2018

Quantifying the Short-term Impacts of Cover Crops and Grazing on Soil Health Under an Integrated Crop-Livestock System in South Dakota

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QUANTIFYING THE SHORT-TERM IMPACTS OF COVER CROPS AND GRAZING ON SOIL HEALTH UNDER AN INTEGRATED CROP-LIVESTOCK SYSTEM IN SOUTH DAKOTA

BY

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A thesis submitted in partial fulfillment of the requirements for the

Master of Science

Major in Plant Science

South Dakota State University

2018

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

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ACKNOWLEDGMENTS

First and foremost, I offer my sincerest gratitude to my advisor, Dr. Sandeep Kumar, who has supported me throughout my dissertation with his excellent guidance, patience, encouragement, and financial aid. Without him, this dissertation would not have been possible. His outstanding way of training helped me to grow my skills as an individual and provided an enduring experience.

I would like to thank my thesis committee members, and all the faculty and staff members of the Department of Agronomy, Horticulture and Plant Sciences, South Dakota State University for sharing their knowledge and providing support when needed.

Thank you to my friends and senior fellows – Liming Lai, Jasdeep Singh, Atilla Polat, Abdullah Alhameid, Navdeep Singh, Shikha Singh, Saroop Sandhu, Hanxiao Feng, Ekrem Ozlu, Kunal Sood, Colin Tobin, Brant Douville, and David Ussiri for all the experience and work shared and for making the lab environment cheerful to work.

Last but not least, I would like to thank my family, parents and brother for their endless support and love during my study.

(Vishal Seth)

Place: Brookings, SD
Date: February 20th, 2018

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ABBREVIATIONS

ACE	Acid Extracts
ANOVA	Analysis of Variance
ρ_b	Bulk Density
$CaCl_2$	Calcium Chloride
$C_3Cl_2N_3NaO_3$	Sodium Dichloroisocyanurate
CFDE	Chloroform Fumigation Direct Extraction Method
$C_7H_5NaO_3$	Sodium Salicylate
CS	Conventional System
CWE	Cold-water Extracts
DAS	Dry Soil Aggregate Stability
DF	Dilution Factor
DW	Dry Weight
EC	Electrical Conductivity
HCl	Hydrogen Chloride
HWC	Hot-water Extractable Carbon
HWE	Hot-water Extracts
ICLS	Integrated Crop-livestock System
KCl	Potassium Chloride
K_2SO_4	Potassium Sulfate
MBC	Microbial Biomass Carbon
MBN	Microbial Biomass Nitrogen
MUB	Modified Universal Buffer
NaOH	Sodium Hydroxide
NH_4Cl	Ammonium Chloride
PNG	p-nitrophenyl-β-D-glucoside
RCBD	Randomized Complete Block Design
SOC	Soil Organic Carbon
SWR	Soil Water Retention
TC	Total Carbon
TN	Total Nitrogen
OM	One Molar HCl
SM	Six Molar HCl
C	Carbon
N	Nitrogen

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ABSTRACT

QUANTIFYING THE SHORT-TERM IMPACTS OF COVER CROPS AND GRAZING ON SOIL HEALTH UNDER AN INTEGRATED CROP-LIVESTOCK SYSTEM IN SOUTH DAKOTA

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Cover crops and grazing under Integrated Crop-Livestock System (ICLS) can impact the rhizosphere nutrient cycling and may have potential to increase the long-term sustainability and economic profitability of agricultural production system. In South Dakota, crop production practices and livestock husbandry are both common which provide the opportunity for ICLS to be successful in this region. However, little is known about the impacts of the cover crops and grazing under ICLS on soil properties in this region. The present study was conducted at South Dakota State University Research Farm (44°20'34.8"N, 96°48'14.8"W), Brookings, SD, to quantify the impacts of cover crops and grazing on soil bulk density (BD), soil penetration resistance (SPR), soil water retention (SWR), pore size distribution (PSD), total nitrogen (TN), carbon and nitrogen fractions (labile, stable, and inert), microbial biomass carbon/nitrogen, urease and beta-glucosidase enzyme activity. Study treatments included grass leaf and broad leaf dominated cover crop mixtures, both with and without grazing, and the cover crop control field with no cover crop or grazing. The experimental site was established on June 2016 by planting of cover crops and soil samples were collected three times i.e., pre-grazing, post-grazing and summer phase at 0- to 5-cm for bulk density, SPR, SWR and PSD; 5- to 15-cm for soil microbial and enzymatic properties.

The results showed that one year of cover crops did not significantly impact the soil bulk density, SPR, carbon and nitrogen fraction (labile, stable, and inert), microbial biomass carbon (MBC), microbial biomass nitrogen (MBN) or urease activity. However, beta-glucosidase enzyme activity significantly increased under broad leaf dominated cover crop mixtures as compared to grass leaf dominated cover crops and no cover crop (control) treatments at the 5- to 15-cm depth. Additionally, cold water extractable nitrogen (CWN) significantly increased under grass leaf dominated cover crops for the 0- to 5-cm depth. Broadleaf and grass leaf dominated cover crop mixtures had higher microbial and enzymatic activities as compared to the no cover crop (control) treatment, but, the differences were non-significant. Grazing treatment significantly impacted soil BD and SWR, PSD, carbon and nitrogen fraction (labile, stable, and inert), MBC, MBN, and urease but beta-glucosidase enzyme activity showed no significant differences at either depth. Sampling time significantly impacted the ρ_b, SWR, PSD, CWC, HWC, SMC, MBC, MBN, urease enzyme, and β-glucosidase enzyme activities.

The present study concluded that one year of cover crops significantly impacted the selected soil properties i.e., CWN increased under grass leaf dominated cover crops and soil beta-glucosidase enzyme activity increased under broad leaf dominated cover crop mixtures as compare to grass leaf dominated cover crops and cover crop control treatments at 5- to 15-cm depth. One episode of grazing only significantly impacted only soil BD. Sampling time significantly impacted soil BD, SWR, PSD, CWC, HWC, CWN, HWN, OMN, SMN, MBC, MBN, urease and beta-glucosidase enzyme activity. Since most soil properties showed no significant differences by cover crop and grazing treatments during this short-term study, because they require longer timeframe to respond

under different management practices, further long-term research may be required to detect impacts of cover crops and grazing management practices under ICLS on soil health.

CHAPTER 1

INTRODUCTION

Conversion of grassland to cropland deteriorates soil health, water quality, human and wildlife. The conversion rate of grassland to cropland in South Dakota is around 5% per year during 2006 to 2012, and a total of 4.6 million acres of grassland has been converted into croplands (Reitsma et al., 2015). The Northern Great Plains (NGP) region of the USA accounts for 18% of all US arable land and 57% conversion of grassland to cropland occurred during 1997 to 2007 (Wright and Wimberly, 2013). In South Dakota, the corn (*Zea mays* L) and soybean (*Glycine max*) land utilization have been doubled from 2.5 million acres in 1995 to 5 million acres in 2015 resulting in the conversion of grasslands to cropland (Johnston, 2014; Wright and Wimberly, 2013). Intensive application of fertilizers, manure herbicides and pesticide have been used to increase crop yields, resulting in environmental problems (Peyraud et al., 2014) such as water pollution, soil contamination with pesticides, heavy metal contamination and depletion of soil fertility (Franzluebbers and Stuedemann, 2013). Therefore, alternative management systems are needed that can improve soil and environmental quality.

Integrated crop-livestock systems (ICLS), the practice of managing crops and animals on a single farm, is considered as one of the several alternatives that can alleviate some of above problems (Hilimire, 2011). The ICLS can improve the nutrient use efficiency and soil health, and enhances the economic benefits (Russelle et al., 2007). Cover crops and crop residues under the ICLS systems provide livestock forage, and in-return, livestock deposit manure and provide nutrients for the plant growth. Potential benefits of ICLS system include reducing fertilizer cost for the subsequent cash crop,

reduced cost of supplemental hay, recycling of soil nutrients to enhance soil fertility (Soussana and Lemaire, 2014), improving livestock health, reducing cost of feeding to livestock. However, if ICLS is managed inappropriatly, it can result in negative impacts such as soil compaction when grazing is allowed under moist soils (Sanderson et al., 2013). Soil compaction can reduce the air movement into the soil pores leading to poor crop producitivty (Hamza and Anderson, 2005)

Cover crops under ICLS are the key factor in impacting the soil properties. Incorporating cover crops into existing cropping systems provides economic and environmental benefits (Thornton, 2010). For example, cover crops planted after harvesting of the main crop can reduce N loss (Huntington and Huntington, 1985). Cover crops help in reducing the soil erosion when planted in fallow season, using cover crop like hairy vetch leads to addition of N into the soils and help in building soil organic matter. There are numerous cover crops species that can provide excellent source of forage for livestock grazing and directly helps in overall profitability (Franzluebbers, 2007; Sulc and Tracy, 2007b). Cover crops increase crop diversity and enhance more photosynthesis assimilations which lead to increase carbon sequestration (Lehman et al., 2015).

Grazing is another key factor in an ICLS which has positive and negative impacts depending upon soil types, crops used for grazing, environmental conditions, and the way it is managed in the system. Grazing can increase soil fertility because of the animal excrement and urine input on the soil surface, therefore, more available nutrients can be supplied to crops for their growth and production (Russelle et al., 2007). However, if grazing is managed inappropriately, it could cause soil compaction problem at surface

depth which can negatively affect the soil physical and hydrological properties (Liebig et al., 2011). The synergistic effect may be found between crops and livestock under the ICLS as the livestock supply nitrogen and other nutrients for crop growth while, crop residue and the cover crop supply livestock forage. However, grazing impacts on soil properties are not consistent across environments due to the complex interactions of climate, grazing time, grazing intensity, soil moisture content, soil structure and soil condition (Savadogo et al., 2007).

The objectives of this study were to (i) quantify the impacts of broad leaf and grass leaf dominated cover crop blends on soil health parameters in South Dakota, and (ii) evaluate and understand the impacts of grazing on soil health parameters in eastern South Dakota.

CHAPTER 2

LITERATURE REVIEW

Integrating crop production with animal husbandry is known as an Integrated Crop-Livestock System (ICLS). The ICLS has been practiced for eight to ten thousand years (Halstead, 1996; Smith, 1995). However, this system is not being practiced intensively in the USA (Russelle et al., 2007). The integration of crop and livestock systems occurs at two scales: (i) within the farm, where spatial and temporal integration are performed in the same field, and (ii) among the farms, or it is also known as regional level integrations where both spatial and temporal work on a contract basis (Bonaudo et al., 2014; Russelle et al., 2007; Sulc and Franzluebbers, 2014).The ICLS includes crops, cover crops, forage crops, and livestock grazing, all of these impacting the soil properties. This literature review focuses on the response of soils to cover crops and livestock grazing under ICLSs.

2.1. Integrated Crop-Livestock System (ICLS)

Conversion from grassland to cropland in the Northern Great Plains of the USA has been degrading soils and environmental quality (Higgins et al., 2002). Agricultural production practices during the last few decades in North America is dominated by energy intensive operations (Sulc and Franzluebbers, 2014). The current intensification of cropping system practices including intensive application of chemical fertilizers, herbicides, pesticides and tillage deteriorate water quality, soil health, air quality, wildlife and human habitat (Reganold et al., 2011), and create negative impacts on the agroecosystem (Tilman, 1999; Tilman et al., 2001). Therefore, there is a strong need for

sustainable intensification. Integrated crop-livestock system is an alternative management approach that can enhance environment quality and farm profitability (Thornton, 2010).

2.1.1. Soil Problems with Specified Crop Production System

During the last two decades, intensive application of synthetic fertilizers and pesticides led to deterioration of water quality, soil quality, wildlife and human habitat (Pretty, 1995). This has resulted in a reduction of soil organic matter (Tiessen et al., 1982), deterioration in soil physical properties and enhanced soil erosion (Sulc and Tracy, 2007b) (Karlen et al., 1994). Continuous monocropping decreases soil pH, enzymatic activity and soil organic matter content (Xiong et al., 2015). Continuous corn monocropping had negative impact on soil quality and crop yield (Lal, 1997). Intensive cropping practices lead to significantly lower microbial biomass carbon and nitrogen as compared to diversified system (Moore et al., 2000). A study conducted in a dryland region of the Northern Great Plains (NGP) reported that conventional practices increased the CO_2 losses, thus decreasing the carbon sequestration in the soils as compared to diversified systems (Halvorson et al., 2002). Intensified crop production systems require more soil disturbance and hence reduces the soil microbial communities, resulting in poor soil health (Islam and Weil, 2000).

2.1.2. Encountering Concurrent Challenges Through ICLS

Integration of crop production with animal husbandry has neutral to positive impacts on soil health. Expanding ICLS use in the US can be economical and environmentally beneficial compared to current agricultural production practices (Sulc and Tracy, 2007a). Livestock manure is directly added in the soil, enhancing the nutrient cycling, and is the primary source of nutrients for crop production (Lemaire et al., 2014).

Cover crops and grasses used in ICLS can diversify the cropping system, benefiting soil microorganisms (Blanco-Canqui et al., 2015), while reducing winter feed costs for livestock (Lawrence et al., 1999). Therefore, synergistic integration of crop production with livestock husbandry system promotes multiple and temporal use of marginally-productive lands leading to an improvement in soil quality, water quality, and wildlife habitat and can be beneficial in maintaining the soil health by providing a variety of ecosystem services (Sulc and Franzluebbers, 2014). However, if the ICLS is not managed properly, it can deteriorate soil quality, water quality and several ecosystem services provided by the cover crops. For example, winter grazing of cover crops and crop residues can result in soil compaction which could reduce yields in the subsequent cropping system (Clark et al., 2004; Liebig et al., 2011; Sulc and Franzluebbers, 2014).

2.2. Role of Cover Crop Under Integrated Crop-Livestock System on Soils

According to SSSA (2008), the term cover crops is defined as a "*close-growing crop that provides soil protection, seeding protection, and soil improvement between periods of normal crop production, or between trees in orchards and vines in vineyards*". Cover crops can improve soil C, microbial properties, nutrient retention, reduce soil erosion and enhance crop yield (Blanco-Canqui and Lal, 2009). Many studies concur that incorporating cover crops into existing cropping systems has positive impacts on soil properties, and these cover crops can be considered as the back bone of the annual cropping system for sustainable production (Blanco-Canqui et al., 2015). Although cover crops can benefit multiple soil parameters, a better understanding of cover crops is required to optimize its potential (Blanco-Canqui and Lal, 2009). Several studies report that continuous monoculture cropping reduces soil organic carbon (Guo and Gifford,

2002), and incorporation of cover crops into an existing cropping system will help to improve the soil physical, chemical, and biological properties and enhance economic benefits (Blanco-Canqui et al., 2015). The impacts of cover crops on soil health parameters are listed below as:

2.2.1. Soil Bulk Density

Soil bulk density is an important parameter impacting crop growth (Baibay et al., 2017). It depends on many factors such as soil texture, soil structure, soil moisture, organic carbon, and crop residue (Luo et al., 2017). Some evidences indicate that cover crops help in alleviating compaction problems via development of roots (Rorick and Kladivko, 2017), and roots help create the macropores that may decrease soil compaction and soil bulk density (Patrick et al., 1957; Steele et al., 2012). A study reported that contrary to the cereal rye, brassica cover crops were more effective than rye in alleviating the effects of soil compaction (Chen, 2009). Therefore, selection of crop species is important and using cover crop or mixtures of cover crop under ICLS diversifies the cropping system, reduces soil compaction, helps building soil health, and enhances economic returns (Sentürklü et al., 2016).

2.2.2. Soil Water Retention

Diversified crop rotation system has the potential to improve soil organic matter content, nutrient cycling, and soil water retention as compared to those under less diversified cropping system (Davinic et al., 2013). A study conducted in Midwest Corn Belt reported that cover crops did not impact total organic C and soil water retention (Beehler et al., 2017). Similar results were observed in a study conducted to evaluate the

impacts of cereal rye cover crops on soil physical properties in southeastern Indiana under no-till corn and soybean rotation (Rorick and Kladivko, 2017). However, a study was conducted to evaluate the soil water improvements with the long-term use of a winter rye cover crop in central Iowa, reported that cover crop treatments have significantly higher soil water storage at 0-30 cm depth when compared to no cover crop treatments (Basche et al., 2016). Researchers reported that continuous living cover significantly increases the total porosity (Basche and DeLonge, 2017; Carof et al., 2007; Głąb and Kulig, 2008). A study conducted at University of Arkansas Delta Branch Experiment Station to evaluate the effect of winter cover crops on selected soil properties (rye + vetch) reported that soil water retention and porosity have measurable changes due to winter cover crops (Keisling et al., 1994). A study was conducted to evaluate the soil hydrological properties impacted by prairie restoration, native prairie, grass and row-crop management on Mexico silt loam soils showed that native prairie had significantly higher water retention at saturation while restored prairie had the highest water retention at − 33 kPa, − 100 kPa and − 1500 kPa (Chandrasoma et al., 2016).

2.2.3. Soil Organic Carbon

The SOC is affected by soil type, cropping system, environmental conditions and management practices (Letey, 1958). A study was conducted to evaluate the impacts of winter cover crops on SOC under leguminous and non-leguminous cover crops and showed that winter cover crops may increase the SOC levels and help in reducing the depletion rate. This study also observed that winter cover crop (shepherd's-purse) helps building more SOC than Austrian winter pea (*Lathyrus hirsutus* L.), hairy vetch (*Vicia villosa* Roth), and canola (*Brassica napus* L.) (Kuo et al., 1997). An experiment was

conducted to demonstrate the long-term effect of cover crops on SOC in Norfolk sandy loam (fine-loamy, siliceous, thermic, Typic Kandiudults) reported that concentration of SOC and nitrogen were greater with rye, hairy vetch, crimson clover than with the control without cover crop (Sainju et al., 2002). Cover crops helps in building the SOC and a study was conducted to examine the long term impacts of cover crop on SOC and nitrogen content on a loam soil (Typic Xerofluvent) in Central Italy with four cover crop treatments (C – no cover crop; NL – non-legume CC; LNL – low nitrogen supply legume CC, and HNL – high nitrogen supply legume CC) reported that NL, LNL and HNL cover crops increased SOC content by 0.17, 0.41 and 0.43 Mg C ha^{-1} $year^{-1}$ (Mazzoncini et al., 2011). In several studies it has been reported that incorporation of cover crops or the blend of cover crops may increase the biomass production and soil organic matter which is one of the important parameter of soil health (Havlin et al., 1990). Soil organic carbon is important soil health indicator and plays important role in improving soil health (Franzluebbers and Stuedemann, 2008).

2.2.4. Soil Carbon Fractions

Commonly three types of carbon and nitrogen fractions are available in the soil, namely labile, stable and recalcitrant. The hot water, cold water and hydrogen chloride is used for the extraction, respectively (Ghani et al., 2003). Hot water extractable carbon is very sensitive parameter to land management practices and affected by the season and soil variability (Ghani et al., 2003). The impact of land use change is more on hot water extractable carbon (HWC) than the SOC. Climatic conditions affect the HWC, as dry summer and mild winter showed drop in the HWC and summer soil showed higher HWC than winter season (Ghani et al., 2003).

2.2.5. Microbial Biomass Carbon/Nitrogen (MBC/N)

Soil microbial biomass C and N is considered as an early indicator of changes in nutrient cycling and SOM dynamics because soil microbial biomass carbon and nitrogen pools have been shown more sensitive to agriculture management than soil bulk carbon and nitrogen pool (Joergensen et al., 1995). A review paper documented that adding one or more crops in monoculture increased soil C by 36% and soil microbial biomass carbon by 20% while increasing the total nitrogen by 5.3% and MBN by 26% (Carter, 1986; Motta et al., 2007). A study findings indicated that the plant diversity was increased from one species to 16 species resulting increase of TC by 13% and MBC by 31% (McDaniel et al., 2014). A study was conducted to compare the differences between rye-cotton and continuous corn rotation showed that soil MBC was higher in rye-cotton as compared to continuous corn (Omeke et al., 2016). In several studies it has been reported that incorporation of cover crops or the blend of cover crops may increase the biomass production (Havlin et al., 1990).

2.2.6. Soil Urease Enzyme Activity

Soil urease enzyme plays a major role in releasing inorganic N in the N cycle (Dick, 1994). Microbial activity and microbial extracellular enzyme activity are much sensitive to climatic conditions (Ren et al., 2017). The microbial enzymes are considered to be an important agent and rate-limiting step in SOC decomposition (Bardgett et al., 2008). Enzymes help in catalyzing biochemical reactions and they play a key role in the nutrient cycling. Soil enzymes are believed to be considered primarily as microbial origin and also found to be originated from plants and animals (Dick, 1994). Continuous grassland showed higher enzyme activity as compared to cultivated field due to reduction

in soil disturbance (Gupta et al., 1988). In a study, enzyme activities were compared between winter fallow and cereal and legume, and found that cereal crop have significantly higher enzyme activities than winter fallow but not significant differences were observed (Bandick and Dick, 1999). Cover crop have wide range of effects on soil urease enzyme activities. A study was conducted to investigate the effect of cover crop on soil urease on a Marvyn sandy loam soil found that cover crop had no significant impact on urease enzyme (Hamido and Kpomblekou-A, 2009). Plants roots impact the soil rhizosphere and imposes changes in soil microbial communities. Increasing the proportion of hairy vetch in comparison to oats resulted in higher urease activity (Mukumbareza et al., 2016). Diversification of cropping system impact the soil urease activities, and urease activity was reported highest in soils under 4-year oat-meadow rotations and lowest under continuous corn (Klose and Tabatabai, 2000). Activities of soil urease enzyme was found to be greater in surface layer than lower depths (Bandick and Dick, 1999). The urease enzyme is very sensitive to sampling depths which is reflected by both plant and microorganisms (McCarty et al., 1998). Wide C: N ratio depress the soil urease enzyme which is primarily being synthesized by microorganisms and plants residues can be a source of soil urease enzyme (Martens et al., 1992).

2.2.7. Soil Beta-glucosidase Enzyme Activity

A study was conducted to evaluate the effect of oats and grazing vetch cover crops on soil enzymatic avtivities reported that cover crops resulted in higher MBC and β-glucosidase enzyme activities than the weedy fallow (Mukumbareza et al., 2015). Bandick and Dick (1999) reported an increase in soil β -glucosidase activity in more intensive cropping system as compared to less intensive cropping system. Acosta-

Martinez et al. (2007b) reported increase in soil β-glucosidase activity with a decrease in the fallow periods, and an increased level of soil β-glucosidase activity was observed under pasture as compared to agricultural rotations at 0- to 5-cm. Beta-glucosidase activity plays an important role in plant decomposition and SOC cycling. Microbial degradation of cellulose to glucose and carbon cycle is affected by a rate-limiting enzyme soil β-glucosidase.

2.3. Grazing under ICLS

In the US, a little less than 1/3 of all land is considered as grazing land (Follett and Kimble, 2000). About 58.7 Mha of grassland pasture and range are in federal ownership, around 30 Mha are in public ownership, and 150.6Mha are in private holdings (Sobecki et al., 2001), with much of this land being degraded or poorly managed (Follett and Kimble, 2000). However, grazing can have small to large impacts on ecosystems depending on several factors (Milchunas, 2006). Grazing lands contain up to 30% of the world's soil organic carbon (SOC) (Eswaran et al., 2001) and 5% of the world's SOC is in the soils of US grazing lands (Waltman and Bliss, 1997) (Lubowski et al., 2006). Overgrazing or poor grazing management can cause a loss of carbon and decreased soil productivity (Lecain et al., 2000). Grazing every alternate year may reduce the soil loss due to water erosion (Sulc and Tracy, 2007b). The acreage of these crops has increased at a significant rate (i.e. in 2.5 million acres in 1995 to 5 million in 2015) in South Dakota (Johnston, 2014; Wright and Wimberly, 2013). Based on the available literature, grazing lands play an important role in improving soils. Furthermore, grazing impacts on some selective soil properties under ICLS are describes below:

2.3.1. Soil Bulk Density

Heavy grazing leads to soil compaction due to trampling, resulting in higher bulk densities and reduced soil pore space which restricts water and oxygen movement in soils (Zhao et al., 2011). Livestock grazing winter residues, weeds or cover crops did not significantly increase soil bulk density (Fernandez-Rivera et al., 2002). Livestock trampling leads to soil compaction and increases bulk density at shallow depths, but the damage is not severe and can be rectified with appropriate measures (Laycock and Conrad, 1967). Infiltration and compaction impact from grazing can be minimized by grazing when soils are dry (Maughan et al., 2009). A study conducted to compare soil surface bulk density between 1) sites not grazed by cattle > 26 years; 2) sites not grazed for 6 years, 3) sites grazed for 15 years with different residual dry matter of >1100 kg/ha, 670-900 kg/ha and <450 kg/ha in California on coarse sandy loam soils reported that bulk density was not significantly different between not grazed > 26 years and sites not grazed for 6 years (Tate et al., 2004). An experiment conducted in the Missouri Coteau reported that different levels of grazing pressure affected the bulk density and porosity of clay loam soils (Engels, 2009). A 4-year grazing trial studied the response of soil bulk density of sandy soils in Sahelian rangelands to two stocking rates (62·5 and 125 kg live weight ha^{-1}) and four sheep:goat ratios (0:6, 2:4, 4:2 and 6:0 animals per pasture), and multiple surface and subsurface soil depths (0–2, 2–6, 6–14 and 14–30 cm), and reveals that soil compaction due to grazing was observed only at the soil surface and soil bulk density was not affected by the grazing (Hiernaux et al., 1999). Many studies, however, reported a significant increase in bulk density with grazing especially in fine textured soils and in surface layers (Abdel-Magid et al., 1987; Hamza and Anderson, 2005; Hunt et al., 1995; Orr, 1960).

2.3.2. Soil Water Retention

A study conducted in semiarid rangelands of southeast Idaho reported that in addition to a variety of other factors, management decisions (grazing and rest) can have substantial influence on soil-water content and it can vary substantially as a result of animal impact and the duration of grazing (Weber and Gokhale, 2011). A study was conducted by Kumar et al. (2008) to evaluate the effect of grazing on soil hydraulic properties under rotationally grazed (RG) pasture, continuously grazed (CG) pasture, grass buffers (GB), and agroforestry buffers (AgB) treatments. Soil water content at high soil water potentials (0 and –0.4 kPa) was greater in the buffer treatments relative to the other treatments for the 0 to 10 cm soil depth (Kumar et al., 2008).

2.3.3. Soil Organic Carbon

Grazing affected soil organic carbon and nitrogen in contrast to un-grazed grassland (An and Li, 2015). Grazing appears to exert a negative effect on soil carbon and nitrogen in desert grassland (An and Li, 2015). In Israel, disturbance of the soil organic carbon pool was smaller for wheat stubble grazing as compared to soils under stubble retention grazing (Stavi et al., 2015). SOC was higher under rotational grazed system as compared to heavy continuous grazing system and excessive grazing that removed crop biomass and litter that exposed soil caused degradation (Jacobo et al., 2006). In Florida sandy soils, grazing reduced the SOC in the first year while an opposite trend was reported in subsequent years (George et al., 2013). A 5-year grazing trial on a native pasture in south-eastern Australia showed that removing grazing pressure may lead to lower SOC in native pastures over time and grazing management practices are required to increase SOC (Orgill et al., 2016). A study was conducted to evaluate the impact of

grazing on microbial biomass and soil carbon in subtropical grassland under grazed and un-grazed plots reported that grazing effects on SOC depend on root system biomass and optimizing grazing management to enhance SOC (Wilson et al.). In Wyoming, 40-years of grazing exclusion resulted in no significant differences in SOC and microbial biomass between grazed and un-grazed treatments. However, Milchunas and Lauenroth (1993) did a detailed review of the literature of grazed and un-grazed sites around the world and reported both a decrease (40%) and increase (60%) in soil carbon as a result of grazing exclusion.

2.3.4. Soil Carbon Fractions

An experiment was conducted to evaluate the impacts of grazing and cultivation on hot water extractable carbon at 52 different sites and under four sampling times i.e., spring, summer, autumn and winter for 2 years in the Waikato region of the North Island, New Zealand (38 S and 175 E) with two grazing treatments i.e., intensively (dairy) and less intensive (sheep/beef). This study showed that in both years of the monitoring period, the amounts of HWC in sheep/beef soils were consistently higher than in dairy soils in all seasons and observed that hot water carbon is more sensitive to grazing as compared to SOC (Ghani et al., 2003). A study conducted to evaluate the sensitivity of water extractable soil organic carbon fractions to land use in three soil types reported that water extractable carbon is highly and positively correlated with SOC and mean weight diameter (Ćirić et al., 2016).

2.3.5. Soil Microbial Biomass Carbon and Nitrogen (MBC and MBN)

Yang et al. (2016) reported that MBC and MBN content decreased significantly under grazing compared to no grazing. In China, four-decades of grazing and cultivation

in a semi-arid grassland decreased MBC by 20% under grazing and cultivation and reduced fungi by 40% to 71% as compared to grassland (He et al., 2017). A study was conducted to evaluate the impact of grazing exclusion on soil respiration in a Meadow grassland and reported that exclusion significantly increased soil moisture and aboveground biomass but decreased soil temperature, microbial biomass carbon (Chen et al., 2016). Researches have shown that soil MBC decreased with increasing in the grazing intensity (Acosta-Martínez et al., 2010). A study was conducted to evaluate the impacts of grazing on soil microbial biomass in Inner Mongolia with different stocking rates (ungrazed, UG; lightly grazed, LG; moderately grazed, MG; heavily grazed, HG) and found that LG increases the soil microbial biomass carbon and SOC (Liu et al., 2012). The effects of grazing on soil properties is very complex and need to sufficiently be understood.

2.3.6. Soil Urease Enzyme

Grazing intensities significantly influence the MBC. In Australia, grazing pressure had no effect on MBC, SOC and enzymes, but a significant reduction was reported in soil microbial biomass carbon levels (approximately 24% and 51%) after heavy grazing (Holt, 1997). A study conducted to quantify the impacts of seasonal changes on urease enzyme reported that enzymes activities were higher under grassland sites regardless of grazing intensities (Dormaar et al., 1984). A study conducted in Northern China to evaluate the changes in soil properties under different grazing pressures (i.e., light grazing, 0.45 sheep unit/ha, moderate grazing, 0.75 sheep unit/ha, heavy grazing, 1.50 sheep unit/ha with no grazing treatments) reported that as the grazing intensities increased, the urease activity decreases significantly, and the urease activity under light

grazing was reported the highest (Jiao et al., 2016). In a study conducted in semi-arid grasslands of China to evaluate the impacts of long term grazing on soil urease activity under grazing and un-grazing treatments reported that urease activity under the grazing treatment was higher as compared to un-grazing treatment (He et al., 2017). Grazing activities induce changes in soil enzymatic activities (Mukumbareza et al., 2016).

2.3.7. Soil Beta-glucosidase Enzyme

Soil beta-glucosidase enzyme is strongly correlated with soil pH (Eivazi and Tabatabai, 1990). Studies have shown that beta-glucosidase activity decreases with the increase in soil depth (Acosta-Martinez et al., 2007a). A study conducted by Acosta-Martínez et al. (2003) to evaluate the enzymatic activities in semiarid agricultural soils reported that the enzyme activities were higher in loam and sandy clay loam as compared to sandy soils. A study showed that soil beta-glucosidase is positively correlated with cumulative N mineralization in soils (Acosta-Martinez et al., 2007a). Plant species especially roots play an important role in triggering the enzymatic activities in soils, and soil beta-glucosidase activity reaches its peak at the booting stages (Hai-Ming et al., 2014).

2.4. Research Gaps

Previous studies have evaluated the impacts of cover crops and grazing on soil physical, chemical and biological properties under various environmental conditions. However, there are some research gaps among these studies. Impacts of cover crops and grazing on soil properties were evaluated broadly across the world. However, quantifying the impacts of cover crops and grazing on soil bio-chemical properties, especially on soil enzymatic activities were very few under the Northern Great Plains (NGP) region of

USA. Little information is known about the interaction of cover crops and grazing activities on soil microbial properties in NGP. Additionally, the role of cover crops for enhancing the economic or environmental benefits need to be studied rigorously in NGP.

CHAPTER 3

MATERIALS AND METHODS

3.1. Study Site

The present study was conducted at the research farm of South Dakota State University, located in Brookings, South Dakota (44°20'34.8"N, 96°48'14.8"W). Soil type in the study area was Brookings (*fine-silty, mixed, superactive, frigid Pachic Hapludolls*) and Kranzburg (*fine-silty, mixed, superactive, frigid, Calcic Hapludolls*). The experiment was initiated in 2016 to explore the short-term impacts of cover crops and grazing under ICLS on soil health. The plots were established in nearly flat areas with a slope less than 1%. The average annual rainfall was 617.5 mm and the average temperature ranges from -9.94°C in January to 20.1°C in July.

3.2. Experimental Design and Treatments

Before establishing the experiment, the study site was countiniously cropped with alfalfa (*Medicago sativa L.*) from 1995 to 1999. Oats were planted in 2016 and killed in June, and cover crops were planted in July 2016. Texture of the soil was silty clay loam. Initial values of soil bulk density, soil organic carbon (SOC), and pH were 1.35 Mg m^{-3}, 13.5 g kg^{-1}, and 6.7 respectively.

There were 20 plots and each plot size was of 60 feet (width) × 90 feet (length). A total of 8 plots were grazed. The experimental design was a Randomized Complete Block Design with 4 replications. Two cover crops blends included a grass leaf dominated cover crop and a broad leaf dominated cover crop. The broad leaf dominated cover crops included radish (15%, 1.2 lb/acre), turnip (10%, 0.3 lb/acre), kale (10%, 0.4 lb/acre), pea (10%, 6 lb/acre), lentil (15%, 3 lb/acre), cowpea (15%, 6.75 lb/acre), g. millet (10%, 2

lb/acre) and oats (15%, 10.5 lb/acre). The grass leaf dominated blend included radish (5%, 5 lb/acre), pea 5%, 3 lb/acre), sorghum (25%, 5 lb/acre) and oats (25%,17.5 lb/ acre). These cover crops treatments are generally used to reduce the impacts of soil compaction that may be created by cattle grazing at the upper depths. All treatments were managed with conservation tillage. The rotation includes cover crops (planted in May 2016)-corn (2017)- Oats (2017) – Cover crops (after killing Oats in May 2017). Grazing was applied in November for 1 week. The Aberdeen Angus cattle breed (common for beef production found in South Dakota) was used for the grazing of cover crops. A total of six rows of corn per plot were planted in May 2017.

3.3. Data Measurements

3.3.1. Soil Sampling

Soil samples were collected three times: pre-grazing, post-grazing, and summer in 2016-2017**.** Baseline soil samples were collected in fall 2016 after the harvest of oats from 0- to 5-, 5- to 15-, 15- to 30-, 30- to 45- and 45- to 60-cm depths of every replicated plots using a hydraulic probe unit. Pre-grazing soil samples were collected on September 2016. These samples were collected to analyze the basic soil properties. Intact soil cores samples were collected in September 2016 before grazing from 0- to 5-cm depths of every replicated plot using 5cm (diameter) × 5cm (height) core for analyzing the bulk density (ρ_b) and measuring soil water retention using pressure plate apparatus. During the same time, soil samples were collected using hand soil auger unit to analyze electrical conductivity (EC), pH, SOC concentration, total nitrogen (TN), and soil carbon and nitrogen fractions. Soil samples were put in the ziplock bags and transported to the laboratory in cool and dry place. Moreover, during the same time, the soil samples were

collected to analyze the soil microbial (soil microbial biomass carbon and nitrogen) and enzymatic activity like urease and Beta-glucosidase. For this, soil samples were put in the ziplock bags and placed in the mini cooler containing dry ice and then transported to the lab and stored at 4°C. Soil samples were air dried, ground, and sieved to pass through a 2mm sieve. In addition, soils were ground to <0.25mm in size for analyzing the soil carbon fractions.

Post-grazing soil samples were collected using hand auger on November 17, 2016, one day after cattle had been removed from 0- to 5- and 5- to 15-cm soil depths of every replicated plots using hand soil probe unit to analyze soil microbial activities (soil beta-glucosidase activity, soil urease activity, soil microbial biomass carbon and soil microbial biomass nitrogen), soil carbon and soil nitrogen fractions like soil labile fractions, soil stable fractions and soil recalcitrant fractions.

Summer phase soil samples were collected on July 5, 2017 before the planting of maize crops at 0- to 5-cm depths from each replicated plots using a 5cm diameter and 5cm height core for analyzing the soil bulk density, soil water retention and pore size distribution by using pressure plate appratus. Furthermore, soil samples were collected for measuring soil microbial (soil microbial biomass carbon and nitrogen) and soil enzymatic activities (soil urease enzyme and soil beta-glucosidase) at 0- to 5- and 5- to 15-cm depths using hand auger unit. Four replication of samples were collected from each plot and mixed together to make a composite sample that represent the plot. The composite were sealed in a plastic zip-locks bags and shipped in the cold boxes to the labouratory for immediate analysis.

3.3.2. LabAnalysis

3.3.2.1. Soil Bulk Density (ρb)

Intact soil core samples were collected two times, first at pre-grazing in September, 2016 and second at summer phase on July 5, 2017 before the planting of maize at 0- to 5-cm depths from each treatments. These samples were used for analyzing the soil bulk density using core method Grossman and Reinsch (2002). The soils samples were dried in the oven at 105°C for 24 to 48 hours. The ρ_b was calculated by dividing the oven-dry soil weight with the volume of the core.

3.3.2.2. Soil Water Retention (SWR) and Pore-Size Distribution (PSD)

At same time soil intact core samples were used to measure the soil bulk density and soil water retention. SWR was measured using intact soil core samples and these samples were saturated for 24 to 48 hours depending upon the soil types. SWR was measured at 0, −0.4, −1.0, −2.5, −5.0, −10.0, −30.0 kPa matric potential using pressure plate and tension table apparatus. Gravimetric water content was converted to volumetric water content by multiplying with soil bulk density and dividing with the density of water and used for calculating soil water content (g g-1). Capillary rise equation was used to from SWR data's for all pore size classes (micropores, fine mesopores, coarse mesopores and macropores) as explained by (Jury et al., 1991). The sizes of each pore varies for example micro-pores (<10 μm equivalent cylindrical diameter; ecd), fine-mesopore (10- to 60- μm ecd), coarse measopore (60- to 1000-μm ecd), macro-pores (>1000 μm ecd).

3.3.2.3. Soil Organic Carbon (SOC) Total Nitrogen (TN)

Dry combustion method was used to determine the soil organic carbon using CN elemental analyzer. CN analyzer gives the percentage of total carbon and total nitrogen. Soils were reacted with hydrochloric acid was used to determine the soil inorganic carbon

(Schumacher, 2002). The SOC was calculated by subtracting the soil inorganic carbon from total soil carbon.

3.3.2.4. Soil Carbon and Nitrogen Fractions

The water extractable organic carbon (WEOC) and acid hydrolysis was carried out by schematic procedure described by Ghani et al. (2003) and Silveira et al. (2008). The extraction was done with distilled water in a soil-to-solution ratio of 1:10. A 3 g of soil was poured with 30 ml of water and put for shaking on vortex and rotatory shaker for 10 sec. and 30 min. at 40 rpm respectively. After extraction, the suspension was centrifuged at 3000 rpm for 25 min. at 4°C. The filtrate obtained is cold water extractable organic carbon (CWEC). A further 30 ml of water is added to the remaining residue and put on a vortex shaker for 10 sec. The suspension was left in hot-water bath at 80°C for 12-15 h. After extraction, the suspension was again put on vortex shaker for 10 secs and then, centrifuged at 3000 rpm for 25 min. at 25°C. The filtrate obtained is hot water extractable organic carbon (HWEC). After CWEC and HWEC, the same soil sample was air-dried and at first used for carrying out acid hydrolysis with 1M HCl and then, with 6M HCl at 105°C for 6 h in a soil-to-solution ratio of 1:30. Both hydrolysis was centrifuged separately at 3000 rpm for 25 min. and the supernatant's obtained are termed as 1M and 6M acid extractable carbon fractions. In this process all the extracts were considered as organic carbon and organic nitrogen because the pH of the soil solution was less than 6. Cold water, hot water and acid extractions of carbon and nitrogen were determined for the 0- to 5- and 5- to 15-cm depths using the TOC-L analyzer (Shimadzu Corporation, model-TNM-L-ROHS).

3.3.2.5. Microbial Biomass Carbon (MBC) and Nitrogen (MBN)

Chloroform fumigation and direct extraction method were used to determine the MBC and MBN (Beck et al., 1997; Kaiser and Heinemeyer, 1993). We divided each sample into 3 subsamples; one for non-fumigated; one for gravimetric soil moisture content and one for fumigated samples. 10g oven dry equivalent wait was used for both fumigated and non-fumigated samples. The non-fumigated samples were placed in a centrifuge tube with 40 ml of 0.5 M K_2SO_4. Samples were shacked for an hour, it was filtered through pre-leached with with 0.5 M K_2SO_4 Whatman No. 1 filter paper, and then soil extract was kept at 4ºC until further analysis. The fumigated samples were fumigated in vacuumed desiccators with 50 ml of beaker containing 20 ml of chloroform. After chloroform boils the samples were kept in dark for 24 hours. After releasing the vacuum and excess chloroform, the soil sample was extracted with 40 ml of K_2SO_4 and shook it for one hour and then filtered it through Whatman No 1 filter paper. The difference between C in the fumigated and non-fumigated samples is the chloroform - labile C pool (EC), and is proportional to microbial biomass C (C):

$$C = EC/kEC$$

where kEC is soil specific, but is often estimated as 0.45 (Beck et al., 1997).

Determination of the microbial biomass C and N.

Total weight of extractable C in the fumigated (C_F) and unfumigated (C_{UF}) soil samples:

C_F, C_{UF} (µg g^{-1} soil) = organic C * [(WT - DW) + EV] / DW

Total weight of extractable N in the fumigated (NF) and unfumigated (NUF) soil samples: N_F, N_{UF} (µg g^{-1} soil) = total N * [(WT - DW) + EV] / DW

Where WT is the soil fresh weight, DW is the soil dry weight, EV is extractant volume.

Microbial biomass C in the soil (MBC): MBC (µg g^{-1} soil) = (C_F - C_{UF}) / K_{EC}

Where K_{EC} = 0.35 and represents the efficiency of extraction of microbial biomass C. Values for K_{EC} range from 0.25 to 0.45 (Joergensen and Mueller, 1996; Wu et al., 1990).

Microbial biomass N in the soil (MBN):

MBN (µg g^{-1} soil) = (N_F - N_{UF}) / K_{EN}

where K_{EN} = 0.5 and represents the efficiency of extraction of microbial biomass N. Values for K_{EN} range from 0.18 to 0.54 (Joergensen and Mueller, 1996).

3.3.2.6. Soil Urease Enzyme

Colorimetric determination of ammonium was used to determine the urease enzyme activity described by (Kandeler and Gerber, 1988). In 50 ml beaker, 5g soil was placed in three flasks, and in first two flask 2.5 mL of urea solution was added. Then 20 mL borate buffer was added in all the flasks. All the flask was incubated for 2 hours at 37˚C. After the incubation process in incubator, 2.5 mL of urea solution was added in the third flask. 30 mL of 2M Potassium Chloride, KCl was added which act as an extractor in all flask and shook it for 30 minutes. After the filtration the color reaction was done by adding 1mL of filtrate with 9mL of water and 5mL of sodium salicylate ($C_7H_5NaO_3$)-sodium hydroxide (NaOH) solution as well as 2mL of Oxidation agent - sodium dichloroisocyanurate ($C_3C_{l2}N_3NaO_3$) was mixed in all the flasks. Spectrophotometer was used to determine the absorbance of the soil samples at 660 nm wavelength and a standard curve was prepared with standards of 0, 1, 1.5, 2, and 2.5 µg N mL^{-1} of ammonium chloride (NH_4Cl). The calculation of urease activities was done using following equations:

Urease Activity (µg NH_4-N g^{-1} soil $2h^{-1}$) = (NCS – NCC) × DF× V× T/DW

where, NCS is the NH_4-N concentration of the sample average (µg NH_4-N mL^{-1}), NCC is the NH_4-N content of the control (µg NH_4-N mL^{-1}), DF is dilution factor (10), V is the volume of urea solution used (2.5 mL), T is incubation time (2 h), and DW is the dry weight of the soil taken (5 g).

3.3.2.7. Soil Beta-glucosidase Enzyme

Determination of beta-glucosidase enzyme activity was done described by (Eivazi and Tabatabai, 1988). Standard stock solution p-nitrophenol 0, 100, 200, 300, 400, or 500 nmol was used to develop calibration curve. 1g of soil was taken separately in three 50 mL Erlenmeyer flasks in which one was used as a control and toluene was added (0.2 mL), mixed properly, and let them settle for 15 minutes in the fume hood. After taking out from hood add 4mL of modified universal buffer (MUB) pH 6.0 and 1 mL of p-nitrophenyl-β-D-glucoside (PNG) solution, mixed well, and incubated it for 1 hour at 37˚C. After incubation add, 0.5M Calcium Chloride (CaCl2) (1mL), 0.1M Tris (hydroxyethyl) Aminomethane (THAM) buffer (pH 12) (4mL), then mixed thoroughly, and filter the suspension through Whatman filter paper (No. 2V). Spectrophotometer was used to determine the yellow color intensity at 405 nm wavelength and the amount of p-nitrophenol released by reference to a calibration curve was calculated. Determination of amount of p-nitrophenol released from the soil was calculated by using references to calibration curve by the following equations:

Beta-glucosidase activity (µmol p-nitrophenol g^{-1} soil h^{-1}) = (NCS-NCC) *V*T/DW

where, NCS is p-nitrophenol content of sample average (µg NH_4-N mL^{-1}), NCC is p-nitrophenol content of control (µg NH_4-N mL^{-1}), V is volume of pNG solution used (1 mL), T is incubation time (1 h), and DW is dry weight of soil taken (1 g).

3.3.3. Soil Penetration Resistance (SPR) Measurement

Eijkelkamp-type hand penetrometer was used to measure the soil penetration resistance for 0- to 5- cm depth for all treatments including cover crop (broadleaf dominated cover crop and grass leaf dominated cover crop), grazing (yes and no) and time (summer phase) (Herrick and Jones, 2002). From each treatment a total of four SPR was taken at 0- to 5-cm depth and the average value was used to represent the SPR. To avoid the impact of soil moisture content it was measured four times using portable soil moisture meter for each treatment and at 0- to 5-cm depth and results were standardize using following equation developed by (Busscher and Bauer, 2003):

$$SPR_c = SPR_0 \times e^{\frac{x-0.1}{0.132}}$$

3.4. Statistical Analysis

The selected soil properties were statistically compared using pairwise differences method (adjusted by Tukey) by a mixed model in which the cover crop, grazing, time, cover crop × grazing, cover crop × time, grazing × time, and crop × grazing × time were considered as fixed effects and the replication as random effects. The models were conducted using GLIMMIX procedure in SAS 9.4 (SAS, 2013). Transformation of data was completed when necessary. The transformations were determined using the Box-Cox method (Box and Cox, 1981; Box and Cox, 1964) and SAS TRANSREG procedure. Significance level was determined at $\alpha = 0.05$ for all statistical analysis.

CHAPTER 4

RESULTS

4.1. Soil Bulk Density (ρ_b) and Soil Penetration Resistance (SPR)

Data of soil bulk density at 0- to 5-cm depth under different cover crops and grazing treatments, collected at different time intervals are presented in Table 1. The time (T) had a significant impact on soil ρ_b at the 0- to 5-cm depth. The mean ρ_b value under post-grazing was significantly lower than that for the pre-grazing. Grazing significantly impact the soil ρ_b where an increase of 1.53% in ρ_b was observed under the grazing compared with that under un-grazing treatment. The cover crop treatments did not significantly impact the ρ_b. However, the mean ρ_b value under the G-CC was higher than that for the B-CC and CT. The lowest ρ_b was observed under the CT (1.29 Mg m^{-3}) and the highest was under the G-CC (1.32 Mg m^{-3}). The interaction effects among cover crop (R), grazing (G) and time (T) on ρ_b were not significant.

Data of SPR at 0- to 5-cm depth under different cover crop and grazing treatments, collected at different times are provided in table 1. Cover crop treatment (R) did not significantly impact the SPR. The highest value of SPR was observed under grass leaf dominated cover crop (1.66MPa) and lowest under broad leaf dominated cover crop treatment (1.60MPa).

4.2. Soil Water Retention (SWR)

Data on soil water retention measured at the 0- to 5-cm depth under different cover crop and grazing treatments, collected at different times are provided in table 3.

The time (T) had a significant impact on SWR at all pressures. The mean SWR values of summer soils significantly increased by 55% at 0 kPa, 54% at -0.4 kPa, 55% at -1.0 kPa, 54% at -2.5 kPa, 54% at -5.0 kPa, 58% at -10.0 kPa and 55% at -30.0 kPa as compared to pre-grazing soil samples. Cattle grazing (G) and cover crop treatments (R) did not significantly impact the SWR at all pressures. The cover crop control treatment had the lowest water retention capacity among three cover crops at the 0- to 5-cm depth. The B-CC and G-CC followed almost similar trend (i.e., soil water retention pattern was same) for all pressures. The effects of cover crops (R) × time (T), grazing (G) × time (T), and cover crop (R) × grazing (G) × time (T) on SWR were not significant.

4.3. Pore-Size Distribution (PSD)

Data on soil pore-size distribution (PSD) for the 0- to 5-cm depth under different cover crop and grazing treatments, collected at different times are provided in table 2. The time (T) had a significant impact on soil PSD at the 0- to 5-cm depth for coarse mesopores and micropores, however, no significant differences were observed for macropores and fine mesopores. Cattle grazing (G), and cover crop treatments (R) did not significantly impact soil PSD for all pore sizes. The effects of cover crops (R) × time (T), grazing (G) × time (T), and cover crop (R) × grazing (G) × time (T) on PSD were not significant.

4.4. Cold Water Carbon Fraction (CWC) and Hot Water Carbon Fraction (HWC)

Data on soil cold water carbon and hot water carbon fractions measured at the 0- to 5- and 5- to 15-cm depths under different cover crop and grazing treatments, collected

at different times are provided in table 4. The time (T) had a significant impact on soil CWC at the 0- to 5- and 5- to 15-cm depths. The mean soil CWC under the post grazing increased by 62% and 41% at the 0- to 5- and 5- to 15-cm depths, respectively, compared with the pregrazing. Cattle grazing (G) and cover crop treatments (R) did not significantly impact the CWC for both depths. Under the cover crop treatment, the highest value of soil CWC was observed under control (22.51 μg C g^{-1} soil). No significant impact of R×T, G×T and R×G×T on CWC were observed.

The time (T) had a significant impact on soil HWC for the 0- to 5- and 5- to 15-cm depths. The HWC under the post grazing numerically increased by 52.47% and 37.79% at 0- to 5- and 5- to 15-cm depth respectively compared to the pregrazing. Cattle grazing (G) and cover crop treatments (R) did not significantly impact HWC for either of the depths. However, after short-term cattle grazing the mean values of soil HWC was found to be numerically decreased at both depths. In general, the soil HWC decreased with increase in depth. Under the cover crop treatment, the highest value of soil HWC was observed under G-CC (97.48 μg C g^{-1} soil) and the lowest under the cover crop control (86.79 μg C g^{-1} soil) at the 0- to 5-cm depth. No significant impact of R×T, G×T and R×G×T on HWC were observed.

4.5. 1M HCl Carbon (OMC) and 6M HCl Carbon (SMC) Fractions

Data on soil 1M HCl carbon and 6M HCl carbon fractions measured at 0- to 5- and 5- to 15-cm depths under different cover crop and grazing treatments, collected at different times are provided in table 5. The time (T) did not significantly impact the soil OMC at the 0- to 5- and 5- to 15-cm depths. Cattle grazing (G) and cover crop treatments (R) did not significantly impact the OMC for both depths. Under the cover crop

treatments, the highest value of soil OMC was observed under the G-CC (439.5μg C g^{-1} soil) and the lowest under the cover crop control (358.3μg C g^{-1} soil) at the 0- to 5-cm depth. No significant impact of R×T, G×T and R×G×T on OMC were observed.

The time (T) significantly influenced the soil SMC fraction for the 0- to 5-cm depth. The mean soil SMC under the post grazing significantly decreased by 29% at the 0- to 5-cm depth as compared to pre-grazed, but no significant differences were observed at the 5- to 15-cm depth. Cattle grazing (G) and cover crop treatments (R) did not significantly impact the SMC for both depths. Under the cover crops treatment, the highest mean value of soil SMC was registered under the G-CC (93.79 μg C g^{-1} soil) and the lowest under the B-CC (90.94 μg C g^{-1} soil) at the 0- to 5-cm depth. No significant impact of R×T, G×T and R×G×T on SMC were observed at both depths.

4.6. Cold Water Nitrogen (CWN) and Hot Water Nitrogen (HWN) Fractions

Data on soil cold water nitrogen and hot water nitrogen fractions measured at 0- to 5- and 5- to 15-cm depths under different cover crop and grazing treatments, collected at different times are provided in Table 6. The time (T) had a significant impact on soil CWN fraction for the 0- to 5- and 5- to 15-cm depths. The mean soil CWN under the pre-grazing was found to be significantly increased by 21% at the 0- to 5-cm depth and 28% at the 5- to 15-cm depth as compared to pre-grazing. Cattle grazing (G) did not significantly impact CWN for both depths. Cover crop had a significant impact on soil CWN at 0- to 5-cm depth. Under cover crops treatment the highest value of soil CWN was observed under G-CC (5.56 μg N g^{-1} soil) and lowest under B-CC (4.89 μg N g^{-1} soil) at 0- to 5-cm depth. No significant impact of R×T, G×T and R×G×T on CWN were observed at both depths.

The time (T) had a significant impact on soil HWN fraction at 0- to 5- and 5- 15-cm depth. The mean soil HWN was found to be significantly increased by 33% at 0- to 5-cm and 37% at 5- to 15-cm depth as compared to pre-grazed. Cattle grazing (G), and cover crop treatments (R) did not significantly impact the HWN for both depths. Under cover crops treatment the highest value of soil HWN fractions was observed under G-CC (14.96 μg N g^{-1} soil) and lowest under CT (13.50 μg N g^{-1} soil) at 0- to 5-cm depth. No significant impact of R×T, G×T and R×G×T on HWN were observed at both depths.

4.7. 1M HCl Nitrogen (OMN) and 6M HCl Nitrogen (SMN) Fractions

Data on soil 1M HCl nitrogen and 6M HCl nitrogen fractions measured at the 0- to 5- and 5- to 15-cm depths under different cover crop and grazing treatments, collected at different times are provided in table 7. The time (T) had a significant impact on soil OMN at 0- to 5- and 5- to 15-cm depth. The mean soil OMN was found to be significantly decreased by 31% at 0- to 5-cm and 36% at 5- to 15-cm depths as compared to pre-grazing. Cattle grazing (G), and cover crop treatments (R) did not significantly impact OMN for both depths. Under cover crop treatments the highest value of soil OMN was observed under G-CC (57.95 μg N g^{-1} soil) and lowest under CT (45.53 μg N g^{-1} soil) at 0- to 5-cm depth. No significant impact of R×T, G×T and R×G×T on OMN were observed at both depths.

The time (T) had a significant impact on soil SMN at 0- to 5- and 5- to 15-cm depth. The soil SMN was found to be significantly decreases at 0- to 5- and 5- to 15-cm depths as compared to pre-grazing. Cattle grazing (G), and cover crop treatments (R) did not significantly impact SMN for both depths. Under cover crop treatments the highest value of soil SMN was observed under CT (9.05 μg N g^{-1} soil) and lowest under G-CC

(8.81 µg N g^{-1} soil) at 0- to 5-cm depth. No significant impact of R×T, G×T and R×G×T on SMN were observed at both depths.

4.8. Microbial Biomass Carbon (MBC) and Microbial Biomass Nitrogen (MBN)

Data on soil microbial biomass carbon and microbial biomass nitrogen measured at the 0- to 5- and 5- to 15-cm depths under different cover crop and grazing treatments, collected at different times are provided in Table 8. The time (T) had a significant impact on soil MBC at the 0- to 5- and 5- to 15-cm depths. The mean soil MBC value under the summer (217.4 µg C g^{-1} dry soil) was significantly higher than that for the pre-grazing (118.3 µg C g^{-1} dry soil) and the post-grazing (103.9 µg C g^{-1} dry soil) at the 0- to 5-cm depth. The mean soil MBC of the summer (130.8) was significantly higher than that for the post grazing (75.28), which was significantly higher than the pre-grazing (42.63) at the 5- to 15-cm depth. Cattle grazing did not significantly impact the soil MBC at both depths, but the mean values of soil MBC decreased after the grazing at both depths. The cover crops treatments did not significantly impact the MBC at both depths. The highest mean value of soil MBC was observed in the grass leaf cover crops (158.9 µg C g^{-1} dry soil) and the lowest in broad leaf dominated cover crops (134.8 µg C g^{-1} dry soil) at 0- to 5- cm depth. No significant impact of R×T, G×T and R×G×T on MBC were observed at both depths.

Time (T) had a significant impact on soil MBN at the 0- to 5- and 5- to 15-cm depths. The mean soil MBN under the post-grazing and summer decreased by 46% and 44% than that for the pre-grazing at the 0- to 5-cm and trend was same for 5- to 15-cm depth, respectively. However, no significant differences among the post-grazing and the summer were observed on MBN for both the depths. Cattle grazing, and the cover crops did not

significantly impact the soil MBN at both depths. The highest mean value of soil MBN under the cover crop treatments (R) were observed in grass leaf cover crops (31.87 µg N g^{-1} dry soil) and the lowest in broad leaf cover crops (29.15 µg N g^{-1} dry soil). However, no significant impact of $R \times T$, $G \times T$ and $R \times G \times T$ on MBN were observed at both depths.

4.9. Soil Urease and β-glucosidase Enzyme Activities

Soil urease enzyme activity measured at the 0- to 5- and 5- to 15-cm depths under different cover crop and grazing treatments, collected at different time interval are provided in table 9. Time (T) had a significant impact on the urease enzyme activity at the 0- to 5- and 5- to 15-cm depths. The mean soil urease enzyme activity under the summer was significantly higher than that for the post-grazing (123%), which was significantly higher than the pre-grazing (195%) at the 0- to 5-cm depth. The mean urease enzyme activity under the summer was significantly higher than the post- and pre-grazing at the 5- to 15-cm depth. Grazing did not show any significant impact on the soil urease enzyme activity at the 0- to 5- and the 5- to15-cm depths. However, there was a decrease in the mean values of soil urease enzyme activity under the grazing was observed, compared with the un-grazing. Cover crop treatments (R) did not significantly impact the mean value of soil urease enzyme activity at the 0- to 5- ($P=0.31$) and 5- to 15-cm ($P=0.30$) depths. The highest soil urease enzyme activity was observed under the grass leaf cover crops (205.3 µg NH_4-N g^{-1} soil $2h^{-1}$) and the lowest in cover crop control (152.3 µg NH_4-N g^{-1} soil $2h^{-1}$). No significant effects of the R×T, G×T, and R×G×T on the soil urease enzyme activity were observed at both depths.

The data of soil β-glucosidase enzyme activity measured at 0- to 5- and 5- to 15-cm depths at three times (i.e., pre-grazed, post-grazed, and post-grazed summer phase) as to

evaluate the influence of cover crop under the grazing and ungrazing treatments in ICLS (Table 9). The time (T) had a significant impact on the soil β-glucosidase enzyme activity at the 0- to 5- and 5- to 15-cm depths. The mean value of soil β-glucosidase enzyme activities under the summer phase was significantly lower than under the post-grazing and was significantly lower than the pre-grazing at the 0- to 5-cm depth. At the 5- to 15-cm depth, the mean β-glucosidase enzyme activity under the summer was significantly lower than that under the post-grazing and the pre-grazing. Cattle grazing did not significantly impact the soil β-glucosidase enzyme activity at both depths. Cover crop treatments (R) did not significantly impact the mean soil β-glucosidase enzyme activity at the 0- to 5-cm depth, but significantly influenced this enzyme activity at the 5- to15-cm depth (the mean under the B-CC was significantly higher than that for the CT). The highest soil β-glucosidase enzyme activity was observed in grass leaf cover crop (54.01 µmol pNP g^{-1} dry soil h^{-1}) and the lowest in control treatment (46.77 µmol pNP g^{-1} dry soil h^{-1}) at the 0- to 5-cm depth. No significant impact of R×T, G×T and R×G×T on the β-glucosidase enzyme observed at both depths.

CHAPTER 5

DISCUSSION

5.1. Impact of Cover Crops on Soil Properties

The findings from this study showed that cover crops did not significantly impact soil BD, SPR, soil water retention, soil urease enzyme, MBC, MBN, and all carbon and nitrogen fractions (labile, stable and recalcitrant), but significantly impacted the soil β-glucosidase activity at the 5- to 15-cm depth and CWN at 0- to 5-cm depth (Table 1-9). The mean β-glucosidase in the B-CC was significantly higher than that for the CT (no cover crops) (Table 9). This is in accord with the previous studies that showed that soil β-glucosidase activity increased by including winter cover crops as compared to no cover crops in the South Dakota, USA (Abbasi et al., 2002; Hai-Ming et al., 2014). This is likely due to avability of plant residues which are consumed by the soil microorganism leading to decomposition of organic matter through the seceretion of enzymes mediated by both plants and soil microorganisms.

Cover crops had no significant impact on soil bulk density and soil penetration resistance (Table 1). This is in accord with a previous study conducted at Lincoln University's Freeman farm during 2011 and 2012 to assess the effects of cover crop management on soil physical and biological properties reported a non significant decrease of 3.5% in soil bulk density observed under cover crop plots as compared with no-cover crop plots (Haruna and Nkongolo, 2015). Similar to our findings, a previous study conducted at typical midwestern Indiana reported that bulk density showed no significant changes between cover crop treatments (Rorick and Kladivko, 2017). Similar to our findings, a study in California reported that there was no difference in bulk density,

soil moisture, soil resistance due to cover crops (Karlen et al., 1997). No significant differences in this study is likely due to the reason that various blends of cover crops help in elevating the problem of soil compaction created by grazing due to differences in root growth pattern which break the soil and helps in movement of soil air and water.

Cover crops did not significantly impact soil water retention at the 0- to 5-cm depth (Table 2). The cover crop control treatment had the lowest water retention capacity and the B-CC and G-CC have almost similar trend for all pressures in this study, and no significant differences were observed under different cover crops. Similar trend was reported by Beehler et al. (2017) in the Midwest Corn Belt where cover crop effects on both total organic C and soil water retention levels were not statistically significant. Control treatment was found to have lowest water retention capacity. In this study, soil water retention of broadleaf dominated cover crop and grass leaf dominated cover crop shows no significant differences and hence in short term period the type of cover crop had no significant impact on soil water retention, but the retention capacity was higher as compare to cover crop control treatment. Similar to our results, a previous study reported that a diversified crop rotation system increased the multiple use of land and may have the potential to improve soil organic matter content, nutrient cycling, and soil water retention capacity as compared to less diversified cropping system (Davinic et al., 2013). The reason behind no significant differences may be because this was short-term study and it is apparent that a long-term experiment would be required to detect changes in soil physical properties because of the soil management practices.

Cover crops had no significant impact on soil MBC and MBN (Table 8). This differs from McDaniel et al. (2014) who found that cover crop rotation increases the soil

microbial biomass C by 20.7%. In contrast to our study findings, Jonasson et al. (1996) reported that diversifying the monoculture by adding one or more crops increased the soil C by 3.6% and MBC by 20% while increasing N by 5.3% and MBN by 26.1%. No significant differences in this study is likely because of the short-term impact of cover crops on soil microbial biomass was evaluated under which the nature and biochemistry of plant materials are important, a long-term study needed to be conducted to quantify the impact of cover crops.

Cover crops significantly impact the cold-water nitrogen at 0- to 5-cm depth and rest soil C and N fractions were not significantly impacted by cover crops at both the depths (Table 4 -7). A similar study found that the hot water extractable carbon showed higher biodegradability rate than the cold water carbon extraction (Gregorich et al., 2003). The grass leaf cover crop showed significantly higher cold water nitrogen fractions and it is likely because the grass leaf cover crop helps in reducing the nitrogen losses through leaching. Significant increase in grass leaf dominated cover crop is likely due to the properties of grass to decrease the nitrogen leaching loss in soils from surface to subsurface horizon due to nature of their roots. No significant differences in this study is likely due to effect of several environmental and land management practices, long term study is needed to monitor the changes in different fractions of carbon and nitrogen, availability differences in temperature, water content and the nature of plant residues may be other reason associated with it.

Cover crops had no significant impact on soil urease enzyme activity for both soil depths (Table 9). The urease activity increased under cover crop treatment as compared with the control cover crop treatments but was not significant. This is in contrast with a

previous study counducted in southwestern part of USA by Hamido and Kpomblekou-A (2009) who reported that incorporation of cover crops such as black oat (*Avena strigosa*), crimson clover (*Trifolium incarnatum* L.), or crimson clover–black oat mixed into rotations may increase enzyme activities in soils. A similar trend was reported in a study conducted in South China that reported that incorporation of winter cover crops into rotations may increase enzyme activities and microbial community in soil and therefore improve soil quality (Hai-Ming et al., 2014). Non significant differences in this study are likely due to impact of land management practices i.e., nature of plant residues and climatic conditions etc for a short-term period. Furthermore, incorporation of plant rasidues (cover crops) into the soils which helps in promoting soil nutrient cycling, and temperature and moisture plays a critical role to activate the enzimatic processes.

Cover crops had no significant impact on soil beta-glucosidase enzyme activity for both the soil depths (Table 9). Beta-glucosidase enzyme activity increases under cover crop treatmenst as compared to that under no cover crop treatments but the differences were non-significant. This is in accord with previous study conducted in Southern China to evaluate the winter cover crop residues impact on soil enzymes which showed that beta-glucosidase activities reached peak at booting stage of crop and found that incorporation of winter cover crops into the exesting cropping system may increase the enzymes activities in soil (Hai-Ming et al., 2014). This is in accord with previous study conducted to compare a fallow-winter wheat (Triticum aestivumL.) rotation to several cover crop-winter wheat rotations under rainfed and irrigated conditions in the semiarid US High Plains reported that cover crop had no significant impact on soil beta-glucosidase enzyme activity (Calderon et al., 2016). Non significant differences are likely

due to short-term cover crop study treatments, however, changes can be observed under different mixtures of cover crop and it is apperent that long term study is required to detect the changes.

5.2. Impact of Grazing on Soil Properties

The findings from this study demonstrated that cattle grazing did not significantly impact soil water retentation, soil urease enzyme, soil betaglucosidase enzyme, MBC, MBN, carbon and nitrogen fractions (labile, stable and recalcitrant) (Table 2 – 9) except soil bulk density (Table 1).

Soil bulk density was found to be significantly increased by 1.58% after the grazing. This is in accord with a previous study that reported grazing significantly impacted soil bulk density, high values of soil bulk density values generally found at the 0- to 10-cm soil depth when heavy grazing is applied (Pulido et al., 2016). Similarly, livestock trampling led to soil compaction and increased soil bulk density at shallow depths and however the damage is not severe and can be rectified with appropriate measures (Hamza and Anderson, 2005). This is in contrast with previous results reported under livestock grazing winter residues, weeds or cover crops did not significantly increases soil bulk density and these measures helps in elevating the problem (Fernandez-Rivera et al., 2002). The significant differences in the study is likely due to changes in land management practices and prevailing weather conditions, most importantly soil moisture content. This part of South Dakota comes under arid region receives little autumn rainfal and in this study the grazing was applied during November when the soil was dry which helps to reduce soil compaction problems. A similar finding was reported

where infilteration and compaction is minimized when the soils are dry (Maughan et al., 2009).

Grazing did not significantly impact the soil water retention for all the soil water pressures at the 0- to 5-cm depth (Table 2). This is in contrast with previous study conducted to eveluate the long-term overgrazing-induced changes in topsoil water-retaining capacity in a typical steppe reported that water retention capacity of the grassland soil decreased significantly (by 23.5%) after long-term over-grazing and long-term-grazed soil had significantly lower water-retaining capacity compared with ungrazed soil (Li et al., 2017). A study conducted at southeast Idaho reported that in addition to a variety of other factors, management decisions (grazing and rest) can have substantial influence upon soil-water content and that soil-water content can vary substantially as a result of animal impact and the duration of grazing (Weber and Gokhale, 2011). This is in contrast with a previous study showed that grazing intensity influenced changes in available water holding capacity at 0- to 5-cm depth (Mapfumo et al., 2000). The non significant differences is likely due to external meteorological factors, temperature most strongly governed grassland soil water evaporation. Long term grazing needs to be conducted to know its impact on soil water retention. Soil texture (fraction of sand, silt and clay) is an important parameter which influences soil water retention (Zhuang et al., 2001). Grazing intensities affect SWR more than that of season of grazing (Naeth et al., 1991).

Grazing did no affect soil MBC and MBN (Table 8). This is in accord with a previous finding in Mongolia by Liu et al. (2012). This is in accord with a previous study conducted to acesses the effects of grazing and nitrogen fertiliser on the soil microbial

biomass under permanent pasture reported that values for microbial C under cutting and grazing were not significantly different (Bristow and Jarvis, 1991). This contrasts with a previous four decades long-term grazing impact on soil MBC study conducted in China that reported significant decreases in MBC under grazing treatment (He et al., 2017). Aditionally, previous study in Brazil reported that high grazing intensity during the pasture cycle may cause a decrease in soil MBC and have a negative effect on the microbial biomass during the succeeding crop in a ICLS (Silva et al., 2015). In contrast with our study result findings, a previous study reported the high rate of microbal activity at surface layer is due to availibility of plant residues, low chemical degradibility of N, increase in soil temperature and water vapour movement (Schimel and Parton, 1986). The reason behind no significant differences may be due to short-term study and the grazing time, grazing intensities and nature of grazing materials plays an important role. It is apparent that long term experiment would be required to detect changes in soil microbial properties as a result of the soil management practices under ICLS.

This study demonstrated that grazing did not significantly impacted all types of carbon and nitrogen fractions i.e., labile, stable and recalcitrant, for both depths (Table 4-7). Similar to our findings, Gregorich et al. (2003) reported the changes in soil CWC is associated with land use and management practices. Contrary to our findings, Ghani et al. (2003) reported that under a two year experiment consist of different grazing intensities on allphanic soils in New Zealand showed that intensively grazing reduces the soil stable carbon under dairy grazed system as compare to sheep grazing i.e., less grazing intensities. To describe the reason behind the no significant changes in our findings, Belić et al. (2011) reported that the differences between the cold and hot water soil carbon are

due to changes in land use and management practices and importantly hot water is more sensitive (indicative) to SOC as compared to CWC. Higher clay content and absorption capacity of the soil like vertisols prevent leaching of SOC hence enhancing the soil extractable carbon content in the soils. This is likely due to negative correlation of grazing with SOC/MBC in initial years of study (He et al., 2017).

Grazing had no significant impact on soil urease enzyme (Table 9). This agrees with a previous study conducted to understand the seasonal changes in urease activities in mixed prairie and fescue grassland Ah horizons reported that enzymatic activities are highest in samples from the grassland site regardless of grazing intensity (Dormaar et al., 1984). In contrast to our findings, Acosta-Martínez et al. (2007), conducted a stuty in Texas, reported that urease activity was higher in grazed plots as compare to ungrazed plots. No significant differences is likely due to nature of plant residues and its degredation in soils and other possiable reason may be due to short-term period of grazing treatment and grazing intensity thus it is apperent that long term study is needed to monitor the changes in urease activity (Reddy et al., 1987). Urease activity is strongly related with vegetation, quality of organic materials and fluctuation in nutrient levels (Palma and Conti, 1990; Speir et al., 1984; Stott and Hagedorn, 1980). Maximum catalytic activity of soil urease enzyme occures at 65 degree celcius (Blakeley and Zerner, 1984) and it is inactive above 70 degree celsius (Frankenberger and Tabatabai, 1982). The optimum pH for urease lies between 6.0 to 7.0 (Boyd and Mortland, 1985; Lai and Tabatabai, 1992). Urease activity increased under vegetation as compared to vegetation free sites (Reddy et al., 1987).

Grazing had no significant impact on soil beta-glucosidase enzyme activity (Table 9). In contrast to our findings, George et al. (2013) reported that grazing treatment had 47% higher beta-glucosidase activity as compared to that under the ungrazed treatment. A study conducted to evaluate the correlations of soil enzyme activity and carbon and nitrogen mineralization reported that soil β-glucosidase activity in the integrated crop–livestock system was significantly lower than the other systems and reducing sugar C was negatively correlated with β-glucosidase activity and positively with exoglucanase activity (Tian et al., 2010). No significant differences in soil enzymes in this study is primarily due to climatic conditions like deposition of snow on soils which can alter soil microbial properties and nature of grazing materials, time and intensity of grazing are important factors.

5.3. Soil Properties Changes With Sampling Time

The finding from this study showed that sampling time significantly impacted the soil BD, soil water retentation, soil urease enzyme, MBC, MBN, and on all carbon and nitrogen fractions (labile, stable and recalcitrant) except for pore size distribution (macropores and fine mesopores) and soil acid fractions. The result from this study reveals that time significantly decreased soil BD. This is in accord with a previous study conducted in Pana, Illinois, from 2002 to 2005 by the Tracy and Zhang found the winter grazing is more prone to soil compaction issues, as time of grazing is important factor which impact the soil compaction (Tracy and Zhang, 2008). In Brookings, South Dakota the soils were dry on September, 2016 (first sampling time), and little bit moist as compare to first sampling time, collected on November 21st 2016 (Second sampling time), and wet in July 1st 2017 due to snow melting (3rd samplign time). Summer soil (3rd

sampling time) showed decreased in soil BD, the reason behind this was high soil moisture content condition due to snow melting. After the grazing the above ground biomss was removed and below ground biomass reduced due to cattle grazing. Snow melts and increase in soil moisture content cause reduction in soil BD at a significant level. This is in accord with a previous findings which showed that high moisture containing soil is more prone to compaction issue (Bell et al., 2011). Similar to our findings, a two-year study in Florida in 2013 on an Ultisol, Dothan sandy loam soil involving two grazing treatments (i.e. grazed and ungrazed) by (George et al., 2013) concluded that grazing significantly impacted soil BD only at 0- to 5- cm depth, few differences were detected at depths lower than 5 cm (George et al., 2013). Contrary to our results, a study in Argentina reported that cattle grazing will not cause the soil compaction if the grazing component under ICLS is managed properly (Fernández et al., 2015).

Sampling time significantly impacted the soil carbon fractions (labile, stable and recalcitrant) except OMC at both the soil depths. The mean values of soil CWC increases by 62% and 41% at 0- to 5- cm and 5- to 15- cm depth, respectively, as compaed to the pregrazed and this is likely due to the changes associated with land use and management practices, soil moisture and temperature also impact the processes. The differences between the cold and hot water soil carbon fractions are likely due to several factors associated with change in land use and management practices and soil rhizosphere microbial community also play important role (Chantigny, 2003; Ćirić et al., 2016; Gregorich et al., 2003). Similar trend was reported under HWC which was found to be numerically increases by 52.47% and 37.8% at 0- to 5- and 5- to 15-cm depths,

respectively, as compaed to the pregrazed. Higher clay content and absorption capacity of the soil like vertisols prevent leaching of SOC hence enhancing the soil extractable carbon content in the soils (Belić et al., 2011). As the depth increases the value of HWC decreases, similar trend was reported in a study where decrease in the soil cold water carbon as we move to lower depths into the soils Hamkalo and Bedernichek (2014).

Sampling time significantly impacted the soil MBC and MBN (Table 8). The MBC significantly increases as the time increases and MBN decreases because nitrogen is used by the soil microorganism as a source of energy. The summer soil sample had significantly higher MBC as compared to pre-grazing which may be due to the increase in soil temperature and the activation of metabolic processes of soil microorganism. This is in accord with a previous study conducted in western China reported that snow removal increases the soil MBC (Tan et al., 2014). Similar to our findings, a study conducted to evaluate the warming effects on microbial community found that warming significantly enhanced the microbial metabolic activity (Schindlbacher et al., 2011).

This study revealed that time significantly impacted soil urease enzyme activity for both the soil depths (Table 9). Differences are likely due to changes in climatic conditions. In Brookings, South Dakota, the soils collected on the first sampling were dry soils with high temperature. The second soil samples were collected under high soil moisture and low temperature as compared to the first sampling and the third sample was collected under wet soil conditions with high temperature. The urease activity was higher under summer soil sample due to high soil moisture content and soil temperature which are low under first sampling. This is in accord with a previous study reported that urease activity is highly influenced by moisture and temperature fluxes and urease activity found

to be highly variable parameters when the spatial varibility and chemical parameters are tested (Bonmati et al., 1991). Similar to our findings, researcher reported that urease activity was higher in surface depth as compare to subsurface(McGarity and Myers, 1967; Myers and McGarity, 1968).

Time significantly impacted the soil beta-glucosidase activity for both depths (Table 9). It is considered as a predictor of soil organic matter decomposition and plays a key role in providing energy for microorganisms. The soil beta-glucosidase enzyme activity during summer was significantly ($P<.0001$) lower than that of pre-grazing and post-grazing at the 0- to 5-cm depth. Potential reason behind this may be due to soil moisture and temperature fluxes which affect the rate of decomposition. Stott et al. (2010) observed the factors that affect the expected rage of β-glucosidase were inherent soil organic matter, soil texture, and climate. BG activity plays an important role in plant decomposition and SOC cycling and reported that soil and climate type has more impacts on β-glucosidase enzyme activity. Microbial degradation of cellulose to glucose and carbon cycle is affected by a rate-limiting enzyme soil β-glucosidase.

CHAPTER 6
CONCLUSIONS

Soil bulk density, SPR, SWR, PSD, carbon and nitrogen fractions (labile, stable and recalcitrant), MBC/MBN, urease and beta-glucosidase activities were quantified to determine the short-term impacts of cover crops and grazing in an ICLS on soil properties. The present study site was located at 44°20'34.8"N, 96°48'14.8"W, near Brookings, South Dakota, USA. The experiment was designed as a randomized complete block design with four replications. Cover crop blends (grass leaf and broad leaf dominated cover crops), grazing, and control treatments were used in 2016.

The main findings of this study are as follows:

Cover crop treatments did not significantly impact the soil ρ_b. However, grazing significantly increased the soil ρ_b. Time significantly decreased the soil ρ_b. No significant interactions of grazing by cover crops on ρ_b were observed. Cover crop treatments did not significantly impact the SPR. Cover crop and grazing treatments did not significantly impact the SWR. However, the time (T) had a significant impact on SWR for all pressures and no significant interactions were observed on SWR. The cover crop and grazing treatments had no significant impact on soil PSD. The time (T) had a significant impact on soil PSD at the 0- to 5-cm depth for coarse mesopores and micropores, however, no significant differences were observed for macropores and fine meso pores. No significant interaction was observed on PSD. The cover crop treatments significantly impacted the CWN at 0- to 5-cm depth, however, significant differences were observed on the rest all forms of soil carbon and nitrogen fractions. Grazing did not affect the soil carbon and nitrogen fractions. The sampling time (T) had a significant impact on soil

CWC and HWC at the 0- to 5- and 5- to 15-cm depths and SMC for only 0- to 5-cm depth. No significant interactions were observed on soil C and N fractions. The cover crop and grazing treatments had no affect on soil MBC. The time (T) had a significant impact on soil MBC at the 0- to 5- and 5- to 15-cm depths. The cover crop treatments had no impact on soil MBN. Grazing did not significantly impact the soil MBN. The time (T) had a significant impact on soil MBN at the 0- to 5- and 5- to 15-cm depths. No significant interaction was observed on soil MBC and MBN. The cover crop and grazing treatments had no significant impact on soil urease activity. The time (T) had a significant impact on the urease enzyme activity at the 0- to 5- cm and 5- to 15- cm depths. No significant interactions were observed on soil urease activity for both depths. Cover crop significantly impacted soil β-glucosidase activity only for 5- to 15-cm depths but no significant differences were observed at 0- to 5-cm depth. Grazing did not significantly impact the soil β-glucosidase activity. The time (T) had a significant impact on the soil β-glucosidase enzyme activity at the 0- to 5- and 5- to 15-cm depths. No significant interactions were observed on soil β-glucosidase activity.

I conclude from above results that the cover crop treatments did not significantly impact all soil properties that were studied. Grazing significantly impacted ρ_b but not others. Time significantly impacted the ρ_b, SWR, PSD, CWC, HWC, SMC, MBC, MBN, urease enzyme, and β-glucosidase enzyme activities. Since, under time the weather conditions (i.e., temperature, precipitation and snow cover) directly influence and alter the soil habitat. Since some of the soil properties responded negatively to grazing and cover crop treatments under ICLS during this short-term period, it is apperant that long-

term experiments are needed to detect changes in soil properties because of the soil management practices under ICLS.

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TABLES AND FIGURES

Table 1. Mean soil bulk density (ρb) and soil penetration resistance (SPR) at the 0- to 5-cm depth under different cover crop, grazing, and time treatments in ICLS.

Treatments[†]	ρ_b	**SPR**
	Mg m^{-3}	MPa
Cover Crops (R)		
B-CC	1.30[a††]	1.60[a]
G-CC	1.32[a]	1.66[a]
CT	1.29[a]	1.65[a]
Grazing (G)		
Yes	1.32[a]	-
No	1.30[b]	-
Time (T)		
Pre	1.35[a]	-
Summer	1.28[b]	-
	P>F	
R	0.88	0.66
G	0.64	-
R×G	0.44	-
T	0.008	-
R×T	0.80	-
G×T	0.84	-
R×G×T	0.51	

[†]B-CC, Broadleaf dominated cover crops; G-CC, Grassleaf dominated cover crops; CT, Control; Pre, Pre-grazing, soil samples were taken before grazing on Sep 2016; Post, post-grazing, soil samples were collected after grazing on Nov 21st, 2016; Summer, Summer phase, soil samples were collected next year in summer on June 2017.

[††]Means within the same column followed by different small letters are significantly different at $P<0.05$ for the cover crop, grazing, and time.

Table 2. Mean soil water retention (m^3 m^{-3}) at the 0- to 5-cm depth under different cover crop, grazing, and time treatments in ICLS.

Treatments[†]	Soil Water Pressure (-kPa)						
	0.01	0.4	1	2.5	5	10	30
	0-5 cm						
	Soil Water Content (m^3 m^{-3})						
Cover crops (R)							
B-CC	0.434[a††]	0.432[a]	0.431[a]	0.431[a]	0.429[a]	0.418[a]	0.400[a]
G-CC	0.433[a]	0.432[a]	0.431[a]	0.430[a]	0.429[a]	0.419[a]	0.416[a]
CT	0.410[a]	0.408[a]	0.407[a]	0.406[a]	0.405[a]	0.397[a]	0.393[a]
Grazing (G)							
Yes	0.442[a]	0.441[a]	0.440[a]	0.439[a]	0.438[a]	0.427[a]	0.408[a]
No	0.420[a]	0.418[a]	0.417[a]	0.416[a]	0.415[a]	0.407[a]	0.402[a]
Time (T)							
Pre	0.336[b]	0.335[b]	0.334[b]	0.334[b]	0.333[b]	0.321[b]	0.317[b]
Summer	0.521[a]	0.519[a]	0.518[a]	0.517[a]	0.516[a]	0.508[a]	0.492[a]
	Analysis of Variance *(P>F)*						
R	0.88	0.88	0.88	0.88	0.89	0.90	0.79
G	0.50	0.50	0.49	0.49	0.48	0.53	0.97
R×G	0.10	0.10	0.10	0.09	0.09	0.08	0.35
T	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
R×T	0.29	0.29	0.29	0.29	0.30	0.31	0.26
G×T	0.57	0.58	0.58	0.59	0.59	0.65	0.38
R×G×T	0.44	0.44	0.44	0.44	0.44	0.49	0.89

[†]B-CC, Broadleaf dominated cover crops; G-CC, Grassleaf dominated cover crops; CT, Control; Pre, Pre-grazing, soil samples were taken before grazing on Sep 2016; Post, post-grazing, soil samples were collected after grazing on Nov 21st, 2016; Summer, Summer phase, soil samples were collected next year in summer on June 2017.

[††]Means within the same column followed by different small letters are significantly different at $P<0.05$ for the cover crop, grazing, and time.

Table 3. Mean soil pore size distribution (m^3 m^{-3}) at the 0- to 5-cm depth under different cover crop, grazing, and time treatments in ICLS.

Treatments[†]	Pore Size Distribution			
	Macropores (> 1000 µm)	**Coarse mesopores (60-1000 µm)**	**Fine mesopores (10-60 µm)**	**Micro pores (< 10 µm)**
	---------- (m^3 m^{-3}) ----------			
	---------- 0-5-cm ----------			
Cover crops (R)				
B-CC	0.001[a††]	0.003[a]	0.029[a]	0.400[a]
G-CC	0.001[a]	0.003[a]	0.013[a]	0.416[a]
CT	0.001[a]	0.002[a]	0.013[a]	0.392[a]
Grazing (G)				
Yes	0.001[a]	0.002[a]	0.029[a]	0.408[a]
No	0.001[a]	0.003[a]	0.012[a]	0.402[a]
Time (T)				
Pre	0.001[a]	0.001[b]	0.016[a]	0.317[b]
Summer	0.001[a]	0.004[a]	0.023[a]	0.492[a]
	Analysis of Variance *(P>F)*			
R	0.38	0.37	0.48	0.79
G	0.31	0.35	0.22	0.97
R×*G*	0.89	0.41	0.26	0.35
T	0.92	0.0005	0.49	<0.0001
R×*T*	0.07	0.42	0.60	0.26
G×*T*	0.31	0.26	0.35	0.38
R×*G*×*T*	0.87	0.81	0.25	0.89

[†]B-CC, Broadleaf dominated cover crops; G-CC, Grassleaf dominated cover crops; CT, Control; Pre, Pre-grazing, soil samples were taken before grazing on Sep 2016; Post, post-grazing, soil samples were collected after grazing on Nov 21st, 2016; Summer, Summer phase, soil samples were collected next year in summer on June 2017.

[††]Means within the same column followed by different small letters are significantly different at $P<0.05$ for the cover crop, grazing, and time.

Table 4. Mean soil cold water carbon (CWC) and hot water carbon (HWC) fraction at the 0- to 5- and 5- to 15-cm depth under different cover crop, grazing, and time treatments in ICLS.

Treatments†	CWC		HWC	
	0-5-cm	5-15-cm	0-5-cm	5-15-cm
	μg C g^{-1} soil			
Cover crops (R)				
B-CC	21.29[a††]	21.47[a]	90.74[a]	69.78[a]
G-CC	20.74[a]	21.26[a]	97.48[a]	66.18[a]
CT	22.51[a]	20.27[a]	86.79[a]	69.25[a]
Grazing (G)				
Yes	20.56[a]	21.31[a]	88.06[a]	65.94[a]
No	21.81[a]	21.04[a]	95.70[a]	69.77[a]
Time (T)				
Pre	16.62[b]	17.55[b]	73.39[b]	57.39[a]
Post	26.00[a]	24.74[a]	111.9[a]	79.08[b]
	Analysis of Variance *(P>F)*			
R	0.66	0.74	0.06	0.06
G	0.42	0.92	0.09	0.09
R×G	0.81	0.34	0.83	0.83
T	<0.0001	<0.0001	<0.0001	<0.0001
R×T	0.60	0.63	0.64	0.64
G×T	0.98	0.45	0.14	0.14
R×G×T	0.79	0.64	0.62	0.62

†B-CC, Broadleaf dominated cover crops; G-CC, Grassleaf dominated cover crops; CT, Control; Pre, Pre-grazing, soil samples were taken before grazing on Sep 2016; Post, post-grazing, soil samples were collected after grazing on Nov 21st, 2016; Summer, Summer phase, soil samples were collected next year in summer on June 2017.

††Means within the same column followed by different small letters are significantly different at $P<0.05$ for the cover crop, grazing, and time.

Table 5. Mean soil acid hydrolysis carbon fraction measured using the 1M HCl (OMC) and 6M HCl (SMC) methods at the 0- to 5- and 5- to 15-cm depth different cover crop, grazing, and time treatments in ICLS.

Treatments[†]	**OMC**		**SMC**	
	0-5-cm	5-15-cm	0-5-cm	5-15-cm
	μg C g^{-1} soil			
Cover crops (R)				
B-CC	370.6[a††]	321.5[a]	90.94[a]	79.90[a]
G-CC	439.5[a]	343.0[a]	93.79[a]	92.07[a]
CT	358.3[a]	221.4[a]	91.86[a]	63.04[a]
Grazing (G)				
Yes	403.0[a]	339.8[a]	90.93[a]	84.19[a]
No	390.8[a]	290.3[a]	93.15[a]	79.53[a]
Time (T)				
Pre	421.3[a]	319.2[a]	108.3[a]	75.57[a]
Post	370.1[a]	301.0[a]	76.15[b]	87.22[a]
	Analysis of Variance *(P>F)*			
R	0.26	0.13	0.95	0.95
G	0.93	0.71	0.79	0.79
R×G	0.96	0.21	0.93	0.93
T	0.14	0.58	0.007	0.08
R×T	0.63	0.42	0.61	0.61
G×T	0.17	0.35	0.29	0.29
R×G×T	0.20	0.41	0.78	0.78

[†]B-CC, Broadleaf dominated cover crops; G-CC, Grassleaf dominated cover crops; CT, Control; Pre, Pre-grazing, soil samples were taken before grazing on Sep 2016; Post, post-grazing, soil samples were collected after grazing on Nov 21st, 2016; Summer, Summer phase, soil samples were collected next year in summer on June 2017.

[††]Means within the same column followed by different small letters are significantly different at $P<0.05$ for the cover crop, grazing, and time.

Table 6. Mean soil cold water nitrogen (CWN) and hot water nitrogen (HWN) fraction measured at the 0- to 5-cm cm and 5- to 15-cm depth under different cover crop, grazing, and time treatments in ICLS.

Treatments[†]	**CWN**		**HWN**	
	0-5-cm	5-15-cm	0-5-cm	5-15-cm
	---------- μg C g^{-1} soil ----------			
Cover crops (R)				
B-CC	4.89[b††]	4.34[a]	13.83[a†]	10.05[a]
G-CC	5.56[a]	4.38[a]	14.96[a]	9.59[a]
CT	5.47[ba]	4.45[a]	13.50[a]	9.83[a]
Grazing (G)				
Yes	5.20[a]	4.32[a]	13.48[a]	9.46[a]
No	5.33[a]	4.41[a]	14.70[a]	10.06[a]
Time (T)				
Pre	4.77[b]	3.84[b]	12.00[b]	8.33[b]
Post	5.78[a]	4.92[a]	16.43[a]	11.31[a]
	Analysis of Variance *(P>F)*			
R	0.06	0.97	0.06	0.58
G	0.83	0.81	0.18	0.18
R×G	0.19	0.55	0.35	0.78
T	0.0008	0.0004	<0.0001	<0.0001
R×T	0.09	0.52	0.11	0.48
G×T	0.85	0.98	0.65	0.90
R×G×T	0.14	0.92	0.25	0.60

[†]B-CC, Broadleaf dominated cover crops; G-CC, Grassleaf dominated cover crops; CT, Control; Pre, Pre-grazing, soil samples were taken before grazing on Sep 2016; Post, post-grazing, soil samples were collected after grazing on Nov 21st, 2016; Summer, Summer phase, soil samples were collected next year in summer on June 2017.

[††]Means within the same column followed by different small letters are significantly different at $P<0.05$ for the cover crop, grazing, and time.

Table 7. Mean soil acid hydrolysis nitrogen fraction measured using the 1M HCl (OMN) and 6M HCl (SMN) methods at the 0- to 5- and 5- to 15-cm depth under different cover crop, grazing, and time treatments in ICLS.

Treatments[†]	OMN		SMN	
	0-5-cm	5-15-cm	0-5-cm	5-15-cm
	μg N g^{-1} soil			
Cover crops (R)				
B-CC	46.99[a††]	41.87[a]	8.94[a††]	6.25[a]
G-CC	57.95[a]	40.35[a]	8.81[a]	7.16[a]
CT	45.53[a]	27.19[a]	9.05[a]	6.53[a]
Grazing (G)				
Yes	48.99[a]	40.47[a]	7.95[a]	6.05[a]
No	52.47[a]	36.90[a]	9.55[a]	7.09[a]
Time (T)				
Pre	60.22[a]	46.96[a]	15.94[a]	11.39[a]
Post	41.94[b]	29.70[b]	1.88[b]	1.95[b]
	Analysis of Variance *(P>F)*			
R	0.35	0.35	0.93	0.87
G	0.47	0.47	0.29	0.55
R×G	0.63	0.63	0.82	0.88
T	0.024	0.024	<0.0001	<0.0001
R×T	0.59	0.59	0.88	0.93
G×T	0.24	0.23	0.38	0.67
R×G×T	0.09	0.09	0.94	0.85

[†]B-CC, Broadleaf dominated cover crops; G-CC, Grassleaf dominated cover crops; CT, Control; Pre, Pre-grazing, soil samples were taken before grazing on Sep 2016; Post, post-grazing, soil samples were collected after grazing on Nov 21st, 2016; Summer, Summer phase, soil samples were collected next year in summer on June 2017.

[††]Means within the same column followed by different small letters are significantly different at $P<0.05$ for the cover crop, grazing, and time.

Table 8. Mean soil microbial biomass carbon (MBC) and soil microbial biomass nitrogen (MBN) at the 0- to 5- and 5- to 15-cm depth under different cover crop, grazing, and time treatments in ICLS.

Treatments[†]	**MBC**		**MBN**	
	0-5-cm	5-15-cm	0-5-cm	5-15-cm
	μg C g^{-1} dry soil		μg N g^{-1} dry soil	
Rotation (R)				
B-CC	134.8[a††]	85.84[a]	29.15[a††]	18.41[a]
G-CC	158.9[a]	89.57[a]	31.87[a]	13.56[a]
CT	145.2[a]	63.76[a]	30.50[a]	13.24[a]
Grazing (G)				
Yes	146.2[a]	81.79[a]	29.50[a]	16.28[a]
No	146.8[a]	83.67[a]	31.18[a]	14.88[a]
Time (T)				
Pre	103.9[b]	42.63[c]	43.21[a]	19.80[a]
Post	118.3[b]	75.28[b]	23.94[b]	13.78[b]
Summer	217.4[a]	130.8[a]	24.38[b]	12.72[b]
	Analysis of Variance *(P>F)*			
R	0.40	0.14	0.84	0.08
G	0.94	0.33	0.67	0.79
R×G	0.35	0.29	0.28	0.91
T	<0.0001	<0.0001	0.0006	0.008
R×T	0.37	0.65	0.53	0.32
G×T	0.37	0.88	0.86	0.36
R×G×T	0.85	0.54	0.65	0.65

[†]B-CC, Broadleaf dominated cover crops; G-CC, Grassleaf dominated cover crops; CT, Control; Pre, Pre-grazing, soil samples were taken before grazing on Sep 2016; Post, post-grazing, soil samples were collected after grazing on Nov 21st, 2016; Summer, Summer phase, soil samples were collected next year in summer on June 2017.

[††]Means within the same column followed by different small letters are significantly different at $P<0.05$ for the cover crop, grazing, and time.

Table 9. Mean soil urease and β-glucosidase activity at the 0- to 5- and 5- to 15-cm depth under different cover crop, grazing, and time treatments in ICLS.

Treatments[†]	Urease		β-glucosidase	
	0-5-cm	5-15-cm	0-5-cm	5-15 cm
	μg NH_4-N g^{-1} soil $2h^{-1}$		μmol pNP g^{-1} dry soil h^{-1}	
Cover crops (R)				
B-CC	205.3[a††]	176.8[a]	47.31[a]	36.51[a]
G-CC	209.6[a]	157.6[a]	54.01[a]	33.30[ab]
CT	189.9[a]	152.3[a]	46.77[a]	30.40[b]
Grazing (G)				
Yes	197.4[a]	162.7[a]	50.04[a]	34.87[a]
No	208.3[a]	165.2[a]	49.77[a]	33.43[a]
Time (T)				
Pre	116.0[c]	103.8[b]	67.76[a]	45.81[a]
Post	153.6[b]	115.7[b]	59.30[b]	45.32[a]
Summer	342.2[a]	273.0[a]	22.59[c]	10.88[b]
	Analysis of Variance *(P>F)*			
R	0.31	0.30	0.09	0.11
G	0.18	0.62	0.68	0.97
R×*G*	0.06	0.81	0.31	0.07
T	<0.0001	<0.0001	<0.0001	<0.0001
R×*T*	0.92	0.49	0.64	0.39
G×*T*	0.37	0.09	0.87	0.15
R×*G*×*T*	0.47	0.74	0.99	0.35

[†]B-CC, Broadleaf dominated cover crops; G-CC, Grassleaf dominated cover crops; CT, Control; Pre, Pre-grazing, soil samples were taken before grazing on Sep 2016; Post, post-grazing, soil samples were collected after grazing on Nov 21st, 2016; Summer, Summer phase, soil samples were collected next year in summer on June 2017.

[††]Means within the same column followed by different small letters are significantly different at $P<0.05$ for the cover crop, grazing, and time.

APPENDIX 1

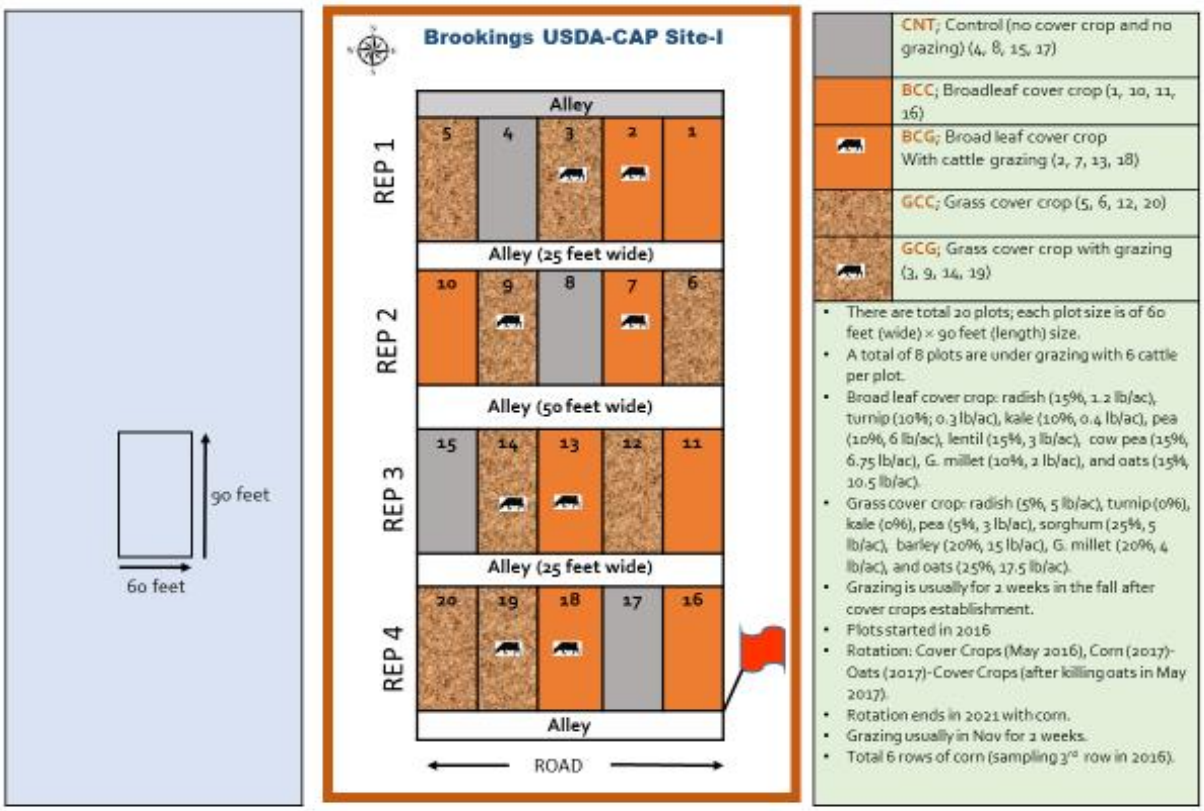

Fig. A1. Experimental design consisting randomized complete block design (RCBD) under no-till with different cover crop and grazing treatments with four replications.

Fig. A2. ICLS plots showing broadleaf dominated cover crop (right) and grass leaf dominated cover crop (left) at Brookings site.

Fig. A3. Soil core samples (left) preparation for the analysis of soil water retention (right).

Fig. A4. Grinding of soil samples for the analysis of soil organic carbon and total nitrogen

Fig. A5. ICLS plots showing control cover crop treatment at Brookings site.

Fig. A6. Measurement of soil moisture with soil moisture meter (left) and penetration resistance using hand penetrometer (right).

Fig. A7. Soil sample collection at various depths using hand auger at Brookings site.

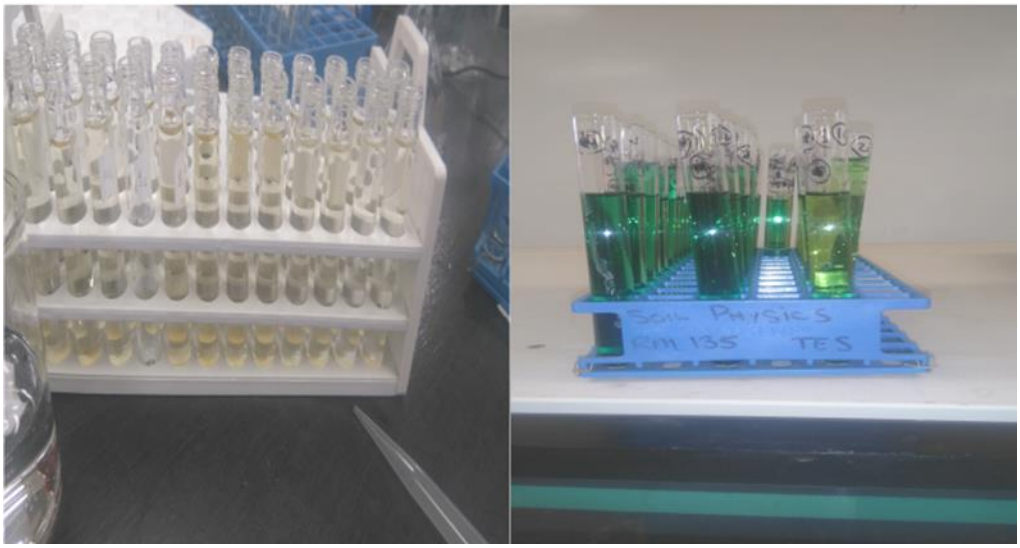

Fig. A8. Filtered soil urease enzyme extract (left) and color reaction (right)

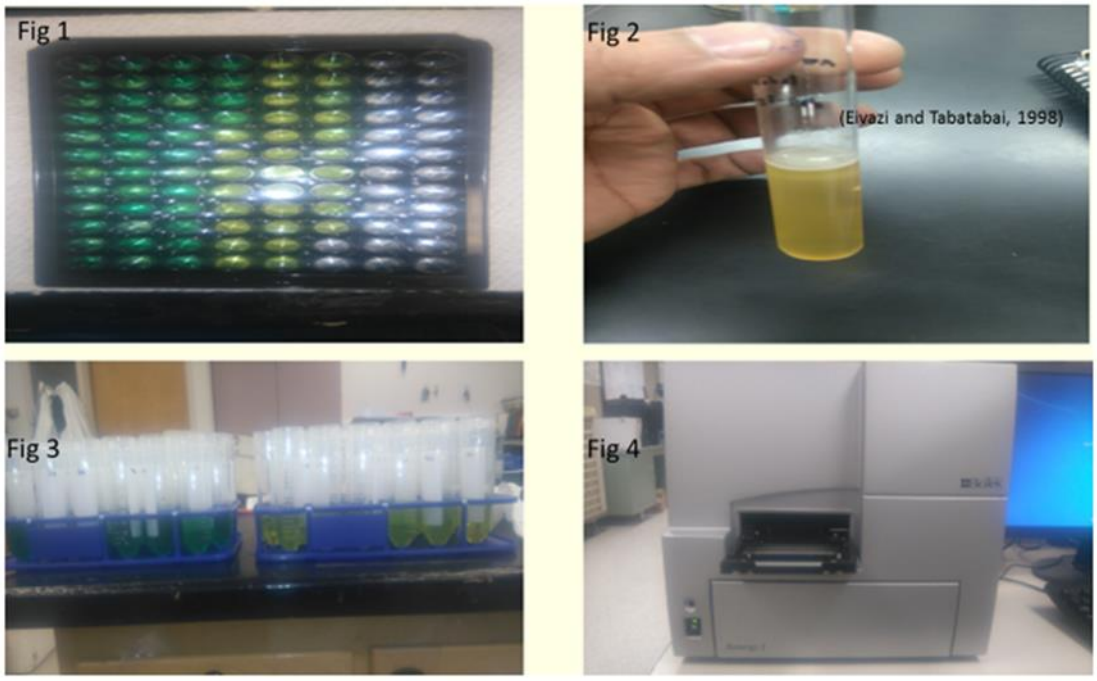

Fig. A9. Enzymes samples in microplate (1), Soil extract (2), color reaction (3) and spectrophotometer (4)

Fig. A10. Desiccator in fume hood for fumigation (1), soil cores samples (2), pressure plate apparatus for soil water retention measurement (3,4)

Fig. A11. Cattle grazing at Brookings site.

Fig. A12. Soil core sampler (left) and collection of soil core samples (right).

Fig. A13. Centrifuge machine used for carbon fraction.

VITA

Vishal Seth was born in Varanasi city of Uttar Pradesh province, India. He received his Bachelors in Agricultural Sciences from Institute of Agricultural Sciences, Banaras Hindu University. He joined the Soil Physics and Hydrology Lab to work with Dr. Sandeep Kumar in the Department of Agronomy, Horticulture and Plant Science at South Dakota State University in fall 2016 academic session as a master student. His master work focuses on Soil Science at South Dakota State University.