and root exudates contain soluble fractions of organic C (Chantigny et al., 2002; Lu et al., 2004; Sekaran et al., 2019). Our results are similar to those reported by Benbi et al. (2015), Wijanarko and Purwanto (2017), Xu et al. (2011), and Liang et al. (2011), who reported increase in water soluble organic C and N as a result of manure or crop residue application. Organic manure significantly increased the water soluble fractions of C and N, indicating that organic matter contains more water-soluble organic fractions (Gong et al., 2009b).

Repeated manure application accumulates organic matter and increases soil carbon stock which acts as a substrate to enhance soil microbial activity and biomass (Xu et al., 2018). In our study, HM treatment has higher MBC and MBN as compared to the CK and inorganic fertilizer treatment for both depths. The increased soil MBC and MBN in the manure-applied treatments may be due to the addition of organic matter, which activates the soil indigenous microbiota and addition of microbial populations in the organic manure. Manure application enhanced soil microbial biomass with the additional C sources those are beneficial for the growth of soil microbes and increasing the soil fertility (Juan et al., 2008; Li et al., 2015). Furthermore, application of inorganic fertilizers can also show positive as well as negative effects on soil microbial biomass. Some researchers reported decrease in microbial biomass with the addition of mineral fertilizer (Abbasi and Khizar, 2012). In our study, these effects were not observed, and even the higher rate of inorganic fertilizer input treatment had a similar microbial biomass with that of CK. Li et al. (2015) also did not found any increase in MBC due the higher inorganic fertilizer.

The HM treatment enhanced soil enzymatic activities significantly compared to that under MF and CK treatments at both the soil depths in either year. It might be due to the fact that, continuous application of organic manure for 16 years improved the SOC content (Ozlu and Kumar, 2018). The SOC is the main substrate for enzyme activities in the soil, therefore, higher SOC under HM treatment could be the possible reason for higher enzyme activities in the manure treatment as compared to that under chemical fertilizer application. The higher organic C fractions observed in HM treatment showed that there was enough and favorable substrate available in this soil, which triggered the microbial activity. High microbial biomass C and N, and organic C fractions represent high microbial growth and activity. It is well known that β-glucoside enzyme acting as the catalysts in the hydrolysis of cellobiose. These reactions produce products those are important sources of energy for soil microbes (Tabatabai, 1994). Medina et al. (2004) reported that more than 2 times in the organic amended soil as compared to the non-amended soil increased β-glucosidase activity. Urease enzyme acts as a catalyst for hydrolysis of urea and urea-associated compound into CO_2 and NH_3 (Das and Varma, 2010). It originates from microorganisms, and presence of urea and alternative N sources enhance the urease activity, whereas, presence of NH_4^+ in the cell of microorganism depresses the urease enzyme production (Geisseler et al., 2010). The increase in urease activity under manure application shows the close relationship of this enzyme with soil organic matter and N cycling. Whereas, the decrease in activity of urease in soils with long-term nitrogen fertilization, can be a result of the absorption of mineral N by soil microorganisms due to higher accumulation of ammonia (Konig et al., 1966). Phosphatases enzymes (acid and alkaline) play a major role in P cycling for release of bioavailable inorganic phosphorus (P) from organic form of P in soil (Nannipieri et al., 2011). Studies reported that long-term chemical N fertilizer addition results decrease in P availability as well as suppress some valuable bacterial phoD gene community (Chen et al., 2019), whereas, addition of manure can enhance P availability as well as bacterial phoD gene community. Long-term manure application increases enzyme activities and legacy effect of manure was observed even after 29 years of manure application (Lupwayi et al., 2019). Generally, enzyme activities decrease with the increase in the depth of soil (Ma et al., 2010). Similar trend was observed in our study where lower enzyme activities were observed in the 10-20 cm soil depth as compared to that in the 0-10 cm depth (Figure 4.1, 4.2 and 4.3). Enzyme activities are generally higher on top soil than the lower soil depth due to higher content of soil organic matter and microbial biomass C, which would stimulate the activity of microorganism, and accelerate the rate of enzyme activities (Ma et al., 2010). Since the plot were minimum tilled, the manure application may have least impacted the soil organic matter and microbial biomass C at lower depth, hence, we did not find much differences among the treatments at the lower (10-20 cm) depth.

Several studies have reported that fertilizer management affects microbial diversity (Böhme et al., 2005; Zhang et al., 2012). Our results clearly demonstrated that organic manure addition had significant effects on the size and structure of soil microbial communities in 2018 and 2019 (Table 4.1a and 4.1b). The HM treatment significantly increased the PLFA biomarkers for bacteria, actinomycetes, total fungi, AMF, and saprophytes, while the inorganic fertilizer decreased the PLFA biomass (Table 4.1a and 4.1b). Compared to CK, the microbial biomass that was marked by total PLFAs significantly increased with manure addition, and the value was higher in 2019. Whereas, total PLFA decreased in CK plot in 2019 as compared to the 2018. There were significantly more PLFA biomass at 0-10 cm under HM treatment in 2018, whereas, HM and MM treatments significantly improved the PLFA biomass at 0-10 cm depth in 2019 as compared to CK.

Organic fertilizers release the nutrient slowly during decomposition. The continued effect after suspending application of organic fertilizer are called legacy effect or residual effect (Zhang et al., 2018). This indicates that organic manures significantly improved the soil fertility status, which enhanced the microbial community biomass and activity (Stark et al., 2007). In the present study, the percentage of Gram-positive bacteria was higher than the Gram-negative bacteria in both the years. This shift in microbial community structure is indicative of a more copiotrophic community, i.e. a higher level of abundance of Gram-positive bacteria when more organic matter derived soluble C is available. Similarly, Fanin et al. (2014) and Kramer and Gleixner (2008) showed that Gram-positive bacteria preferentially uses more complex sources like older soil organic matter derived C, and that Gram-negative bacteria use recent plant-derived C sources. Several studies have documented the effects of nutrient management on microbial community composition using PLFA analysis (Böhme et al., 2005; Lupwayi et al., 2018; Weitao et al., 2018). Manure application enhanced PLFA biomass, whereas, nitrogen fertilizer had no effect (Lupwayi et al., 2018). Soil management practices, such as manuring, which result in accumulation of organic carbon can result in increased microbial biomass and changes in community structure (Peacock et al., 2001). Stark et al. (2007) also reported that addition of organic matter ultimately enhanced the soil microbial biomass and activity. Effects of inorganic fertilizers on soil microbial community structure varied; they can have positive effect directly because of nutrients being added to the soil (Lupwayi et al., 2012) as well as indirect positive effect because of increased root exudates by crops or crop biomass which adds organic C (Geisseler and Scow, 2014). Inorganic fertilization can have direct negative effect due to acidification which can lead to changes in soil microbial community composition (Peacock et al., 2001). Organic manure application is rich in organic matter, N, P, and K, and other nutrients. Therefore, long-term application of manure not only increase the nutrient status and organic matter content of the soil, but also increase the abundance of certain bacteria beneficial to the nutrient solubilization, biochemical activities, and organic matter decomposition.

Long-term manure application has been reported to improve soil quality indicators (Ozlu et al., 2019). Improvement in the soil quality indicators results in higher value of SMAF scores, thereby, increasing SQI values. Jokela et al., (2009) reported that 4 year of manure application did not change the soil quality index. Since, SQI index is sensitive to soil function, a higher value of SOM and improved physical properties can increase the value of SQI index (Cherubin et al., 2016). The SQI for 10-20 cm was lower than that for the 0-10 cm soil depth. Similar findings were reported by Cherubin et al. (2016) in Brazil. This was attributed to the less C accumulation and lower microbial activities on lower depth.

4.5 Conclusions

A long-term study was conducted to assess the impacts of manure application and inorganic fertilization on selected soil biochemical and microbial parameters for two depths (0-10 and 10-20 cm). The following conclusions were drawn from this study and those are mentioned below as:

- In general, HM increased the carbon fractions and β-glucosidase enzyme activity as compared to CK for 0-10 cm, indicating the carbon stability and carbon cycling in manure applied system. Carbon and nitrogen fractions increased with the higher manure application for both depths, but were not affected by fertilization; however, only cold water nitrogen (CWN) was increased by MF treatment as compared to the CK for 0-10 cm depth, whereas, both MF and HF increased the CWN by 1.21 and 0.86 times, respectively, for 10-20 cm soil depth.
- Higher manure application increased microbial community structure, whereas, fertilizer application did not alter microbial community compared to CK in 2018 and 2019.

- Alkaline phosphatase activity was higher under MM and HM when compared to CK for 0-10 cm soil in both years.
- Manure application enhanced the soil quality index (SQI), however, fertilizer application did not impact the SQI.

We can conclude from this study that manure addition positively influences the C and N dynamics as well as microbial community structure as compared to the mineral fertilizer and control treatments. However, further investigation needed that can study the environmental and economic benefits associated with manure and fertilization application in South Dakota. This research may be beneficial in improved understanding of the relationship between soil fertilization strategy, soil biochemical properties, and overall soil health which can contribute to the development for effective nutrient management system toward sustainability.

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Table 4.1 Response of total, total bacterial, actinomycetes, Gram-negative bacterial, Gram-positive bacterial, total fungi, arbuscular mycorrhizal fungi (AMF) and saprophytes biomass PLFAs as influenced by manure (low, LM, based on P requirement; medium, MM, based on N requirement; and high, HM, double rate of manure based on N), and fertilizer (medium, MF, recommended rate; and high, HF, higher dose) application, and the control (CK) treatments (TRT) at 0-10 cm soil depth in 2018 and 2019.

TRT	**Total**	**Total Bacterial**	**Actinomycetes**	**Gram (-ve)**	**Gram (+ve)**	**Total Fungi**	**AMF**	**Saprophytes**
	ng PLFA-C g^{-1} soil							
	2018							
CK	3147^{b}[†]	1532^{b}	302^{b}	512^{b}	1019^{b}	298^{b}	117^{bc}	182^{b}
MF	2611^{b}	1250^{b}	263^{b}	389^{b}	861^{b}	222^{b}	66^{c}	155^{b}
HF	2902^{b}	1431^{b}	310^{b}	493^{b}	938^{b}	277^{b}	88^{c}	188^{b}
LM	3713^{b}	1860^{b}	334^{b}	634^{b}	1226^{ab}	358^{b}	108^{bc}	234^{b}
MM	3754^{ab}	1854^{b}	327^{b}	691^{b}	1163^{b}	382^{ab}	148^{ab}	295^{ab}
HM	5355^{a}	2815^{a}	497^{a}	1066^{a}	1749^{a}	651^{a}	225^{a}	430^{a}
	Analysis of Variance (*P*>*F*)							
Trt	0.017	0.012	0.032	0.005	0.025	0.027	0.005	0.034
	2019							
CK	2563^{c}[†]	1369^{b}	282^{c}	444^{b}	926^{c}	205^{bc}	90^{bc}	115^{b}
MF	2770^{c}	1496^{b}	309^{c}	519^{b}	977^{c}	252^{bc}	103^{bc}	149^{b}
HF	2404^{c}	1389^{b}	287^{c}	390^{b}	999^{c}	87^{c}	20^{c}	67^{b}
LM	3171^{c}	1729^{b}	355^{c}	635^{b}	1094^{c}	362^{ab}	168^{ab}	194^{ab}
MM	4448^{b}	2501^{a}	489^{b}	1007^{a}	1494^{b}	539^{a}	248^{a}	291^{a}
HM	5694^{a}	3009^{a}	605^{a}	1217^{a}	1792^{a}	571^{a}	250^{a}	321^{a}
	Analysis of Variance (*P*>*F*)							
Trt	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	<0.001	0.005

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

Table 4.2 Response of total, total bacterial, actinomycetes, Gram-negative bacterial, Gram-positive bacterial, total fungi, arbuscular mycorrhizal fungi (AMF) and saprophytes biomass PLFAs as influenced by manure (low, LM, based on P requirement; medium, MM, based on N requirement; and high, HM, double rate of manure based on N), and fertilizer (medium, MF, recommended rate; and high, HF, higher dose) application, and the control (CK) treatments (TRT) at 10-20 cm soil depth in 2018 and 2019.

TRT	**Total**	**Total Bacterial**	**Actino mycetes**	**Gram (-ve)**	**Gram (+ve)**	**Total Fungi**	**AMF**	**Sapro phytes**
	ng PLFA-C g^{-1} soil							
	2018							
CK	1315^{b}	653^{b}	170^{b}	148^{b}	506^{b}	57.3^{b}	19.7^{b}	37.6^{b}
MF	1430^{b}	655^{b}	187^{b}	117^{b}	493^{b}	43.1^{b}	11.1^{b}	32.0^{b}
HF	1954^{ab}	908^{b}	260^{ab}	208^{b}	700^{ab}	91.2^{b}	26.4^{b}	64.8^{b}
LM	1574^{b}	797^{b}	231^{ab}	176^{b}	620^{b}	97.3^{b}	31.3^{b}	66.0^{b}
MM	1780^{b}	905^{b}	253^{ab}	190^{b}	715^{ab}	76.7^{b}	22.6^{b}	54.1^{b}
HM	2715^{a}	1395^{a}	356^{a}	444^{a}	951^{a}	264^{a}	84.0^{a}	181^{a}
	Analysis of Variance (*P*>*F*)							
Trt	0.021	0.024	0.039	0.020	0.039	0.012	0.021	0.011
	2019							
CK	1920^{a}	761^{a}	179^{a}	266^{a}	494^{a}	111^{a}	33.5^{a}	77.8^{a}
MF	1158^{a}	556^{a}	144^{a}	155^{a}	401^{a}	53.3^{a}	16.1^{a}	37.2^{a}
HF	1128^{a}	533^{a}	142^{a}	130^{a}	403^{a}	63.9^{a}	20.7^{a}	43.3^{a}
LM	1344^{a}	627^{a}	176^{a}	179^{a}	448^{a}	110^{a}	36.2^{a}	73.9^{a}
MM	1526^{a}	828^{a}	206^{a}	266^{a}	562^{a}	110^{a}	44.5^{a}	65.1^{a}
HM	1737^{a}	748^{a}	193^{a}	236^{a}	512^{a}	123^{a}	44.0^{a}	79.1^{a}
	Analysis of Variance (*P*>*F*)							
Trt	0.381	0.467	0.687	0.224	0.667	0.604	0.496	0.635

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

Table 4.3 Cold water soluble organic carbon (CWC) and nitrogen (CWN), hot water soluble organic N (HWN), and microbial biomass N (MBN) and hot water soluble organic C (HWC), and microbial biomass C (MBC) concentrations as influenced by manure (low, LM, based on P requirement; medium, MM, based on N requirement; and high, HM, double rate of manure based on N), and fertilizer (medium, MF, recommended rate; and high, HF, higher dose) application, and the control (CK) treatments (TRT) at 0-10 and10-20 cm soil depths in 2018.

TRT	**CWC**	**HWC**	**CWN**	**HWN**	**MBC**	**MBN**
	μg C g^{-1} soil		μg N g^{-1} soil		μg g^{-1} soil	
			0-10 cm			
CK	$15.4^{bc\dagger}$	54.0^{cd}	4.52^{c}	5.91^{bcd}	832^{b}	73.1^{bc}
MF	11.2^{c}	49.5^{d}	10.4^{ab}	4.70^{d}	762^{b}	72.2^{c}
HF	13.9^{c}	50.7^{cd}	9.11^{bc}	5.05^{cd}	788^{b}	75.5^{bc}
LM	18.9^{ab}	62.8^{bc}	8.91^{bc}	6.65^{bc}	883^{b}	90.8^{bc}
MM	19.4^{ab}	71.1^{b}	8.44^{bc}	7.44^{b}	990^{b}	105^{b}
HM	22.5^{a}	109^{a}	14.84^{a}	11.3^{a}	1671^{a}	163^{a}
			Analysis of Variance (*P*>*F*)			
Trt	0.015	<0.001	0.019	<0.001	<0.001	<0.001
			10-20 cm			
Ck	14.8^{ab}	33.0^{b}	2.82^{c}	3.29^{b}	645^{b}	34.8^{b}
MF	8.32^{c}	29.6^{b}	6.24^{a}	3.05^{b}	609^{b}	38.0^{b}
HF	11.9^{b}	33.2^{b}	5.25^{ab}	3.30^{b}	645^{b}	38.0^{b}
LM	13.1^{b}	32.8^{b}	4.93^{abc}	3.48^{b}	650^{b}	37.3^{b}
MM	13.0^{b}	35.3^{b}	4.09^{bc}	3.41^{b}	708^{b}	39.2^{ab}
HM	17.1^{a}	52.3^{a}	6.29^{a}	5.33^{a}	943^{a}	58.4^{a}
			ANOVA (*P*>*F*)			
Trt	<0.001	<0.001	0.017	<0.001	<0.001	0.026

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

Table 4.4 Soil management assessment framework (SMAF) scores of each indicator [pH, electric conductivity (EC), bulk density (BD), beta-glucosidase (BG), wet aggregate stability (AGG), microbial biomass carbon (MBC), soil organic carbon (SOC)], and soil quality index (SQI) as influenced by manure (low, LM, based on P requirement; medium, MM, based on N requirement; and high, HM, double rate of manure based on N), and fertilizer (medium, MF, recommended rate; and high, HF, higher dose) application, and the control (CK) treatments (TRT) at 0-10 and10-20 cm soil depths in 2018.

	SMAF scores of each indicator							
TRT	**pH**	**EC**	**BD**	**BG**	**AGG**	**MBC**	**TOC**	**SQI**
				0-10 cm				
CK	$0.99^{a\dagger}$	0.98^{a}	0.64^{a}	$0.02096^{bcd\ddagger}$	0.02096^{bcd}	0.939^{a}	0.837^{b}	$0.768^{cd\ddagger}$
MF	0.97^{a}	1.00^{a}	0.76^{a}	0.02088^{d}	0.02088^{d}	0.937^{a}	0.797^{b}	0.774^{bcd}
HF	0.99^{a}	1.00^{a}	0.59^{a}	0.02089^{cd}	0.02089^{cd}	0.872^{a}	0.850^{b}	0.755^{d}
LM	0.99^{a}	1.00^{a}	0.86^{a}	0.02113^{ab}	0.02113^{ab}	0.960^{a}	0.925^{a}	0.815^{ab}
MM	0.97^{a}	1.00^{a}	0.76^{a}	0.02106^{abc}	0.02106^{abc}	0.976^{a}	0.926^{a}	0.806^{abc}
HM	0.98^{a}	1.00^{a}	0.95^{a}	0.02115^{a}	0.02115^{a}	1.00^{a}	0.978^{a}	0.829^{a}
				Analysis of Variance ($P>F$)				
Trt	0.434	0.465	0.215	0.017	0.036	0.408	0.001	0.0574
				10-20 cm				
CK	1.00^{a}	0.79^{b}	0.38^{a}	0.02070^{b}	-	$0.729^{b\ddagger}$	0.718^{cd}	$0.607^{c\dagger}$
MF	0.98^{a}	1.00^{a}	0.38^{a}	0.02069^{b}	-	0.737^{b}	0.708^{d}	0.638^{bc}
HF	0.98^{a}	0.96^{a}	0.44^{a}	0.02076^{a}	-	0.761^{b}	0.752^{bc}	0.653^{bc}
LM	1.00^{a}	1.00^{a}	0.45^{a}	0.02076^{a}	-	0.790^{b}	0.756^{bc}	0.668^{ab}
MM	0.99^{a}	0.96^{a}	0.44^{a}	0.02073^{ab}	-	0.783^{b}	0.771^{ab}	0.660^{ab}
HM	0.99^{a}	1.00^{a}	0.44^{a}	0.02079^{a}	-	0.980^{a}	0.807^{a}	0.706^{a}
				Analysis of Variance (P>F)				
Trt	0.368	0.070	0.535	0.009		0.066	0.003	0.0178

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

[‡] Mean values within the same column followed by different small letters are significantly different at $p<0.10$ for treatment for each depth.

Table 4.5 β-Glucosidase, urease and alkaline phosphatase enzyme activity as influenced manure (low, LM, based on P requirement; medium, MM, based on N requirement; and high, HM, double rate of manure based on N), and fertilizer (medium, MF, recommended rate; and high, HF, higher dose) application, and the control (CK) treatments(TRT) at 0-10 cm and 10-20 cm soil depths for 2018.

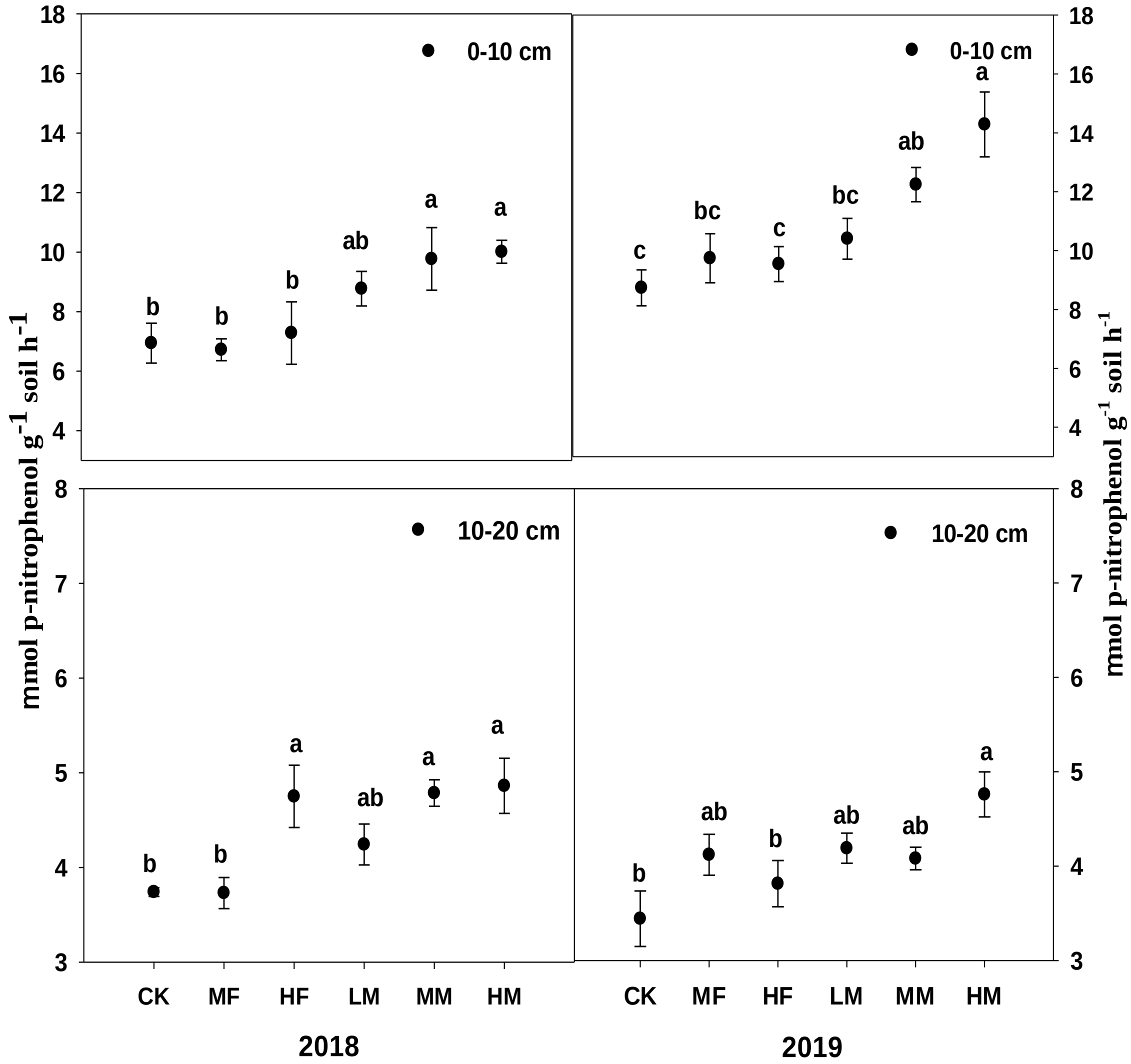
(Trt)	**β-Glucosidase (μg PNP g^{-1} soil h^{-1})**	**Urease (μgNH_4-N g^{-1} soil h^{-1})**	**Alkaline phosphatase (μg pNPg^{-1} soil h^{-1})**
		0-10 cm	
CK	$6.94^{b\dagger}$	4.02^{b}	376^{b}
MF	6.72^{b}	2.95^{b}	228^{c}
LM	8.77^{ab}	3.74^{b}	486^{b}
MM	9.77^{a}	4.04^{b}	498^{b}
HM	10.0^{a}	6.20^{a}	760^{a}
HF	7.28^{b}	2.51^{b}	221^{c}
		Analysis of Variance ($P>F$)	
Trt	0.020	0.003	<0.001
		10-20 cm	
CK	3.74^{b}	4.05^{a}	205^{d}
MF	3.73^{b}	3.69^{a}	296^{c}
LM	4.24^{ab}	5.27^{a}	321^{bc}
MM	4.79^{a}	5.61^{a}	382^{ab}
HM	4.86^{a}	5.25^{a}	423^{a}
HF	4.75^{a}	5.02^{a}	300^{c}
		Analysis of Variance (P>F)	
Trt	0.003	0.428	0.0002

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

Table 4.6 β-Glucosidase, urease and alkaline phosphatase enzyme activity as influenced by manure (low, LM, based on P requirement; medium, MM, based on N requirement; and high, HM, double rate of manure based on N), and fertilizer (medium, MF, recommended rate; and high, HF, higher dose) application, and the control (CK) treatments (TRT) at 0-10 cm and 10-20 cm soil depths for 2019.

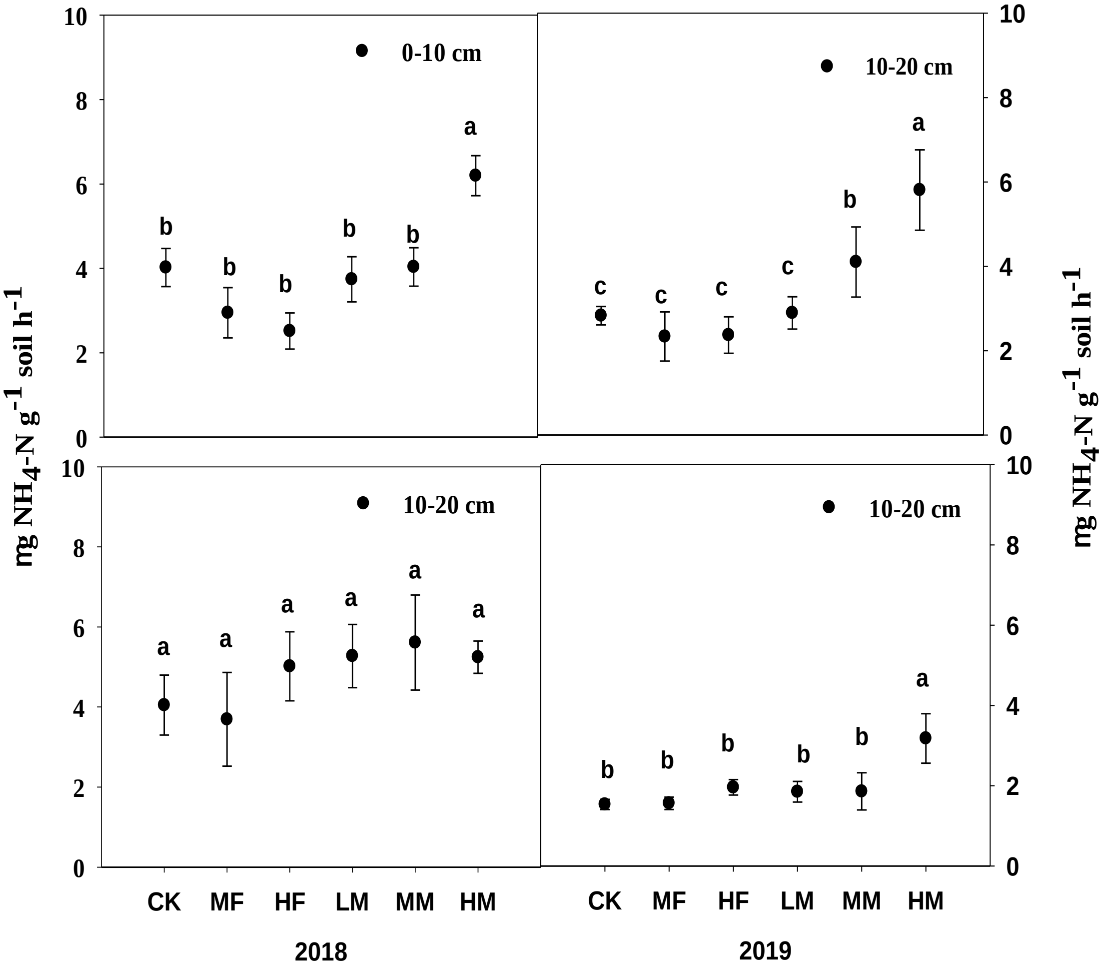
TRT	**β-Glucosidase (µg PNP g^{-1} soil h^{-1})**	**Urease (µgNH$_4$-N g^{-1} soil h^{-1})**	**Alkaline phosphatase (µg pNPg^{-1} soil h^{-1})**
		0-10 cm	
CK	$8.74^{c\dagger}$	2.83^{c}	104^{cd}
MF	9.74^{bc}	2.34^{c}	60^{d}
LM	10.4^{bc}	2.90^{c}	193^{bc}
MM	12.2^{ab}	4.10^{b}	279^{b}
HM	14.3^{a}	5.81^{a}	425^{a}
HF	9.55^{c}	2.37^{c}	230^{b}
		Analysis of Variance (*P*>*F*)	
Trt	0.002	<0.001	<0.001
		10-20 cm	
CK	3.44^{b}	1.53^{b}	40.4^{a}
MF	4.12^{ab}	1.56^{b}	93.2^{a}
LM	4.19^{ab}	1.85^{b}	56.1^{a}
MM	4.08^{ab}	1.86^{b}	78.4^{a}
HM	4.76^{a}	3.18^{a}	75.3^{a}
HF	3.81^{b}	1.96^{b}	101^{a}
		Analysis of Variance (P>F)	
Trt	0.022	0.014	0.832

[†] Mean values within the same column followed by different small letters for different depths are significantly different at $p<0.05$ for treatments.



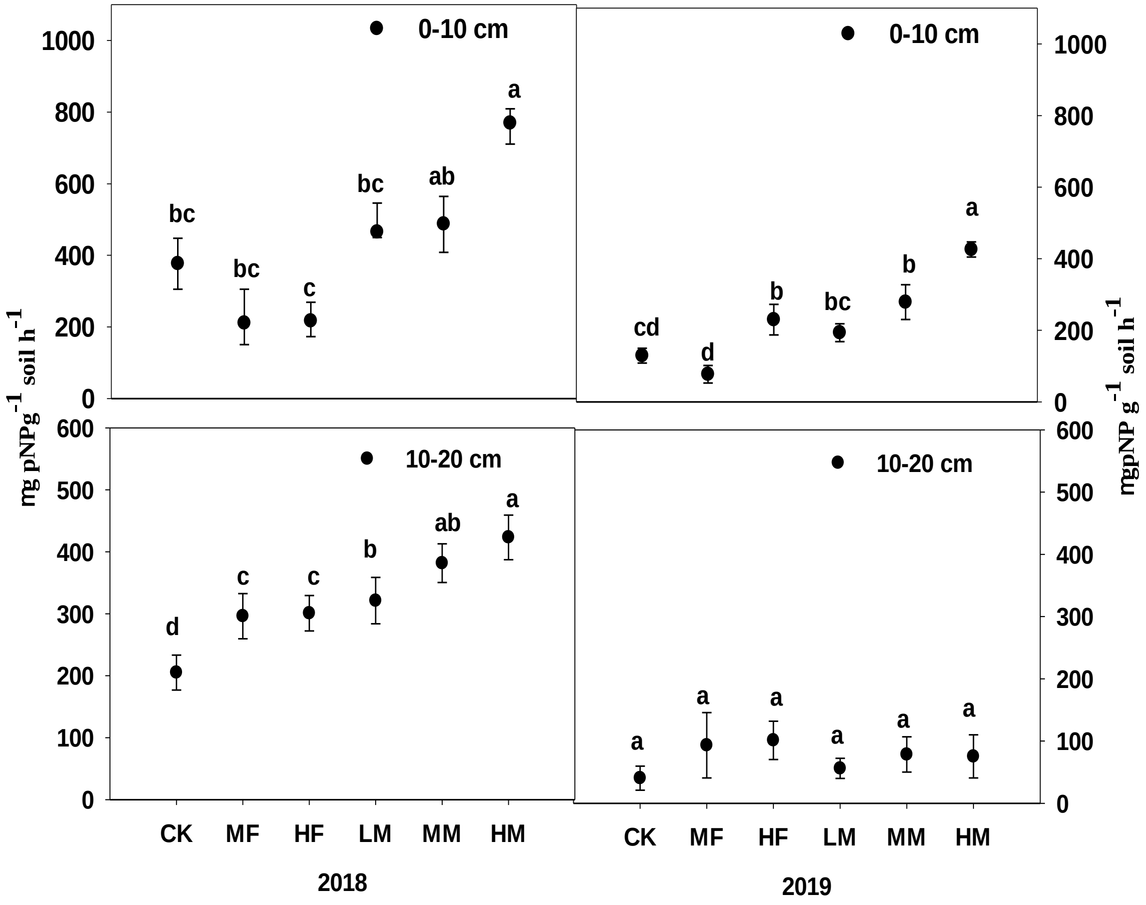
† Different small letters are significantly different for each depth at $p<0.05$ for treatment.

Figure 4.1 β-Glucosidase enzyme activity (μ mol p-nitrophenol g^{-1} soil h^{-1}) as influenced by manure (low, LM, based on P requirement; medium, MM, based on N requirement; and high, HM, double rate of manure based on N), and fertilizer (medium, MF, recommended rate; and high, HF, higher dose) application, and the control (CK) treatment at 0-10 and10-20 cm soil depths in 2018 and 2019.



†Different small letters are significantly different for each depth at p<0.05 for treatment.

Figure 4.2 Urease enzyme activity (μg N-NH_4^+ g^{-1} soil h^{-1}) as influenced by manure (low, LM, based on P requirement; medium, MM, based on N requirement; and high, HM, double rate of manure based on N), and fertilizer (medium, MF, recommended rate; and high, HF, higher dose) application, and the control (CK) treatment at 0-10 cm and 10-20 cm soil depths in 2018 and 2019.



†Different small letters are significantly different for each depth at $p<0.05$ for treatment.

Figure 4.3 Alkaline phosphatase enzyme activity (µg pNP g^{-1} soil h^{-1}) as influenced by manure (low, LM, based on P requirement; medium, MM, based on N requirement; and high, HM, double rate of manure based on N), and fertilizer (medium, MF, recommended rate; and high, HF, higher dose) application, and the control (CK) treatment at 0-10 cm and 10-20 cm soil depths in 2018 and 2019.

CHAPTER 5

CONCLUSIONS

A study was conducted in South Dakota to investigate the long-term manure and inorganic fertilization impacts on soil aggregate stability, organic carbon and nitrogen in different aggregate fractions, and microbial activity. The following conclusions were drawn from this study, and those are mentioned below as:

Study 1- Long-term impact of manure application and inorganic fertilization on soil organic carbon, nitrogen, and aggregate stability.

I. Manure application, in general, increased the aggregate stability, and aggregate associated SOC and TN as compared to the CK in all the aggregate size fractions. Further, higher manure application increased the SOC and TN as compared to the CK.

II. Higher manure application increased the SOM, coarse POM and fine POM, whereas, fertilizer application did not influence these parameters.

III. Higher manure application decreased soil bulk density as compared to the CK and HF for 0-10 cm soil depth in either years.

Study 2- Long-term impacts of manure application and inorganic fertilization on selected soil biochemical and microbial properties.

I. High manure (HM), in general, increased the carbon fractions and β-glucosidase enzyme activity as compared to the CK for 0-10 cm, indicating the carbon stability and carbon cycling in manure applied system.

II. High manure application increased the microbial community structure, whereas, fertilizer application did not alter microbial community compared to the CK in 2018 and 2019.

III. Alkaline phosphatase activity was higher under MM and HM compared to the CK for 0-10 cm soil in both years.

IV. Soil quality index (SQI) was enhanced with manure application, whereas, no differences were observed by inorganic fertilizer application.

We can conclude from this study that manure addition can positively influence the C and N dynamics, microbial community structure, aggregate stability as compared to the mineral fertilizer and control treatments. However, higher application rate of manure and fertilizer can be very detrimental to the soils and the environment. This was not the scope of the present work, and this can be investigated in the future to study the environmental and economic benefits associated with manure and fertilization application in South Dakota.

APPENIX AND SUPPORTING MATERIALS

SUPPORTING MATERIALS

S1. Nutrient applied in each treatment during spring 2018

Nutrient application in different treatments

TRT	avg soil N (kg/ha)	N to add	Available N (g/kg)	Manure Rate (tons/ac)	Available P (g/kg)	Estimated P removal (kg/ha)	P recommended
MF	30.27	182	n/a	n/a	n/a	74.5	60.53
CK	33.63	n/a	n/a	n/a	n/a	74.5	n/a
HF	36.99	176	n/a	n/a	n/a	74.5	n/a
LM	39.24	174	4.4	15916	4.7	74.5	60.53
MM	57.17	158	4.4	40126	4.7	74.5	n/a
HM	93.04	126	4.4	80477	4.7	74.5	n/a

*where available N is estimated as half of organic N plus NH4+NO3 | Olsen P at 8.1, K- 171 ppm

N goal based on 190 bu/ac yield goal times 1.1 lb N per bushel

Used P removal to guide manure rate as it was greater than the P soil test recommendation

Fert recommended dose according to EC 2005 is 204,54.9,0 N, P_2O_5, and K_2O kg/ha

Higher fertilizer recommendation is 224-78.5-67.3 kg/ha for corn, No fert for soybean

S2. Mean Manure nutrient analysis of beef manure in 2018

Manure	**Moisture**	**Dry matter**	**NH4-N**	**Organic N**	**Total P_2O_5**	**Total K K_2O**	**Available N**
	%				g/kg as is		
Beef	51.72	48.28	0.75	7.25	6.7	6.3	4.4

APPENDIX 1

A.1.1 Response of large macroaggregates (LMA>2 mm), small macroaggregates (SMA, 2-0.25 mm), micro aggregates (MI, 0.25-0.053), sand clay (SC,<0.053 mm), mean weight diameter (MWD) and wet stable aggregates (WSA)as influenced by manure (low, LM, based on P requirement; medium, MM, based on N requirement; and high, HM, double rate of manure based on N), and fertilizer (medium, MF, recommended rate; and high, HF, higher dose) application, and the control (CK) treatments (TRT) at 0-10 cm soil depth in 2019.

TRT	**Rep**	**Initial wt**	**LMA**	**SMA**	**MI**	**SC**	**MWD**	**WSA**
		Gm		%			mm	%
CK	1	100	9.2	41.6	22.3	26.9	0.748	50.8
MF	1	100	17.3	43.3	22.5	17.0	1.105	60.5
LM	1	100	27.7	42.5	14.3	15.6	1.499	70.1
MM	1	100	22.5	48.8	17.3	11.4	1.576	71.3
HM	1	100	32.3	54.1	10.2	3.3	1.894	86.5
HF	1	100	21.2	36.6	20.1	22.1	1.237	57.8
CK	2	100	24.1	36.1	14.5	25.3	1.473	60.2
MF	2	100	19.3	41.0	18.5	21.2	1.265	60.3
LM	2	100	32.5	41.4	12.0	14.1	1.978	73.9
MM	2	100	39.1	40.8	9.4	10.6	2.334	79.9
HM	2	100	34.7	49.6	15.1	0.7	2.128	84.2
HF	2	100	19.5	44.8	17.9	17.8	1.258	64.3
CK	3	100	16.8	44.2	16.4	22.6	1.196	61.0
MF	3	100	16.4	40.9	11.6	31.2	1.019	57.2
LM	3	100	30.7	40.4	23.9	5.0	1.658	71.1
MM	3	100	29.3	49.7	14.1	6.9	1.688	78.9
HM	3	100	40.1	47.2	10.4	2.3	2.204	87.3
HF	3	100	12.9	43.2	20.1	23.8	0.990	56.1
CK	4	100	24.0	42.6	19.9	13.5	1.455	66.6
MF	4	100	15.5	44.4	22.3	17.8	1.067	59.9
LM	4	100	28.7	45.7	15.8	9.8	1.700	74.4
MM	4	100	28.8	47.1	14.8	9.4	1.751	75.8
HM	4	100	37.4	47.1	8.4	7.0	2.149	84.6
HF	4	100	16.0	44.0	20.3	19.6	1.091	60.0

A.1.2 Response of large macroaggregates (LMA>2 mm), small macroaggregates (SMA, 2-0.25 mm), micro aggregates (MI, 0.25-0.053), sand clay (SC,<0.053 mm), mean weight diameter (MWD) and wet stable aggregates (WSA)as influenced by manure (low, LM, based on P requirement; medium, MM, based on N requirement; and high, HM, double rate of manure based on N), and fertilizer (medium, MF, recommended rate; and high, HF, higher dose) application, and the control (CK) treatments (TRT) at 0-10 cm soil depth in 2019.

TRT	**Rep**	**Initial wt**	**LMA**	**SMA**	**MI**	**SC**	**MWD**	**WSA**
		gm		%			mm	%
CK	1	100.001	6.179	43.115	19.662	31.045	1.363	49.3
MF	1	100.002	17.862	34.800	14.725	32.615	1.676	52.7
LM	1	100.008	9.161	40.527	20.268	30.052	3.019	49.7
MM	1	100	20.151	45.682	17.288	16.879	2.091	65.8
HM	1	100.004	32.048	50.500	13.256	4.200	3.056	82.5
HF	1	100.01	16.232	36.497	28.903	18.378	1.525	52.7
CK	4	100	16.698	43.786	15.452	24.064	1.627	60.5
MF	4	100.003	13.294	35.986	18.351	32.372	1.747	49.3
LM	4	100.009	21.617	44.181	12.423	21.788	2.122	65.8
MM	4	100.004	29.687	39.907	11.815	18.595	2.124	69.6
HM	4	100.003	35.739	49.743	9.682	4.839	2.945	85.5
HF	4	100.006	11.598	34.399	21.990	32.019	1.379	46.0
CK	3	100	19.735	40.157	19.253	20.855	1.891	59.9
MF	3	100	15.547	31.090	18.401	34.962	1.325	46.6
LM	3	100.005	34.324	37.902	15.599	12.180	3.465	72.2
MM	3	100	35.006	51.008	9.451	4.535	2.922	86.0
HM	3	100	35.202	39.986	14.194	10.618	2.661	75.2
HF	3	100.01	16.353	34.776	23.779	25.102	1.657	51.1
CK	2	100.01	18.790	39.045	17.928	24.247	1.883	57.8
MF	2	100.01	14.885	32.791	22.059	30.275	1.758	47.7
LM	2	100	37.297	40.606	11.564	10.533	2.325	77.9
MM	2	100	22.992	48.001	9.883	19.124	3.154	71.0
HM	2	100	36.804	40.911	16.094	6.191	3.045	77.7
HF	2	100.01	19.691	38.108	14.747	27.464	1.704	57.8

A.1.3 Response of aggregate associated soil organic carbon (SOC, g kg^{-1}) and nitrogen (TN, g kg^{-1}) in 8-4 mm, 4-2 mm and 2-1 mm size water stable aggregates influenced by manure (low, LM, based on P requirement; medium, MM, based on N requirement; and high, HM, double rate of manure based on N), and fertilizer (medium, MF, recommended rate; and high, HF, higher dose) application, and the control (CK) treatments (TRT) in 2019.

		8- 4mm		**4-2 mm**		**2-1 mm**	
TRT	**Rep**	**SOC**	**TN**	**SOC**	**TN**	**SOC**	**TN**
		g kg^{-1}		**g kg^{-1}**		**g kg^{-1}**	
CK	1	25.2	2.5	28.0	2.5	30.4	2.7
MF	1	25.5	2.3	25.1	2.2	26.6	2.3
LM	1	23.6	2.1	29.8	2.7	29.3	2.7
MM	1	27.4	2.4	28.4	2.5	30.0	2.7
HM	1	39.4	3.9	41.6	3.9	40.7	3.8
HF	1	24.6	2.3	25.3	2.4	25.8	2.3
CK	2	22.3	2.3	22.5	2.3	24.0	2.5
MF	2	22.5	2.2	22.6	2.2	22.4	2.2
LM	2	30.3	2.8	30.6	3.0	30.6	3.0
MM	2	28.1	2.8	27.1	2.8	26.9	2.6
HM	2	39.4	3.9	38.7	3.9	39.6	3.9
HF	2	25.0	2.4	23.9	2.3	24.7	2.4
CK	3	25.2	2.5	24.6	2.4	24.3	2.4
MF	3	24.5	2.3	24.0	2.3	23.4	2.3
LM	3	27.0	2.6	26.6	2.6	26.0	2.6
MM	3	27.4	2.4	28.4	2.5	30.0	2.7
HM	3	37.5	3.6	36.9	3.6	42.6	4.2
HF	3	25.4	2.6	25.3	2.4	25.8	2.3
CK	4	21.5	1.9	22.0	2.0	22.0	1.9
MF	4	27.2	2.2	25.9	2.3	26.1	2.3
LM	4	25.5	2.3	28.0	2.6	28.0	2.6
MM	4	28.5	2.5	28.4	2.5	27.0	2.4
HM	4	37.5	3.6	36.9	3.6	36.9	3.5
HF	4	24.6	2.3	23.5	2.2	24.6	2.3

A.1.4 Response of aggregate associated soil organic carbon (SOC, g kg^{-1}) and nitrogen (TN, g kg^{-1}) in 1-0.5 mm, 0.5-0.25mm and 0.25-0.053 mm size water stable aggregates influenced by manure (low, LM, based on P requirement; medium, MM, based on N requirement; and high, HM, double rate of manure based on N), and fertilizer (medium, MF, recommended rate; and high, HF, higher dose) application, and the control (CK) treatments (TRT) in 2019.

		1-0.5 mm		**0.5-0.25 mm**		**0.25-0.053 mm**	
TRT	**Rep**	**SOC**	**TN**	**SOC**	**TN**	**SOC**	**TN**
			g kg^{-1}	**g kg^{-1}**		**g kg^{-1}**	
CK	1	23.4	2.37	29.5	2.68	23.2	2.40
MF	1	24.7	2.22	25.5	2.32	23.5	2.09
LM	1	28.0	2.52	26.8	2.44	25.7	2.34
MM	1	30.5	2.74	29.7	2.64	29.0	2.64
HM	1	41.4	3.71	37.8	3.56	34.9	3.49
HF	1	25.2	2.42	26.0	2.46	23.5	2.32
CK	2	23.4	2.37	22.7	2.32	21.4	2.21
MF	2	22.3	2.31	22.8	2.06	21.8	2.12
LM	2	31.0	3.08	29.6	2.94	27.1	2.72
MM	2	27.0	2.69	26.7	2.59	24.8	2.52
HM	2	38.4	3.83	36.0	3.60	34.2	3.53
HF	2	23.8	2.23	23.9	2.37	24.6	2.41
CK	3	23.8	2.40	24.1	2.52	23.2	2.40
MF	3	23.0	2.26	21.9	2.19	20.7	2.12
LM	3	25.8	2.58	25.7	2.60	24.5	2.46
MM	3	34.2	3.31	31.9	3.21	28.4	2.87
HM	3	40.6	3.86	38.0	3.67	34.9	3.49
HF	3	25.2	2.42	26.0	2.46	23.5	2.32
CK	4	21.8	1.97	20.8	1.86	20.3	1.84
MF	4	25.4	2.12	24.0	2.14	24.8	2.26
LM	4	28.0	2.62	26.3	2.50	24.3	2.38
MM	4	26.3	2.33	25.8	2.40	23.4	2.19
HM	4	38.4	3.83	32.0	3.14	31.7	3.10
HF	4	23.9	2.24	23.3	2.26	22.6	2.26

A.1.5 Response of soil organic carbon (SOC) and total nitrogen (TN) as influenced by manure (low, LM, based on P requirement; medium, MM, based on N requirement; and high, HM, double rate of manure based on N), and fertilizer (medium, MF, recommended rate; and high, HF, higher dose) application, and the control (CK) treatments (TRT) at 0-10 cm, 10-20 cm, 20-30 cm and 30-40 cm soil depths in 2019.

		SOC				TN			
TRT	**Rep**	**0-10 cm**	**10-20 cm**	**20-30 cm**	**30-40 cm**	**0-10 cm**	**10-20 cm**	**20-30 cm**	**30-20 cm**
			g kg^{-1}				**g kg^{-1}**		
CK	1	29.5	27.0	25.0	21.6	3.29	3.03	2.90	2.65
MF	1	27.9	27.7	27.1	23.5	2.73	3.06	3.06	2.79
LM	1	33.5	28.4	28.4	25.1	3.62	3.13	3.21	2.87
MM	1	44.2	28.8	28.1	25.5	4.82	3.17	3.15	2.96
HM	1	57.7	30.7	27.2	22.8	6.36	3.46	3.16	2.76
HF	1	32.8	28.9	27.7	24.4	3.61	3.11	3.12	2.81
CK	2	29.1	25.4	23.0	7.8	3.32	2.90	2.79	2.05
MF	2	28.9	25.8	21.2	13.8	3.27	2.92	2.58	2.07
LM	2	36.6	26.4	23.7	17.7	4.10	3.02	2.82	2.37
MM	2	32.2	27.3	23.6	18.5	3.59	3.02	2.80	2.42
HM	2	38.9	27.2	25.7	20.6	4.35	3.02	2.99	2.67
HF	2	29.3	26.4	21.4	17.3	3.28	3.06	2.58	2.40
CK	3	30.5	25.4	19.3	14.5	3.50	2.96	2.47	2.17
MF	3	27.9	23.7	16.7	13.9	3.22	2.87	2.27	2.08
LM	3	33.6	26.2	21.0	15.7	3.79	3.00	2.59	2.29
MM	3	31.7	26.3	22.4	16.2	3.63	3.04	2.74	2.37
HM	3	42.2	28.2	23.0	16.5	4.59	3.20	2.74	2.68
HF	3	29.0	25.6	23.7	13.5	3.27	2.99	2.52	2.12
CK	4	27.7	25.6	21.6	18.4	3.12	2.87	2.60	2.52
MF	4	27.9	24.7	19.7	24.6	3.20	2.90	2.48	2.12
LM	4	30.4	26.3	23.4	20.1	3.41	3.08	2.65	2.29
MM	4	31.6	25.6	21.2	30.3	3.63	3.06	2.58	2.21
HM	4	38.6	27.1	23.5	23.3	4.33	3.11	2.83	2.75
HF	4	29.6	26.5	23.8	26.2	3.29	3.00	2.82	2.54

A.1.6 Response of soil organic matter (SOM), coarse particulate organic matter (coarse POM), fine POM, total POM and sand as influenced by manure (low, LM, based on P requirement; medium, MM, based on N requirement; and high, HM, double rate of manure based on N), and fertilizer (medium, MF, recommended rate; and high, HF, higher dose) application, and the control (CK) treatments (TRT) at 0-10 cm soil depth in 2019.

TRT	Rep	SOM	Coarse POM	Fine POM	Total POM	Sand
		mg g^{-1}				
CK	1	68.9	0.84	7.93	8.77	11.71
MF	1	65.0	0.63	5.09	5.72	11.73
LM	1	67.1	0.43	5.74	6.17	13.23
MM	1	68.5	0.82	6.92	7.74	11.97
HM	1	87.5	1.94	14.33	16.27	19.91
HF	1	74.7	1.22	10.35	11.57	12.18
CK	2	60.5	0.50	6.12	6.62	12.30
MF	2	61.5	0.46	5.63	6.09	12.22
LM	2	74.1	0.96	10.26	11.22	11.62
MM	2	71.5	1.04	8.89	9.93	14.88
HM	2	85.2	2.29	14.52	16.81	15.49
HF	2	67.2	0.95	8.32	9.27	11.72
CK	3	67.0	1.28	7.53	8.81	12.46
MF	3	62.3	0.94	6.37	7.31	17.13
LM	3	70.6	0.73	7.93	8.66	12.92
MM	3	77.3	1.31	10.88	12.19	13.07
HM	3	95.5	3.10	18.67	21.77	17.10
HF	3	73.0	1.42	8.84	10.26	11.33
CK	4	64.6	0.65	5.89	6.53	11.84
MF	4	73.5	1.67	8.48	10.16	12.53
LM	4	67.7	0.85	7.35	8.20	11.21
MM	4	65.8	1.36	8.49	9.85	15.82
HM	4	79.3	2.01	11.15	13.16	14.56
HF	4	65.3	0.98	6.43	7.41	12.45

A.1.7 Response of bulk density (BD, g cm^{-3}) as influenced by manure (low, LM, based on P requirement; medium, MM, based on N requirement; and high, HM, double rate of manure based on N), and fertilizer (medium, MF, recommended rate; and high, HF, higher dose) application, and the control (CK) treatments (TRT) at 0-10 cm, 10-20 cm, 20-30 cm and 30-40 cm soil depths in 2018.

		Bulk Density (g cm^{-3})			
TRT	**Rep**	**0-10 cm**	**10-20 cm**	**20-30 cm**	**30-40 cm**
CK	1	1.235	1.358	1.277	1.328
MF	1	1.127	1.401	1.276	1.175
LM	1	1.162	1.341	1.219	1.257
MM	1	1.137	1.369	1.274	1.167
HM	1	1.096	1.356	1.244	1.236
HF	1	1.193	1.337	1.267	1.262
CK	2	1.289	1.414	1.295	1.390
MF	2	1.330	1.468	1.372	1.374
LM	2	1.169	1.461	1.393	1.382
MM	2	1.127	1.376	1.360	1.310
HM	2	1.046	1.350	1.284	1.241
HF	2	1.349	1.419	1.323	1.377
CK	3	1.441	1.494	1.388	1.436
MF	3	1.188	1.388	1.370	1.333
LM	3	1.194	1.330	1.353	1.337
MM	3	1.405	1.372	1.349	1.408
HM	3	1.064	1.413	1.414	1.415
HF	3	1.357	1.370	1.264	1.403

A.1.8 Response of bulk density (BD, g cm^{-3}) as influenced by manure (low, LM, based on P requirement; medium, MM, based on N requirement; and high, HM, double rate of manure based on N), and fertilizer (medium, MF, recommended rate; and high, HF, higher dose) application, and the control (CK) treatments (TRT) at 0-10 cm, 10-20 cm, 20-30 cm and 30-40 cm soil depths in 2019.

		Bulk Density (g cm^{-3})			
TRT	**Rep**	**0-10 cm**	**10-20 cm**	**20-30 cm**	**30-40 cm**
CK	1	1.493	1.755	1.609	1.658
MF	1	1.536	1.784	1.559	1.730
LM	1	1.457	1.703	1.547	1.620
MM	1	1.564	1.736	1.629	1.702
HM	1	1.455	1.697	1.628	1.576
HF	1	1.575	1.608	1.616	1.717
CK	2	1.627	1.718	1.701	1.660
MF	2	1.618	1.727	1.722	1.764
LM	2	1.534	1.715	1.683	1.685
MM	2	1.744	1.730	1.709	1.728
HM	2	1.546	1.737	1.815	1.684
HF	2	1.661	1.724	1.709	1.807
CK	3	1.557	1.729	1.640	1.653
MF	3	1.661	1.703	1.738	1.766
LM	3	1.461	1.741	1.706	1.736
MM	3	1.552	1.756	1.701	1.741
HM	3	1.459	1.724	1.664	1.555
HF	3	1.602	1.732	1.675	1.746
CK	4	1.833	1.670	1.689	1.702
MF	4	1.563	1.761	1.775	1.740
LM	4	1.674	1.796	1.718	1.741
MM	4	1.681	1.761	1.671	1.699
HM	4	1.453	1.456	1.747	1.712
HF	4	1.712	1.757	1.639	1.694

APPENDIX 2

A.2.1 Cold water (CWC) and hot water soluble organic carbon (HWC) (µg C g-1 soil), cold water (CWN) and hot water soluble nitrogen (HWN) (µg N g^{-1} soil) concentrations as influenced by manure (low, LM, based on P requirement; medium, MM, based on N requirement; and high, HM, double rate of manure based on N), and fertilizer (medium, MF, recommended rate; and high, HF, higher dose) application, and the control (CK) treatments (TRT) at 0-10 and10-20 cm soil depths in 2018.

		0-10 cm				10-20 cm			
TRT	**Rep**	**CWC**	**HWC**	**CWN**	**HWN**	**CWC**	**HWC**	**CWN**	**HWN**
		µg C g-1 soil		µg N g^{-1} soil		µg C g-1 soil		µg N g^{-1} soil	
CK	1	3.14	64.00	2.70	6.83	19.99	37.81	3.57	3.67
MF	1	8.67	59.41	13.90	6.23	10.60	36.58	8.36	3.35
LM	1	21.74	66.28	7.34	7.50	16.16	37.78	4.88	3.68
MM	1	21.12	73.40	6.61	7.15	15.43	40.91	3.37	3.30
HM	1	22.97	125.70	21.45	14.15	17.50	62.96	7.68	6.63
HF	1	13.92	59.52	9.13	6.09	14.78	32.63	5.67	3.38
CK	2	23.55	56.62	5.45	6.45	17.24	30.51	3.09	3.16
MF	2	16.07	51.47	9.23	4.45	8.62	33.70	5.57	3.46
LM	2	20.34	61.92	9.21	6.18	13.58	32.45	3.76	3.42
MM	2	21.13	86.50	12.43	8.96	13.13	36.95	5.15	3.63
HM	2	21.66	104.51	11.59	9.08	16.36	46.54	4.71	4.61
HF	2	14.41	44.64	11.44	4.46	11.37	25.76	5.05	2.69
CK	3	20.72	52.07	5.87	5.35	10.30	28.51	2.61	3.12
MF	3	10.81	46.18	12.74	3.64	7.01	22.33	4.35	2.34
LM	3	20.80	60.41	6.08	6.72	11.78	29.46	3.69	3.17
MM	3	18.75	66.01	7.51	7.79	12.82	34.73	3.31	3.71
HM	3	23.26	117.71	17.02	12.21	21.58	48.00	6.55	4.98
HF	3	15.86	43.82	9.71	4.32	10.82	30.82	6.68	3.28
CK	4	14.06	43.30	4.05	5.35	11.63	35.25	2.03	3.21
MF	4	9.14	41.05	5.87	4.50	7.05	25.67	6.69	3.03
LM	4	12.69	62.66	13.01	6.22	10.72	31.66	7.39	3.64
MM	4	16.44	58.61	7.22	5.88	10.57	28.51	4.52	3.00
HM	4	22.31	89.48	9.28	9.62	12.95	51.52	6.21	5.10
HF	4	11.45	54.95	6.17	5.32	10.75	43.51	3.61	3.87

A.2.2 Microbial biomass carbon (MBC) and Microbial biomass N (MBN) concentrations (µg g^{-1} soil) as influenced by manure (low, LM, based on P requirement; medium, MM, based on N requirement; and high, HM, double rate of manure based on N), and fertilizer (medium, MF, recommended rate; and high, HF, higher dose) application, and the control (CK) treatments (TRT) at 0-10 cm and 10-20 cm soil depths in 2018.

		0-10 cm		10-20 cm	
TRT	**Rep**	**MBC**	**MBN**	**MBC**	**MBN**
		µg g^{-1} soil		µg g^{-1} soil	
CK	1	677.1	50.0	472.9	28.8
MF	1	884.4	89.9	453.5	37.0
LM	1	802.5	77.5	550.4	55.4
MM	1	859.5	95.0	469.5	47.6
HM	1	1639.2	207.7	899.2	78.7
HF	1	969.0	74.2	523.3	42.1
CK	2	894.3	71.8	607.6	44.5
MF	2	844.6	67.1	726.9	56.1
LM	2	872.7	82.3	661.1	27.4
MM	2	1194.8	115.8	892.2	40.0
HM	2	1448.0	153.5	930.8	61.9
HF	2	766.7	80.2	667.6	36.0
CK	3	1062.4	99.0	721.3	37.2
MF	3	711.5	65.6	651.3	27.8
LM	3	910.0	101.8	682.6	31.8
MM	3	887.7	119.3	682.1	33.7
HM	3	1871.5	160.7	1011.8	47.5
HF	3	570.0	62.5	640.8	31.5
CK	4	695.5	71.7	776.4	28.8
MF	4	607.3	66.3	605.3	31.2
LM	4	945.1	101.5	705.1	34.6
MM	4	1016.3	88.4	786.8	35.3
HM	4	1726.1	129.3	929.8	45.7
HF	4	846.9	84.9	746.3	42.2

A.2.3 Response of total, total bacterial, actinomycetes, Gram-negative bacterial, Gram-positive bacterial, total fungi, arbuscular mycorrhizal fungi (AMF) and saprophytes biomass PLFAs as influenced by manure (low, LM, based on P requirement; medium, MM, based on N requirement; and high, HM, double rate of manure based on N), and fertilizer (medium, MF, recommended rate; and high, HF, higher dose) application, and the control (CK) treatments (TRT) at 0-10 cm soil depth in 2018.

0-10 cm / 2018									
TRT	**Rep**	**Total Biomass**	**Total Bacterial**	**Actinomycetes**	**Gram neg**	**Gram pos**	**Total Fungi**	**AMF**	**Saprophytes**
ng PLFA-C g^{-1} soil									
HM	1	6244	490	1140	2124	667.1	178.6	488.5	490
HM	2	4851	497	1067	1536	693.3	245.8	447.5	497
HM	3	5381	491	1098	1747	592.9	216.7	376.2	491
HM	4	4942	510	960	1590	650.5	257.8	392.7	510
CK	1	1653	114	224	452	91.2	29.8	61.4	114
CK	2	4021	356	606	1253	330.4	129.2	201.2	356
CK	3	4709	511	923	1618	620.8	234.5	386.3	511
CK	4	2206	226	296	755	151.3	73.4	77.8	226
MF	1	1573	169	204	642	29.9	0.0	29.9	169
MF	2	2357	212	253	759	120.4	44.6	75.9	212
MF	3	3843	395	652	1188	420.5	136.2	284.3	395
MF	4	2669	275	448	854	316.0	84.8	231.2	275
MF	1	2462	233	309	765	212.6	58.8	153.8	233
HF	2	2218	253	353	816	168.4	51.4	117.0	253
HF	3	2780	315	479	892	226.0	64.1	161.9	315
HF	4	4147	439	831	1278	499.3	179.5	319.8	439
MM	1	2361	218	367	846	160.0	89.3	70.7	218
MM	2	3767	306	678	1139	373.4	136.2	237.2	306
MM	3	4067	315	782	1131	387.9	143.6	244.3	315
MM	4	4820	468	939	1535	606.5	221.4	385.1	468
LM	1	1963	173	196	724	64.7	12.4	52.3	173
LM	2	5216	439	919	1802	644.9	164.1	480.8	439
LM	3	4063	320	780	1147	347.7	119.9	227.9	320
LM	4	3609	405	641	1230	376.5	134.4	242.1	405

A.2.4 Response of total, total bacterial, actinomycetes, Gram-negative bacterial, Gram-positive bacterial, total fungi, arbuscular mycorrhizal fungi (AMF) and saprophytes biomass PLFAs as influenced by manure (low, LM, based on P requirement; medium, MM, based on N requirement; and high, HM, double rate of manure based on N), and fertilizer (medium, MF, recommended rate; and high, HF, higher dose) application, and the control (CK) treatments (TRT) at 10-20 cm soil depth in 2018.

10-20 cm / 2018									
TRT	**Rep**	**Total Biomass**	**Total Bacterial**	**Actinomycetes**	**Gram neg**	**Gram pos**	**Total Fungi**	**AMF**	**Saprophytes**
		ng PLFA-C g^{-1} soil							
HM	1	2805	1459	365	488	970	318.6	109.0	209.6
HM	3	1795	927	266	254	672	143.6	46.4	97.2
HM	4	3547	1798	438	588	1210	331.2	96.5	234.7
CK	1	939	473	116	69	404	9.6	0.0	9.6
CK	2	1134	588	172	69	519	10.7	0.0	10.7
CK	3	1786	931	228	271	660	93.0	36.1	56.9
CK	4	1402	622	164	181	441	116.1	42.9	73.2
MF	1	1216	610	184	66	544	26.5	13.5	13.0
MF	2	1646	776	207	115	661	35.7	0.0	35.7
MF	3	1146	526	169	63	463	30.0	12.9	17.2
MF	4	1711	708	189	226	482	80.3	17.9	62.4
MF	1	1523	627	161	95	532	19.0	0.0	19.0
HF	2	1655	670	156	151	518	30.0	0.0	30.0
HF	3	2569	1184	367	303	882	159.4	49.8	109.6
HF	4	2070	1153	356	283	870	156.5	55.8	100.7
MM	1	1816	903	234	137	766	36.8	7.8	29.0
MM	2	1417	637	161	89	548	17.6	0.0	17.6
MM	3	1869	995	296	275	720	134.7	43.1	91.6
MM	4	2018	1085	322	260	826	117.7	39.5	78.1
LM	1	1369	717	218	98	619	43.0	19.5	23.6
LM	2	2202	1120	283	349	772	217.0	64.5	152.5
LM	3	1410	682	212	134	548	67.1	23.7	43.4
LM	4	1315	669	211	125	544	62.2	17.6	44.6

A.2.5 Response of total, total bacterial, actinomycetes, Gram-negative bacterial, Gram-positive bacterial, total fungi, arbuscular mycorrhizal fungi (AMF) and saprophytes biomass PLFAs as influenced by manure (low, LM, based on P requirement; medium, MM, based on N requirement; and high, HM, double rate of manure based on N), and fertilizer (medium, MF, recommended rate; and high, HF, higher dose) application, and the control (CK) treatments (TRT) at 0-10 cm soil depth in 2019.

		0-10 cm / 2019							
TRT	**Rep**	**Total Biomass**	**Total Bacterial**	**Actinomycetes**	**Gram neg**	**Gram pos**	**Total Fungi**	**AMF**	**Saprophytes**
		ng PLFA-C g^{-1} soil							
CK	1	2429	1361	266	382	978	160.0	86.5	73.5
MF	1	3188	1681	326	606	1075	259.9	92.7	167.2
LM	1	3996	2141	398	829	1312	444.5	215.4	229.1
MM	1	4889	2764	515	1110	1654	556.8	262.5	294.3
HM	1	4984	2833	575	938	1895	496.3	256.4	239.9
HF	1	2770	1521	319	354	1167	55.1	0.0	55.1
CK	2	3739	2019	431	755	1264	479.5	221.7	257.9
MF	2	3748	2031	411	807	1224	450.4	199.2	251.2
LM	2	3646	1872	376	795	1076	580.2	246.6	333.6
MM	2	4598	2489	500	1054	1435	725.2	299.6	425.6
HM	2	6024	2600	581	855	1745	371.8	154.4	217.4
HF	2	2456	1369	260	432	937	182.0	80.4	101.6
CK	3	2209	1306	264	329	977	54.9	0.0	54.9
MF	3	2363	1296	285	389	907	176.8	65.3	111.5
LM	3	3320	1955	440	613	1341	278.3	136.5	141.8
MM	3	4962	2840	589	973	1868	518.3	265.4	252.9
HM	3	6489	3746	691	1724	2022	642.1	289.0	353.0
HF	3	2624	1593	319	432	1161	60.8	0.0	60.77
CK	4	1875	792	168	308	485	124.6	50.4	74.18
MF	4	1782	976	216	273	703	119.5	55.3	64.19
LM	4	1721	947	205	301	646	145.0	72.0	73
MM	4	3344	1911	351	889	1022	353.9	164.4	189.5
HM	4	5280	2859	575	1353	1505	773.6	301.2	472.4
HF	4	1765	1072	250	340	732	50.2	0.0	50.15

A.2.6 Response of total, total bacterial, actinomycetes, Gram-negative bacterial, Gram-positive bacterial, total fungi, arbuscular mycorrhizal fungi (AMF) and saprophytes biomass PLFAs as influenced by manure (low, LM, based on P requirement; medium, MM, based on N requirement; and high, HM, double rate of manure based on N), and fertilizer (medium, MF, recommended rate; and high, HF, higher dose) application, and the control (CK) treatments (TRT) at 10-20 cm soil depth in 2019.

10-20 cm / 2019									
TRT	**Rep**	**Total Biomass**	**Total Bacterial**	**Actinomycetes**	**Gram neg**	**Gram pos**	**Total Fungi**	**AMF**	**Saprophytes**
		ng PLFA-C g^{-1} soil							
CK	1	2170	928	245.3	280.2	648	162.4	49.22	113.2
MF	1	1236	690	191.1	210.1	480	63.1	22.91	40.19
LM	1	1602	676	194.2	196.3	480	116.9	33.46	83.44
MM	1	1580	881	203.1	296.8	584	93.4	38.3	55.1
HM	1	1625	741	197.6	194.0	547	89.1	36.92	52.21
HF	1	1441	745	192.1	148.1	597	76.6	28.41	48.14
CK	2	2613	922	172.2	358.8	563	116.9	27.13	89.8
MF	2	797	403	99.4	71.9	331	11.9	0	11.85
LM	2	2053	1004	276.7	293.1	711	211.6	74.49	137.1
MM	2	1694	940	234.6	297.6	642	101.8	43.04	58.74
HM	2	719	354	93.0	72.3	282	14.0	0	13.95
HF	2	673	287	74.2	51.2	236	7.1	0	7.1
CK	3	1551	534	101.4	213.4	321	73.3	19.37	53.94
MF	3	1187	440	99.5	159.5	281	62.1	15.64	46.44
LM	3	505	213	66.6	40.6	172	8.4	0	8.37
MM	3	1265	663	170.9	214.9	448	88.3	29.91	58.35
HM	3	2917	1032	237.8	399.4	632	238.9	74.1	164.8
HF	3	1501	762	222.3	216.0	546	150.9	54.31	96.63
CK	4	1348	657	197.6	213.4	444	91.9	38.09	53.77
MF	4	1414	690	187.6	178.1	512	76.1	25.87	50.2
LM	4	1218	614	165.6	185.0	429	103.4	36.78	66.58
MM	4	1564	829	217.1	255.0	574	154.9	66.55	88.35
HM	4	1687	864	244.0	278.2	586	150.5	64.88	85.57
HF	4	897	339	78.0	104.2	235	21.2	0	21.16

A.2.7 Response of urease (µgNH$_4$-N g^{-1} soil h^{-1}) enzyme activity as influenced by manure (low, LM, based on P requirement; medium, MM, based on N requirement; and high, HM, double rate of manure based on N), and fertilizer (medium, MF, recommended rate; and high, HF, higher dose) application, and the control (CK) treatments (TRT) at 0-10 cm and 10-20 cm soil depths in 2018 and 2019.

		2018		**2019**	
TRT	**Rep**	**0-10 cm Urease**	**10-20 cm Urease**	**0-10 cm Urease**	**10-20 cm Urease**
CK	1	5.257	2.474	1.262	2.680
MF	1	2.750	0.890	1.390	1.692
MM	1	4.112	2.224	1.855	5.514
LM	1	2.602	2.749	2.289	4.186
HM	1	7.581	3.520	1.995	4.095
HF	1	2.454	1.310	2.233	3.570
CK	2	3.670	2.939	1.643	2.825
MF	2	3.440	2.349	1.542	5.932
MM	2	3.505	2.460	1.252	5.149
LM	2	3.273	2.583	0.523	3.694
HM	2	5.913	5.015	2.946	5.364
HF	2	2.632	2.128	1.410	3.699
CK	3	4.033	3.406	1.831	5.408
MF	3	1.404	2.364	1.314	1.654
MM	3	3.239	3.950	1.787	3.017
LM	3	4.001	5.509	1.980	6.964
HM	3	5.878	6.978	2.853	5.971
HF	3	3.536	2.704	1.970	5.598
CK	4	3.127	2.509	1.394	5.275
MF	4	4.202	3.748	1.997	5.493
MM	4	5.287	2.957	2.509	8.762
LM	4	5.096	5.573	2.639	6.250
HM	4	5.420	7.723	4.915	5.552
HF	4	1.446	3.346	2.221	7.197

A.2.8 Response of β-glucosidase (μg PNP g^{-1} soil h^{-1}) enzyme activity as influenced by manure (low, LM, based on P requirement; medium, MM, based on N requirement; and high, HM, double rate of manure based on N), and fertilizer (medium, MF, recommended rate; and high, HF, higher dose) application, and the control (CK) treatments (TRT) at 0-10 cm and 10-20 cm soil depths in 2018.

		2018		**2019**	
TRT	**Rep**	**0-10 cm β-glucosidase**	**10-20 cm β-glucosidase**	**0-10 cm β-glucosidase**	**10-20 cm β-glucosidase**
CK	1	6.314	9.634	4.099	3.831
MF	1	6.731	10.375	4.065	3.438
MM	1	12.834	8.799	4.614	4.564
LM	1	9.302	13.571	3.991	4.184
HM	1	10.115	11.565	4.264	5.403
HF	1	7.751	9.253	4.333	4.219
CK	2	7.611	7.869	3.768	3.611
MF	2	6.356	9.888	4.038	3.786
MM	2	9.109	11.738	3.917	4.619
LM	2	7.631	10.734	3.838	3.719
HM	2	9.803	13.553	4.568	4.257
HF	2	4.634	10.225	3.375	4.175
CK	3	8.437	9.928	2.865	3.767
MF	3	6.051	7.401	3.667	3.527
MM	3	8.065	9.724	4.253	4.775
LM	3	10.145	12.255	4.087	4.772
HM	3	10.984	15.458	4.814	5.315
HF	3	7.013	10.703	4.130	5.488
CK	4	5.402	7.521	3.037	3.749
MF	4	7.733	11.312	4.711	4.169
MM	4	9.081	11.363	3.974	5.184
LM	4	8.008	12.411	4.405	4.296
HM	4	9.139	16.572	5.392	4.474
HF	4	9.719	8.003	3.417	5.118

A.2.9 Response of alkaline phosphatase (µg pNPg^{-1} soil h^{-1}) enzyme activity as influenced by manure (low, LM, based on P requirement; medium, MM, based on N requirement; and high, HM, double rate of manure based on N), and fertilizer (medium, MF, recommended rate; and high, HF, higher dose) application, and the control (CK) treatments (TRT) at 0-10 cm and 10-20 cm soil depths in 2018 and 2019.

		2018		**2019**	
TRT	**Rep**	**0-10 cm Alkaline phosphatase**	**10-20 cm Alkaline phosphatase**	**0-10 cm Alkaline phosphatase**	**10-20 cm Alkaline phosphatase**
CK	1	223.7	152.6	78.1	187.1
MF	1	93.2	139.8	239.0	299.5
MM	1	343.4	238.8	27.1	260.4
LM	1	434.8	162.8	4.7	310.1
HM	1	764.1	368.2	5.7	446.5
HF	1	149.2	351.0	77.4	312.2
CK	2	292.2	93.9	12.6	277.4
MF	2	100.3	92.5	98.9	327.6
MM	2	358.0	163.1	40.7	430.9
LM	2	425.6	258.1	130.4	462.0
HM	2	774.7	431.8	133.0	470.8
HF	2	128.7	204.1	36.0	364.8
CK	3	461.7	93.9	2.1	142.5
MF	3	396.5	92.5	31.3	194.1
MM	3	626.8	232.8	101.4	295.9
LM	3	495.9	296.3	113.9	371.9
HM	3	631.0	431.4	25.8	316.3
HF	3	284.6	152.0	109.9	300.2
CK	4	527.3	175.2	68.8	213.3
MF	4	321.8	39.5	3.5	363.3
MM	4	617.7	138.7	55.0	297.8
LM	4	635.1	397.8	64.8	382.6
HM	4	870.1	470.4	136.7	459.7
HF	4	320.2	211.4	181.2	226.3

A.2.10 Response of score of each indicators [pH, electric conductivity (EC), bulk density (BD), beta-glucosidase (BG), wet aggregate stability (AGG), microbial biomass carbon (MBC), soil organic carbon (SOC)], and soil quality index (SQI) as influenced by manure (low, LM, based on P requirement; medium, MM, based on N requirement; and high, HM, double rate of manure based on N), and fertilizer (medium, MF, recommended rate; and high, HF, higher dose) application, and the control (CK) treatments at 0-10 cm soil depth in 2018.

TRT	Rep	pH	EC	BD	BG	AGG	MBC	TOC	SQI
CK	1	1.00	0.93	0.72	0.02	0.93	0.85	0.83	0.75
MF	1	0.93	1.00	0.94	0.02	0.96	0.97	0.79	0.80
LM	1	1.00	1.00	0.89	0.02	0.93	0.94	1.00	0.83
MM	1	0.98	1.00	0.93	0.02	1.00	0.96	0.91	0.83
HM	1	0.98	1.00	0.97	0.02	0.87	1.00	0.99	0.83
HF	1	0.97	1.00	0.82	0.02	0.96	0.98	0.90	0.81
CK	2	1.00	1.00	0.83	0.02	0.99	0.97	0.82	0.80
MF	2	0.99	1.00	0.51	0.02	0.91	0.96	0.82	0.74
LM	2	0.99	1.00	0.87	0.02	0.93	0.97	0.89	0.81
MM	2	0.97	1.00	0.94	0.02	0.98	1.00	0.95	0.84
HM	2	0.98	1.00	0.99	0.02	0.93	1.00	0.97	0.84
HF	2	0.99	1.00	0.48	0.02	0.99	0.92	0.83	0.75
CK	3	0.98	1.00	0.35	0.02	1.00	0.99	0.86	0.74
MF	3	0.98	1.00	0.83	0.02	0.93	0.88	0.79	0.78
LM	3	0.97	1.00	0.82	0.02	1.00	0.97	0.88	0.81
MM	3	0.96	1.00	0.40	0.02	0.99	0.97	0.92	0.75
HM	3	0.98	1.00	0.88	0.02	0.83	1.00	0.98	0.81
HF	3	1.00	1.00	0.46	0.02	0.95	0.71	0.82	0.71

A.2.11 Response of score of each indicators [pH, electric conductivity (EC), bulk density (BD), beta-glucosidase (BG), microbial biomass carbon (MBC), soil organic carbon (SOC)], and soil quality index (SQI) as influenced by manure (low, LM, based on P requirement; medium, MM, based on N requirement; and high, HM, double rate of manure based on N), and fertilizer (medium, MF, recommended rate; and high, HF, higher dose) application, and the control (CK) treatments (TRT) at 10-20 cm soil depth in 2018.

TRT	**Rep**	**pH**	**EC**	**BD**	**BG**	**MBC**	**SOC**	**SQI**
CK	1	0.99	0.64	0.46	0.02	0.53	0.76	0.57
MF	1	0.98	1.00	0.40	0.02	0.49	0.78	0.61
LM	1	1.00	1.00	0.49	0.02	0.68	0.80	0.67
MM	1	0.99	0.87	0.44	0.02	0.52	0.81	0.61
HM	1	0.99	1.00	0.47	0.02	0.97	0.86	0.72
HF	1	0.97	1.00	0.50	0.02	0.63	0.82	0.66
CK	2	1.00	0.72	0.38	0.02	0.77	0.70	0.60
MF	2	0.98	1.00	0.33	0.02	0.90	0.71	0.66
LM	2	1.00	1.00	0.34	0.02	0.84	0.74	0.65
MM	2	1.00	1.00	0.43	0.02	0.97	0.77	0.70
HM	2	0.98	1.00	0.48	0.02	0.98	0.76	0.70
HF	2	0.99	0.88	0.38	0.02	0.84	0.73	0.64
CK	3	1.00	1.00	0.31	0.02	0.89	0.70	0.65
MF	3	0.99	1.00	0.42	0.02	0.83	0.63	0.65
LM	3	0.99	1.00	0.51	0.02	0.86	0.73	0.68
MM	3	0.97	1.00	0.44	0.02	0.86	0.73	0.67
HM	3	0.99	1.00	0.39	0.02	0.99	0.80	0.70
HF	3	0.99	1.00	0.44	0.02	0.81	0.71	0.66

VITA

Asmita Gautam was born in Nepal. She received her Bachelors of Science in Agriculture (B.Sc.Ag) from Institute of Agriculture and Animal Science (IAAS, Rampur, Nepal) in 2016. She joined South Dakota State University in 2018 to pursue her MS in Plant Science department emphasizing in soil science under the supervision of Dr. Jose Guzman, and Dr. Sandeep Kumar.