INTEGRATING LIVESTOCK INTO SMALL-SCALE VEGETABLE FARMING SYSTEMS

by

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TABLE OF CONTENTS

TABLE OF CONTENTS CONTINUED

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

ABSTRACT

Since World War II, modern agriculture systems have shifted to low-diversity monoculture crops, specializing in a singular species and generally separating those crops from livestock. Such systems require high inputs including fertilizers, herbicides and tillage, all of which may reduce ecological potential of farmland. Small-scale farms are becoming more popular due to recent interest in local eating and sustainability. To improve their environmental sustainability, some small-scale farms have incorporated livestock back into cropping systems. Soil health measurements can be valuable in understanding the impacts livestock have on small- scale farming systems as soil is the growth medium for vegetation. The objectives of this study were to evaluate nutrient cycling, microbial communities and compaction in response to grazed versus un-grazed vegetable cropping systems and use this information to understand the interaction between soil biology, nutrient cycling and livestock when integrated in a variety of vegetable production systems. Soil and biomass samples were collected over three years (2017- 2020) before and after sheep grazing occurred on three farm locations in the Northern Great Plains. Soil samples were analyzed for soil microbial diversity, bulk density and soil nutrients. While I found no consistent differences in soil nutrients, bulk density or soil microbial diversity, my results indicate that integrating livestock into small-scale vegetable farming systems did not negatively impact soil quality. Results from this study may help demonstrate to farmers and livestock operators the importance of an integrated approach, for those that already practice this approach there is affirmation that integration is feasible and purposeful and also become the starting point for further research into a little studied topic.

CHAPTER ONE

INTRODUCTION

Integrated livestock cropping systems (ILCS) focus on rotating or concurring crops and livestock in a way that creates productive interactions between the two (Peterson et al., 2020). Livestock integrated cropping systems produce half of the world's food through utilizing cover crops and residue, adding value to crops that would normally be underutilized, and turning it into feed for livestock (Sanderson et al., 2013). Benefits of integrated livestock cropping systems can include increased soil microbial biomass, improved soil structure and fertility, potential decrease in the need for external inputs, and decreased nutrient leaching (Sulc and Tracy, 2007; Hilimire, 2011).

Soil microbes play a major factor in processes such as decomposition, and plant productivity and are controlled by a multitude of biotic and abiotic factors, such as climate, soil, and nutrient inputs (Kaiser et al., 2016; Ishaq et al., 2017). A gram of soil contains about 10 billion organisms. Soil is biologically diverse and may also have approximately 700 different taxa of microbes within the 109 cells per cubic centimeter (Torsvik and Øvreås, 2002; Rainy et al., 2005). These biologically diverse communities are dynamic and can incur changes due to land management (Balser et al., 2010). Increased soil microbial diversity may increase soil resilience to disturbance, elevate, and improve overall soil health (Schnitzer et al., 2011, Schmidt and Waldron, 2015, Ishaq et al., 2017). Soil microbial communities are important in indicating the health of soil and so will be the main focus of this thesis (Ouverson et al., 2021). The objectives of this thesis were to: 1) evaluate microbial communities, nutrient cycling, and

compaction in response to grazed versus ungrazed vegetable cropping systems, and 2) understand how soil biology, nutrient cycling, and livestock interact when integrated in a variety of vegetable production systems.

CHAPTER TWO

LITERATURE REVIEW

Introduction

Since World War II, modern agriculture systems have shifted from small, family-owned farms that sustained individual families to low-diversity monoculture systems; farm and livestock operations separated following the war, becoming specialized in a singular enterprise (Russel et al., 2007; Sulc et al., 2007; Lemaire et al., 2014). Reasons for this shift to monoculture systems include advancement in farm equipment technology, government incentives, and meeting the increasing market demand for food (Sulc and Franzluebbers, 2013). Typically, monoculture systems require high inputs (fertilizers, herbicides, tillage, etc.) which may reduce its sustainability, agriculture sustainability focuses on the social, ecological, and economic aspects of an agriculture system (Yunlong and Smit, 1994; Hendrickson et al., 2008). Soil organic carbon has declined 24-60% in Great Plains' soils since land was first plowed (Krall and Schuman, 1996). Environmental sustainability can be improved by reintroducing diversified systems and incorporating livestock back into cropping systems (Russelle et al., 2007; Sulc and Franzluebbers, 2014).

Livestock integrated cropping systems typically include high diversity and find value in under-utilized crops as a forage base for livestock (Hendrickson et al., 2008). The addition of organic inputs, such as manure from livestock, has many benefits including improving microbial biomass and nutrient cycling, increasing soil organic matter and carbon sequestration, and improved crop yields and soil quality (Clark, 2004; Sulc and Tracy, 2007;

Acosta-Martinez et al., 2010). The following sections will review what livestock integrated systems are and past research looking at the impacts of these types of systems with particular focus on changes to soil health and crop production.

Monoculture Systems

Monoculture or specialized systems focus on the management of one commodity intending to produce a consistent yield (Hendrickson et al., 2008). These systems' lack of diversity may put a strain on the surrounding environment due to nutrient imbalances and removal of vegetation from the landscape (Glendining et al., 2008; Lenssen et al., 2013). Studies have indicated that including crop rotations into monoculture systems can increase productivity; while fertilizers and pesticides can improve production, adding diversity into crop production systems, especially through cover crops, can improve soil moisture and nutrient capture, reduce soil erosion, and decrease nitrogen leaching (Wyland et al.,1996, Fageria et al., 2005).

Smith et al. (2008) reported that increased crop diversity had a positive effect on yield in corn, soybean, and wheat crops. Corn yields, in particular, increased in response to an increase in the number of legume crops incorporated into the rotation. Yields increased exponentially as cover crops were added, with monoculture yields (1 tons[1] per hectare) being lower than one legume crop rotation (1.8 tons[1] per hectare). In the Northern Great Plains, low diversity farming operations have seen a reduction in soil organic carbon. These operations are depleted of nutrients due to a lack of vegetative inputs so require expensive amendments such as fertilizers (Sulc and Franzluebber, 2014; Lemaire et al.,2014, Hendrickson et al., 2008). Glab (2014) conducted a study comparing a crop rotation of sugar beet (Beta vulgaris subsp.

Vulgaris), faba bean (Vicia faba), and triticale (*x Triticosecale*; spring and winter) to monoculture triticale (spring and winter). The rotation alternated crops annually. Researchers determined that monoculture triticale (3.62 tons[1] per hectare spring wheat, 4.53 tons[1] per hectare in the winter wheat) had~ 20% lower grain yields than the crop rotation (4.21 tons[1] per hectare spring wheat, 6.18 tons[1] per hectare winter wheat). Differences were attributed to the degradation of the soil under the monoculture system and the positive effects may have resulted from the incorporation of the faba bean, a legume (Glab, 2014). While monoculture systems can economically produce mass quantities of produce in the short term, soil health may be improved by diversifying crops and integrating livestock, which is important due to soil providing important ecosystem services such as, sustainable plant production, water quality, human health, and climate change mitigation (Lehmann et al., 2020).

Soil Health

The capacity for soil to sustain plant and animal life, function as a living system, and its contribution to nutrient and water cycles is known as soil health (Karlen et al., 1997). Soil health is a criterion for sustainable land management; as management directly impacts the overall function of the soil (Doran and Zeiss, 2000). Two approaches to soil health can be described as "integrated" and "reductionist". The integrated approach is based on the idea that soil health or quality can be measured using physical, chemical, and biological indicators; this approach has been utilized in many studies. The reductionist approach to determining soil health assumes that several processes and soil characteristics result in certain outcomes for the soil. (Kibblewhite et al., 2007). By assessing the health or function of soil we can perhaps better understand the impacts of different management strategies. The three parts of soil health

(sustainable productivity, environmental quality, and biological health) are significant to the function of soil (Doran et al.,1998). Soil chemistry, biology, and physical properties interact to drive the soil's potential function for sustaining plant life, controlling water holding capacity and flow, and providing resistance against environmental contaminants (Doran, 1999; Parr et al., 1992).

Soil health is determined using indicators based on management and assessment goals (Laishram et al., 2012). Indicators may be abiotic and biotic, as soil health can only be inferred by these indicators (Seyold et al., 1997). Examples of biotic indicators include microbial communities, microbial biomass, and enzymes. Abiotic indicators may include pH, cation exchange capacity (CEC), and aggregate stability (Karlen et al., 1997). Examples such as these fall under the integrated approach of soil health determination (Kibblewhite et al., 2007). These indicators can be used to answer different questions related to soil health and management.

Knight et al., (2013) measured several soil health parameters to test the suitability of soil health indicators on lettuce crop productivity and quality in an urban environment. Researchers detected a positive correlation among soil organic matter, percent clay, and microbial biomass nitrogen on dry lettuce biomass (Knight et al., 2013). This research suggests that these indicators are important to overall soil health, which is valuable information when measuring soil health, although this is not indicative of all the food we consume, a majority of food production does not occur in an urban setting. Soil health determinants in a semi-arid setting such as the Northern Great Plains may vary from those found to have positive correlations with dry biomass in an urban setting. Understanding what soil health is and how it can be measured is useful in understanding how livestock integrated systems may impact soil health.

Livestock Integrated Systems

Several types of integrated livestock systems fit various management goals and environments. Some systems include cover crops, crop residue, sod-based crop rotations, dual- purpose cereal crops, and sod intercropping but all utilize livestock to graze vegetation produced through farming (Sulc and Franzluebbers, 2014). Sod based rotations refer to utilizing sod forming grasses and legumes and alternating them with cereal and row crops (NRCS, 2004).

Crop residue grazing refers to livestock grazing residues of crops once they have been harvested. Dual-purpose crops refer to utilizing cereal grains as both a grazing opportunity and for grain production (Sulc and Franzluebbers, 2014). Past researchers have investigated the effects of integrated livestock operations on soil health indicators such as compaction and organic matter, as well as crop yield (Fernández et al., 2010; Sainju et al., 2010; Miller et al., 2014). However, there is a lack of research evaluating how integrated livestock systems may alter soil microbial communities, another important indicator of soil health, as these communities play a large role in soil carbon sequestration, nutrient cycling, and decomposition (Doran and Zeiss, 2000). The following sections will cover descriptions of a few variations of livestock integrated systems.

Cover Crops

Cover crops are used as temporary soil cover when harvestable crops are out of production. Cover crops help retain soil moisture, decrease soil erosion, and improve soil nutrient capture; all of which can improve crop productivity (Fageria et al., 2005). Cover crops may include grasses, forbs, and legumes or mixes of the three. These crops can be terminated

by herbicides, tillage, crimping, rolling, cutting, and grazing but are not typically harvested for grain or seed; crops that are harvested for grain or seed cannot be a cover crop. For crop insurance purposes, cover crops can be used as forage, hay or silage for livestock; unless specified otherwise by a local USDA Risk Management Agency (NRCS, 2011a; USDA, 2019a). Producers typically cannot make revenue from cover crops during the season in which they are grown since the crops are not harvested for seed or grain (Sulc and Franzluebbers, 2007; USDA, 2019a). Financial value may be recovered by using cover crops as feed for livestock. This allows producers to continue to reap the environmental benefits of cover crops while also feeding livestock, saving on feed costs.

McKenzie et al. (2016) investigated the impacts of integrating sheep into a cover crop vegetable cropping system over a three-year period. During all years of the study, there was no difference in cash-crop yields at harvest between the grazed and mowed cover crop treatments. The nutritional value of all four cover crops indicated that these cover crops met the nutritional requirements of sheep with a potential value of $144-482/ha under a 6-month grazing lease. This study indicated that sheep grazing did not negatively impact cash crop yields and may be an economically viable option for terminating cover crops before planting cash crops, as cover crop termination may cost up to $4 per hectare (Myers et al., 2019).

Nitrogen leaching is a well-known concern in agriculture, as nitrates can leach into water systems causing eutrophication (Thapa et al., 2018). In production systems that incorporate fallow rotations, mineralization of nitrogen to nitrate is assisted by these types of rotations and a fallow rotation that occurs once every three years may experience a 43% nitrate loss due from its total nitrate to leaching during fallow years, as seen in models based on data collected in the Judith Basin, Montana (Sigler et al., 2020). Horticulture systems, in particular,

can experience high rates of nitrogen leaching due to frequent tillage, residue incorporation, and low nutrient use efficiency by vegetable crops (Di and Cameron, 2002). However, livestock may also contribute to nitrogen leaching from sources such as livestock holding facilities, waste ponds and livestock manure applied to fields (van der Schans et al., 2009).

Cover crops may help reduce nitrogen leaching. Nitrate leaching was significantly reduced by 65-70% under Merced rye (Secale cereale) and Phacelia (Phacelia tanacetifolia) cover crops when compared to fallow plots in a Salinas Valley California study.by who. The authors speculate that This may have been due to the presence of roots in the cover crop plots, which removed nitrogen and water from the soil. Therefore, incorporating cover crops into intensive cropping systems, such as horticulture systems, may reduce nitrate leaching (Wyland et al.,1996).

Crop Aftermath

Grazing crop aftermath or crop residue is one the simplest and most economical ways to integrate livestock into a cropping system after harvest; crop residue grazing typically involves directly grazing crop stubble (Sulc and Franzluebbers, 2014). Crop residue grazing is common in the Midwest and helps to reduce feeding costs for many livestock farmers (Clark et al.,2004).

Grazing crop residues may improve soil organic matter and has shown to have little impact on soil bulk density (soil compaction can increase soil bulk density) and crop yields (Rakkar and Blanco-Canqui, 2018). For example, Drewnowski et al. (2016) found that grazing corn residue had either a positive or no effect on soybean and corn yields. Grazing livestock in the spring produced significantly greater soybean yields than non-grazed plots

and did not affect corn yields in the first six years of the study (Drewnowski et al., 2016). This research is particularly important because it demonstrates the positive impacts of grazing crop aftermath. Additionally, many producers that incorporate livestock into cropping systems are concerned about the effects grazing animals will have on soil compaction, as soil is impacted by environmental and management factors.

Soil Compaction

Soil compaction occurs when void space between soil aggregates is decreased, bringing aggregates closer together (Hamza and Anderson, 2005). Soil compaction reduces the soil's ability to store and provide water and nutrients ultimately decreasing plant growth (Torbert and Wood, 1992; Hamza and Anderson, 2005; Batey, 2009). Soil compaction can be caused from compression of the soil by vehicles and livestock. Several factors influence the susceptibility of soil to compaction including soil moisture levels, soil texture, and the amount of soil organic matter (Shah et al., 2017).

Macropores in the soil are typically filled with air and supply oxygen to soil fauna and aboveground vegetation but; as soil becomes more compact, the number of macropores is reduced which may alter crop productivity and vigor, reducing yields (Pagliai et al., 2003; Shah et al., 2017). Pagliai et al., (2003) determined that the porosity of macropores was significantly lower in plots that were driven over with tractor tires and tractor tracks as compared to the control, which had no machinery driven on the plots. Porosities were 35%, 20% and 10% for the control, tractor tires, and tractor with tracks, respectively. Soil is considered compacted when porosity is less than 10% (Pagliai, 1988).

Compaction can result in changes to soil microbiology. Torbert and Wood (1992)

reported that soils became more compacted and pore space was filled with water, microbial activity decreased by 60%, and soil respiration was decreased when field capacity (an index of soil moisture after water has been allowed to drain from soil) was at 60%. As bulk density increased (1.4 to 1.8 Mg/cm^3) there was a significant increase in nitrogen loss (90 to 330 g N/m^3). Still Torbert and Wood?). Researchers presumed that the change in soil microbial activity with increasing bulk density could be due to altered conditions in the soil where the soil environment changed from aerobic to anaerobic, changing the number of aerobic microbes in the soil.

Livestock grazing in cropping systems is known to have variable effects on soil compaction. Fernández et al., (2010) found that livestock grazing crop residue decreased bulk density at the beginning of the study, during wet years. Bulk density was lower in the top 0-50 mm layer of the grazed treatment (1.16 Mg/m^3) than the un-grazed treatment (1.32 Mg/cm^3). The decrease in bulk density in the grazing treatment was attributed to a high volume of moisture the soil received that year in conjunction with trampling of the soil. This caused air entrapment in the pores, increasing total soil porosity. Lessen et al. (2013) determined that sheep grazing resulted in a significantly higher bulk density in the tilled treated versus the chemical treated plots in wheat- fallow weed management systems (1.28 vs. 1.21 Mg/m^3). Differences in bulk density between treatments were attributed to freeze and thaw cycles or pre-seed bed tillage.

Freeze and thaw cycles have been shown to alleviate compaction issues in the Northern Great Plains due to soil water freezing and thawing in between soil aggregates, causing contraction and expansion of soil pores (Jabro et al., 2014). Freeze and thaw cycles may allow livestock to be integrated into cropping and farming systems without increased soil

compaction in the Northern Great Plains. Additionally, researchers found no differences in bulk density (g/cm^3) between grazed and ungrazed plots in a study involving four farms in central Montana (Hatfield et al., 2007c). Sheep were utilized in dryland grain production systems to determine the effect sheep grazing would have on wheat stem sawfly (Cephus cinctus Norton) populations. Within the study, fall grazing, tilling, burning and a control were all compared to determine the effect sheep grazing had on soil nutrient profiles and bulk density (Hatfield et al., 2007a; Hatfield et al., 2007c).

Soil Nutrient Cycling

Evaluation of the amount of C and N in the soil has l been used as an indicator of soil health, vegetative productivity, and functionality of biological cycles (Bhowmik et al., 2017; Wang et al., 2016; Doran and Parker, 1994). Soil organic matter (SOM) is the organic material component of the soil and is comprised of approximately 55-60% organic carbon or soil organic carbon (SOC; FAO, 2017; Bernoux and Cerri, 2005). Importantly, soil organic carbon (SOC) may have a positive influence on soil structure and plant growth (Bauer and Black, 1994; Halvorson et al., 2002). SOC changes based on the amount of carbon being added to the system, most often from plant litter, and the amount of carbon being lost from a system. When C inputs exceed the rate of decomposition, C is stored in the form of SOC. As C inputs or the rate of decomposition is disrupted, SOC stocks can also be changed (Janzen et al., 1998). Annual cropping systems that lack cover crops may result in an accumulation of nitrogen that leads to leaching, due in part to a lack of carbon inputs during fallow periods (Lemaire et al., 2014). After water, organic systems, in particular, are limited most by nitrogen, due to soil microorganisms need for a carbon: nitrogen (C: N) ratio of 24:1. As the C: N ratio increases

more than 24:1 in the vegetation in a cropping system, the microbes will immobilize any excess nitrogen making plants unable to access the nitrogen. If the C:N ratio is less than 24:1 though, the soil microbes will leave behind a surplus of nitrogen (mineralization, NRCS, 2011b).

Soil organic matter is heavily relied on as a source of both C and N (Gaskell and Smith, 2007). One possible source of organic matter is from integrating livestock into cropping systems, through livestock excreta inputs (Franzluebbers and Stuedemann, 2010). Sainju et al. (2010) found that N leaching may be decreased, and SOM was maintained, in continuous wheat crops in the Northern Great Plains with reduced-tillage and light grazing by sheep on fallow. These researchers suggest that sheep urine and feces may have increased soil total nitrogen (STN) at subsoil depths (30-60 cm) but did not in the topsoil (0-5 cm) due to crop vegetation removal by grazing. These results indicate that sheep may be integrated into wheat systems without causing detrimental damage to soil C and N cycling. A meta-analysis quantifying the effects of livestock grazing in the Northern Great Plains on carbon and nitrogen cycles showed that grazing increased nitrogen and carbon pools in the upper most layer of the soil (0-15 cm), increased soil nitrogen mineralization and NH_4^+ and NO_3^- concentrations (Wang et al., 2016). Understanding the relationship between livestock grazing and soil nutrient cycles helps in understanding how livestock grazing impacts soil microbial communities, as microbes are essential to nutrient cycling.

Soil Microbial Communities

Soil biological health is one of the three parts of soil health (Parr et al., 1992; Doran et al., 1998). Microorganism communities play an asymmetrically important role in soil biology

and are extremely diverse systems, with one gram of soil containing about 10 billion organisms (Torsvik and Øvreås, 2002). Microorganisms play an important role in carbon sequestration, contribute to nutrient cycling and decomposition, and are essential in the function of soil (Wall et al., 2005; Condron et al., 2010). These organisms can contribute anywhere from 5-80% of the nitrogen and 75% of the phosphorus taken up by plants annually (van der Heijden et al., 2008).

There are three major soil microbial groups: bacteria, fungus, and protozoa; each group plays an important role in maintaining important soil functions (Ingram, 2009). Bacteria, for example, can convert nutrients locked in soil organic matter into forms that are readily available for plant uptake. Species such as Actinomycetes break down substrates such as chitin and cellulose when soil is at basic pH levels (pH of 7-7.5; Ingram, 2009). Actinomycetes are also the reason why soil has its earthy smell (Ingram, 2009). Fungi are capable of living in a wide range of soil conditions but flourish in soils with a low pH (Ingram, 2009; Fraç, 2018). Fungi decompose substrates and convert them into biomass, carbon dioxide, and organic acids, making nitrogen more readily available for plants (Fraç, 2018). Protozoa are a group of microbes that don't fit into any other category. They can be broken up into three groups: flagellates, ciliates, and amoebae (Clarholm et al., 2007). Protozoa feed on bacteria; under conditions where there is a low population of bacteria the majority of the protozoa will be flagellates (Clarholm et al., 2007). While this method is quick and inexpensive, only microbes that can be cultured can be identified with many types of microbes not able to be cultured, so they will be overlooked (Stefanowicz, 2006).

While these techniques are important in understanding soil microbial communities, detecting small changes in these communities can be difficult. One reason for this is because

changes in soil microbial communities can be detected at distances as small as 20 cm. (Schmidt and Waldron, 2015). Since changes in microbial communities can happen at such small distances, they may be overlooked

Soil Microbial Community Influences

Soil microbial communities are influenced by several environmental conditions such as pH, nutrient inputs, climate, and availability of soil nutrients (Kaiser et al., 2016; Ishaq et al., 2017). Kaiser et al., (2016) found that pH was an excellent predictor for function, diversity, and structure of bacterial communities in grassland and forests in Germany (explained 26% of the variance) Management at grassland sites (fertilization, mowing and grazing) and forest sites (one age class, multiple age classes and unmanaged) seemed to have no impact on the bacterial communities. Nonetheless, a canonical correspondence analysis (CCA) showed that pH explained 26% of the variation in bacterial community structure (Kaiser et al.,2016). Alternatively, Schutter et al., (2001) suggested that microbial community changes respond primarily to climate factors such as season, and secondarily to chemical factors such as pH and CEC. Soil samples were collected and used for microbial microscopy counts, FAME profiles and Biolog plates. FAME and Biolog profiles were clustered by field site and not management type (fallow or cover crop), this may indicate that the microbial community structure is site dependent (Schutter et al., 2001). Studies such as these show the difficulty in analyzing changes in soil microbial communities that could be attributed to management changes, soil conditions, and climate.

Soil Microbial Communities and Livestock Grazing

While there have been many studies looking at how grazing affects plant production

and physiology, little has focused on how grazing affects soil microbiology (Yang et al. 2018). It is important to understand the interaction between soil microorganisms and grazing due to soil microbes' ability to regulate nutrient cycling, specifically the regulation of carbon movement from plants to the atmosphere (Zhou et al., 2017). Even though plants can also have an impact on the structure of soil icrobial communities, livestock grazing may facilitate positive feedback when it allows for plant regrowth and increased nutrient and energy movement throughout a grazed area (Hamilton and Frank, 2001; Smith et al., 2018). Grazing can also drive the structure of the soil microbial community, where changes in grazing intensity can impact the spatial heterogeneity and relative abundance of soil microbial communities (Eldridge et al., 2020).

Grazing may alter the community dynamics and dominant species of soil microbes. Yang et al. (2018) found that winter grazing caused soil microbial communities to be less stable and to have lower SOC and SON levels than in comparison to annual grazing and no grazing. Shannon- Wiener's diversity, Pielou's evenness, and species richness did not significantly differ between the no grazing, winter grazing, and annual grazing (Yang et al., 2018). How grazing livestock affects soil microbial communities is dependent on specific site characteristics (Eldridge et al., 2020). Ishaq et al., (2020) found that factors such as seasonality and weed abundance played more of a role in changes to microbial communities than management practices, plant phenology and time of year; they found that there was no difference in bacterial richness or evenness among chemical no-till, USDA-certified till organic, and USDA-certified organic with grazing farming systems. Environmental and physical factors should be considered when evaluating the relationship between grazing livestock and soil microbial communities, as factors such as pH and litter can also play a role

in the dynamics of the microbial communities (Eldridge et al., 2020).

Analyzing Soil Microbial Community Diversity

Soil microbial diversity is measured by species richness (number of species) and evenness (relative abundance of the number of species; Magurran, 1988). Past research has indicated that increased soil microbial diversity may improve soil resilience to disturbance, increase plant productivity, and improve soil health; methods as to how soil microbial diversity does this is unknown (Ishaq et al., 2017; Schmidt and Waldron, 2015; Schnitzer et al., 2011).Differences in microbial communities may be measured in a variety of ways including α-diversity (measuring diversity in a community), and β-diversity (comparing diversity between two or more communities; Lozupone and Knight, 2008).

There are several options for analyzing microbial communities. 16S rRNA sequencing utilizes a phylogenetic marker that is commonly found in most prokaryotic cells and allows for measuring phylogenetic relationships (Srinivasan et al., 2015). Operational taxonomic subunits (OTUs) are generated by 16S rRNA sequencing. Instead of categorizing by species, OTUs are utilized to overcome uncertainty about which species are being measured. For example, in some instances the 16S rRNA method may designate two closely related species to be of the same species (Lozupone and Knight, 2008). Shotgun sequencing includes the entire genome instead of one phylogenetic marker, such as in 16S rRNA sequencing. This method may provide additional information on the functionality, organization, and structure of genes (Roumpeka et al., 2017).

Other ways of profiling soil microbial communities include fatty acid methyl ester (FAME) profiles and Biolog plates. FAME profiles use fatty acids contained in

microorganism lipids. Different microorganisms have a unique combination of fatty acids. Fatty acids are analyzed by volatilizing them in methylation and then analyzing with gas chromatography. FAME profiles are inexpensive, and it can be difficult to distinguish between microorganisms that share common fatty acids (Cavigelli et al., 1995). Biolog plates measure the metabolism of 31 sources of carbon. Different microbial communities have characteristic metabolic properties, which can help in microbial community level profiling. Plates in the Biolog will change color depending upon how the microbes use the carbon substrates and the colors are measured spectrophotometrically. While this method is quick and inexpensive, only microbes that can be cultured can be identified. Because many types of microbes cannot be cultured, so will be overlooked in the Biolog analysis (Stefanowicz, 2006).

While these techniques are important in describing soil microbial communities, detecting small changes in these communities can be difficult. One reason for this is because changes in soil microbial communities can be seen across distances as small as 20 cm. (Schmidt and Waldron, 2015). Since changes in microbial communities can happen at such small distances, they may be overlooked in standard soil health monitoring. Soil may also take decades to transition to a new, stable community (Ishaq et al., 2020). This may make it difficult to see any small changes in soil microbial communities over a few years requiring a longer-term study to detect any changes. Because these communities are influenced by a variety of other environmental and seasonality factors explaining attributing differences in these communities to management factors can be difficult (Ishaq et al., 2020).

Summary and Implications

As concern for food security increases with the rise in the human population, there has been a growth in the number of studies concerning livestock integrated cropping systems (Cole et al., 2018). The literature has mainly studied the impact of livestock on cropping systems in regard to soil health and crop yield in South America, and the midwestern and southern United States with a focus on grain crops, in particular. There is a lack of research looking at the impacts of livestock integrated systems on small-scale vegetable farms in the Northern Great Plains. As we better understand the relationship between livestock grazing in farming systems and soil health, we can use the results to demonstrate to farmers and livestock operators the importance of an integrated approach. To those that already practice this approach, they will have the affirmation that what they are exercising is feasible and purposeful and could provide a model for future food security, both locally and globally.

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CHAPTER THREE

EFFECTS OF INTEGRATING LIVESTOCK INTO SMALL-SCALE VEGETABLE FARMING SYSTEMS

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Effects of Integrating Livestock into Small-scale Vegetable Farming Systems

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INTRODUCTION

The capacity for soil to sustain plant and animal life, function as a living system, and its contribution to nutrient and water cycles is known as soil health (Doran, et al. 2000). Understanding how soil health is impacted by livestock grazing embedded in cropping systems and its impacts on the surrounding ecosystem is important, as healthier soils provide a better environment for plants and animals to grow. With 165,111,742 hectares of cropland in the United States, and farms contributing $1.109 trillion dollars to the U.S. economy, it is important to evaluate how soil health can be improved as it is the growth medium for food products (Nickerson and Borchers, 2012; USDA, 2019b). Since cultivation began on the Great Plains, soil organic carbon has declined by 24-60%, thus soil health has been declining (Sanderman, et al., 2017; Krall and Schuman, 1996).). As farming systems have evolved, more inputs are required for these systems to produce high yielding crops, which may reduce ecological potential of the cultivated area (Hendrickson et al., 2008). While livestock-free monoculture systems allow for reduced food costs and increased availability of foods, integrating livestock may provide a cost- effective nutrient source for crops through animal excreta, plus livestock may consume underutilized crop residues and cover crops while

offsetting feed costs (Hillimire, 2001).

Livestock integrated systems utilize natural nutrient cycles associated with vegetation and animals. With proper management these systems may have a positive impact on plant productivity and the surrounding ecosystem (Kumar, 2019). Integrating livestock increases the biological diversity within a system and allows for additional products to be harvested within a given area (Carvalho et al., 2018). Furthermore, livestock integrated systems may lead to increased microbial carbon, nitrogen, and higher crop yields through the addition of organic inputs from livestock (Poffenbarger, 2010; Tracy and Zhang, 2008). Livestock can be integrated into cropping systems in various ways including grazing cover crops, crop aftermath, sod-based crop rotations, dual-purpose cereal crops, and sod intercropping (Sulc and Franzluebbers, 2014). However, there has been little research into soil health impacts due to livestock grazing on cropping systems in the Northern Great Plains and specifically in small-scale vegetable farms.

From 2009 to 2014, small-scale vegetable farms in Montana increased in value by 32% and increased production by 23% (USDA NASS, 2014a). Additionally, the U.S. had 33,848 horticulture or vegetable systems with approximately 112.6 million acres in production in 2018 (USDA NASS, 2018). Small-scale farms (less than 2 ha) produce 30-34% of the food supply on 24% of total agriculture land (Ricciardi et al., 2018). Without information on the soil health impacts of integrating livestock into small horticulture farms in the Northern Great Plains the interest in sustainable farming and local foods is underserved meaning there is an increased need for studies such as these.

This study investigated three privately owned, certified organic, livestock integrated

small-scale vegetable systems in the Northern Great Plains. The study specifically looked at differences in grazed versus un-grazed sampling sites with an emphasis on soil health including soil microbial diversity. Previous studies of livestock integration have shown a positive impaction soil health (Rakkar et al., 2018) While little has been studied in terms of livestock integrated systems in the Northern Great Plains., this research will as well as add to the body of knowledge in research focused on livestock integrated cropping systems in the Northern Great Plains.

MATERIALS AND METHODS

Field Sites

On-farm studies were conducted at three, privately owned, United States Department of Agriculture (USDA) certified organic farms in the Northern Great Plains. The first site, Black Cat Farms (40° 3'6.07"N, 105°14'14.14"W) is located in northern Boulder, CO. Soils at this location are Renohill silty clay loam (fine, smectitic, mesic Ustic Haplargids) and Valmont clay loam (clayey over loamy-skeletal, smectitic, mesic Aridic Argiustolls; NRCS, 2013).

Precipitation averages 48 cm per year and air temperatures average 10°C. Climate data was collected from US National Center for Atmospheric Research (NCAR) Foothills Lab, in NE Boulder, CO. Black Cat Farm grows a variety of vegetable crops including chili peppers, potatoes, and tomatoes. They also raise sheep, chickens, geese, and hogs that are utilized in their integrated livestock system. The livestock and produce that are harvested from the farm are utilized in two restaurants owned by the producer. This experiment focuses on the sheep

that are overwintered on 2.43 hectacres of cover crops and crop residue.

Strike Farms (45°39'0.19"N, 111° 7'24.95"W) and 13 Mile Lamb and Wool (45°51'57.48"N, 111° 4'36.65"W) are both located in the Gallatin Valley in southwest Montana. 13 Mile Lamb and Wool's soils are Quagle silt loam (coarse-silty, mixed, superactive, frigid Typic Calciustolls) and Enbar loam (Fine-loamy, mixed, superactive, frigid Cumulic Haplustolls; NRCS, 2013).13 Mile Lamb and Wool produces lamb and wool products as well as a variety of vegetables from their small-scale vegetable farm including: squash, beets, and garlic. Sheep are integrated into the vegetable garden wherever possible, grazing both crop residues and cover crops. Strike Farm soils include the Hyalite-Beaverton Complex loam (Fine-loamy, mixed, superactive, frigid Typic Argiustolls; NRCS, 2013). Strike Farm was a certified organic farm focusing on herbs, vegetables, and flowers, all of which were incorporated into a local community- supported agriculture model (CSA). Precipitation averages 36 cm-48 cm/year and air temperatures average 4-7°C per year for both Strike and 13 Mile farms (NRCS, 2013).

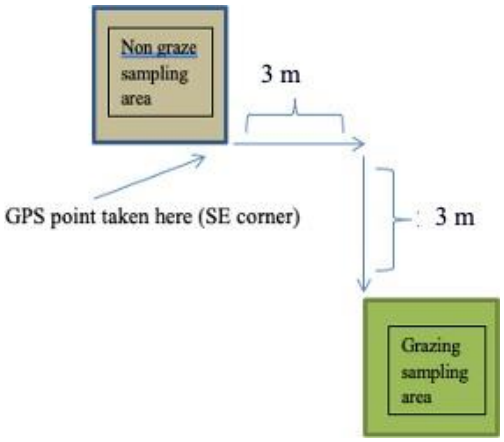

Figure 1. Example of the layout of paired grazing exclosure (UNGRAZE) and grazing sampling area (GRAZED).

Experimental design

This study evaluated the impact of integrating livestock into small-scale farming systems on soil health. Farms were set up in a complete random design with one treatment (livestock grazing) repeated over four years (2017-2020).

Paired grazed and ungrazed plots were randomly placed at each of the farms with six 1-m^2 exclosures (UNGRAZE) and six 1-m^2 plots (GRAZE) in the grazed treatments within a designated field. The six grazed 1-m^2 plots were placed 10 feet to the right and 10 feet below the exclosures, to avoid high traffic areas around the exclosure fences (Fig. 1). Exclosures were removed at the end of each grazing season. Global Positioning System (Garmin Oregon 700 GPS Unit) waypoints were collected in the southeast corner of each grazing exclosure prior to removal of the exclosures to assist in reestablishing the sampling areas the next year

Soil sampling

Five soil core samples were randomly collected and composited using a 30 cm (5 cm in diameter) soil corer before and 1 year after treatment imposition at five randomly selected locations within each of the GRAZED and UNGRAZED 1-m^2 sampling areas to a depth of 15 cm, which was determined by previous studies where soil nutrient analysis was conducted. Soil samples were collected prior to grazing and then again approximately one year later, prior to crop harvest. The soil samples were divided; half were sent to Agvise Labs where nutritional analysis was conducted to quantify pH; organic matter (OM, %); salts (ds/m); potassium (mg/kg) and ammonium (mg/kg), bicarbonate-extractable phosphorus (P-Olsen, mg/kg); potassium, calcium, magnesium, and sodium (all mg/kg); sulfur (mg/kg), total % N, and Cation exchange capacity (CEC, $cmol_+$/kg. The remaining half of the soil sample was stored at -80°C before being sent to Argonne labs for DNA extraction and 16S rRNA amplification.

Three random soil cores (5 cm diameter) were collected to a depth of 7.62 cm (volume

= 150 cm^3) from each grazed and ungrazed sampling area to evaluate the effects of grazing livestock on soil compaction by measuring bulk density (Wills et al., 2018). Soil samples were weighed and then oven dried at 105 ° C in an oven and then weighed again. After a dry weight was collected, bulk density was calculated with the following equation:

$$\frac{\text{Dry weight of the soil sample}}{\text{Volume of soil sample}}$$

DNA Extraction and Sequencing

Soil samples were processed to obtain DNA and sequenced at Argonne National Laboratory (https://www.anl.gov/) by Illumina Miseq using V3 chemistry. Using the earth microbiome project protocol (earthmicrobiome.org), nucleic acids were extracted from 3 grams of soil using the 96-well PowerBead® DNA Plate(s), Garnet kit. PCR was conducted to amplify prokaryotes 16S rRNA of the V4 region using the Quant-IT PicoGreen dsDNA Assay Kit, which includes 13.0 µL PCR-grade water (Sigma or MoBio), 10.0 µL Platinum Hot Start PCR Master Mix (x2) (ThermoFisher), 0.5 µL of Forward Primer, 0.5 µL of Reverse Primer, and 1.0 µL of Template DNA. The earth microbiome protocol using the Quant-IT PicoGreen dsDNA Assay Kit is as follows: 94°C for 3 minutes, 35 cycles at 94°C, 50°C for 60 seconds for 35 cycles, 72°C for 90s at 35 cycles, 72°C for 10 min. and then stored at 4°C (Marotz et al., 2017).

DNA Analysis

In total 21,388,183 sequence reads of variable region 4 (V4) of the 16S ribosomal

RNA (rRNA) gene were collected across 328 samples (average 65010 sequences /sample) and processed using the standardized protocol of DADA2 (Callahan et al,. 2016) amended to trim beyond the first 230nt in both forward and reverse reads to recognize the slightly worse and better overall quality characteristics of these reads, respectively. 3,369,617 total low-quality reads were removed for failing to meet strict quality criteria, including the presence of uncalled bases and an expected error rate of >2 erroneous nucleotide calls. Following this process, five samples were removed due to retaining less than 1000 total sequence reads. Reads were assorted into 58,179 Amplicon Sequence Variants (ASVs), clusters of sequences that are identical across their coverage of the V4 region of the 16S rRNA gene. Of these 1,122 (<0.2 % of all reads) ASVs were determined to be chimeric (artifacts created in PCR) and removed. Data were normalized and log transformed, also known as a Hellinger transformation (Legendre and Gallagher, 2001,), and then beta-diversity was compared using Bray-Curtis (Beals, 1984). One outlier was identified in a preliminary non-metric multidimensional scaling (nMDS) plot and was removed to enhance clustering of the remaining samples. Final Bray-Curtis dissimilarities were presented in nMDS plots, using 100 restarts to find the solution with the lowest stress (Beals, 1984). The ultimate stress value was 0.15583, indicating a fair fit of the data, as per Kruskal, 1964.

Biomass Sampling

Three biomass samples were randomly collected removing all above-ground biomass from each experimental field using a 1-m^2 clipping frame before and after grazing to determine the amount of biomass removed due to grazing. Samples were weighed, oven-dried at 55°C, ground to pass a 1-mm sieve, and sent to Midwest Labs (Omaha, NE) to be analyzed

for neutral detergent fiber and acid detergent fiber concentrations pre- and post-grazing (Goering, K., and P.J. Van Soest., 1970). Sub-samples were analyzed for total nitrogen and crude protein using the LECO carbon and nitrogen analyzer (St. Joseph, MI) in house (Tables 5, 6 & 7).

Data Collection

Black Cat Farms

During the first year of the study (2017) preliminary data for soil nutrients, microbial DNA analysis, and biomass was collected and exclosures were put into place, prior to grazing on a .4 ha spelt (*Triticum spelta*) aftermath field grazed by sheep (.4 Animal unit month; AUM). During the second year of data collection (2018), soil samples and biomass data were collected as a comparison to the grazing treatment from the previous year (2017-2018) as well as data for the start of the new grazing season on 2.42 ha of a mixture of eggplant (*Solanum melongena*), variety of peppers (*Capsicum spp*,), purple potato, *(Solanum tuberosum)*, Tomatillo (*Physalis philadelp*hica), and Kentucky bluegrass (*Poa pratensis*). Soil samples but no biomass data were collected after Black Cat Farms planted a harvest crop of black garbanzo beans (*Cicer arietinum*) and assorted dry beans (*Phaseolus ssp*.) in the third year of the project (2019-2020). After harvesting, Black Cat Farms planted a forage radish (*Raphanus raphanistrum*), buckwheat oats (*Fagopyrum esculentum*), and peas (*Pisum ssp*.) cover crop, which once established was grazed by their flock of sheep. Soil and biomass samples were collected once again in June 2020, after grazing had occurred (Table 1).

13 Mile Lamb and Wool

During the first year of the study (2017) preliminary data for soil nutrients, microbial DNA analysis, and biomass was collected and exclosures were put into place prior to grazing on a .81 ha squash (*Cucurbita ssp*.) aftermath, and mint *(Mentha ssp*.) and garlic (*Allium sativum*) mix with sheep (1.2 AUMs). In 2018 soil and biomass samples were collected on the .81 ha field prior to the sheep over-wintering on the hairy vetch and clover cover crop (1.2 AUMs). The squash residue was grazed (1.2 AUMs) prior to sampling, and data was only collected for the areas of the field that included the cover crops. In 2019, sheep returned to the .81 ha squash field over the winter to graze (1.2 AUMs) the squash crop residual as well as the mixture of hairy vetch and clover cover crop. Soil and biomass samples were collected prior to grazing in month 2019 and were collected again in June 2020 (Table 1).

Strike Farms

During the first year of the study (2017) preliminary data for soil nutrients, microbial DNA analysis, and biomass was collected and exclosures were put into place, prior to grazing 2.43 ha of alfalfa and grass mix pasture (2.4 AUMs). Data was collected then collected prior to and after grazing on a 1.21 ha oat and pea cover crop by sheep (2.4 AUMs), that was previously a section of the 6-acre alfalfa and grass pasture. Data was also collected prior to harvest on the 2.43 ha field that was previously alfalfa and grass mix pasture and converted to a mixture of herb and vegetable crops. The field where data collection occurred was put up for sale in 2019, and no new crops were planted for the 2019 grazing season. Fortunately, sheep were still able to graze (.8 AUMs) the residual pea and oat cover crop from 2018 that had previously been grazed. Soil samples were collected

prior to grazing and were collected again in June 2020 (Table 1).

Statistical Analysis

Statistical analysis for bulk density and soil nutrient data was conducted using the two-way analysis of variance (ANOVA) procedure with repeated measures of R (R Core Team 2017) for each farm separately. Fixed effects in the model were year, treatment, and year × treatment for bulk density and soil nutrient type. Means were separated using the Tukey method with a confidence level of 95%

RESULTS

Soil Microbial Diversity

Soil microbial ASV (Amplicon sequence variant) profiles clustered by location (R = 0.87; F = 9.4; *P* = 0.001; Figure 3a), this explained 24.7% of the total variation observed. While all farms were very distinct from one another, Black Cat was less similar to either 13 Mile (R = 0.952) or Strike (R = 0.926) than 13 Mile and Strike from one another (R = 0.784). Samples also exhibited farm × year effects (R = 0.739, *P* = 0.001; F = 6.2887) which explained 12.4% of the variation (Figure 3b). Weak year effects were detected by ANOSIM between 2020 and either 2017 (R = 0.237; *P* = 0.001) or 2018 (R = 0.229; *P* = 0.001) and between 2019 and 2017 (R = 0.275, *P* = 0.001) and 2018 (R = 0.285, *P* = 0.001). No effects were detected for treatment, farm × treatment, or farm × year × treatment (*P* > 0.05; Figures 5, 6, and 7). No ASV was found to differ with treatment (Figures 5, 6, and 7).

Soil Nutrients and Bulk Density

Of the 14 soil properties we measured across all three farms and four years (14x12=168 combinations), only 19 combinations showed to be statistically significant. For example, 13 Mile Lamb and Wool decreased in NH_4+ (mg/kg, *P* < 0.01) from 2017-2020. Additionally, Sulfur (mg/kg, *P* < 0.01) also decreased from 2017-201. Na (mg/kg) increased in 2018, before decreasing again in 2019 and 2020 (*P* = 0.07). Nitrate-N (kg/mg) increased from 2017-2020 (*P* = 0.03). Black cat farms NH_4+ (mg/kg) and Na (mg/kg) increased from 2017-2018 and then decreased through 2020 (*P* < 0.01, *P* < 0.01) OM % decreased from 2017-2018 and then

increased from 2019-2020 ($P = 0.07$). Nitrate-N (kg/mg) increased from 2017-2018 before decreasing in 2019 ($P = 0.05$). Sulfur decreased from 2018-2020, where CEC ($cmol_+/k$) and Ca (mg/kg) decreased from 2017-2020 ($P < 0.01$, $P < 0.01$, $P < 0.01$). Strike farms Nitrate-N (kg/mg) increased from 2017-2018 ($P < 0.01$). Na (mg/kg) decreased from 2018-2020 ($P = 0.04$). P-Olsen (mg/kg) increased from 2018-2019 ($P = 0.04$). Sulfur (mg/kg) decreased from 2017-2020 ($P < 0.01$). Salts (mmhos/cm) increased from 2017-2020 ($P < 0.02$; Tables 2, 3 and 4). There was no significant difference in bulk density measurements between the grazed and ungrazed treatments at Black Cat ($P = 1.00$), 13 Mile Lamb and Wool ($P = 0.98$), and Strike Farms ($P = 1.00$; Figures 2a-2c). Bulk density (kg/m^3) increased from 2017-2019 at 13 Mile Lamb and Wool ($P < 0.01$). Bulk density increased from 2018-2019, but then subsequently decreased from 2019-2020 at Black Cat ($P < 0.01$). At Strike Farms bulk density (kg/m^3) increased from 2017-2019 and then decreased from 2019-2020 ($P < 0.01$; Figures 2, 3 and 4).

DISCUSSION

Soil chemistry, biology, and physical properties are all invaluable to the overall function of soil, as they drive the soil's ability to hold water, sustain plant life and assist in resisting disturbance (Doran, 1999; Parr et al., 1992). Due to this, we sought to evaluate soil nutrient content, microbial communities, and compaction in response to grazed versus un-grazed vegetable cropping systems and understand the interaction between soil biology, nutrient cycling, and livestock when integrated into a variety of vegetable production systems.

Soil samples were collected at a depth of 0-15 cm, while it may be possible that livestock grazing could impact lower depths in terms of soil compaction, nutrient levels and soil microbial diversity it's more likely that the shallowest depths will be impacted by grazing to a greater extent than deeper depths (Golodets and Boeken, 2006). Integrating livestock into each of the farms studied had no significant effect on bulk density when comparing the grazed and un- grazed sampling areas. This finding is similar to other studies involving integrated livestock operations. In 2011, Liebig et al. (2011) found that at the USDA-ARS Northern Great Plains Research Laboratory southern research station in Mandan, ND soil infiltration rates (another index of soil compaction) were not significantly different among the livestock integrated annual cropping sequence and residue management that was hayed or left in place. Liebig et al. (2011) indicated that infiltration and compaction issues were not of concern when livestock were integrated into winter grazing systems due to the soil being frozen during grazing. Additionally, it was noted that soil in the Northern Great Plains experience freeze and thaw cycles which could mitigate any compaction from grazing (Liebig et al., 2011).

On the contrary, other studies have indicated that integrating livestock into farming systems may lead to compaction, with increased soil bulk density. When studying three farm locations in the Southern Great Plains, Krenzer et al. (1989) reported that cattle increased soil bulk density by as much as 16% when allowed to graze red winter wheat (*Triticum aestivum L.*) during the fall and winter, following summer harvest (Krenzer et al., 1989). Soil compaction may be influenced by soil type, grazing level, precipitation, and location, so all variables must be taken into consideration when developing a grazing plan (Mapfumo et al., 1999).

Bulk density did however significantly change at all three farms between years, specifically in 2019. In 2019, Black Cat received more annual precipitation than in 2017, 2018, and 2020. Both Strike Farms and 13 Mile received greater annual precipitation in 2018, with 2019 annual precipitation being very similar. 2020 was the lowest annual precipitation received across all three farms. Considering that soil bulk density is known to increase with increased soil moisture, it may be possible that the greater amounts of annual precipitation in 2018-2019 compared to 2017 and 2020 may have resulted in significantly higher bulk density across all three farms (Mwendera and Feyen, 1994; NOAA, 2021). As Black Cat Farms received the most annual precipitation in 2019, then 2018 and 2020, where both 13 Mile and Strike Farms received the most precipitation in both 2018 and 2019. All three farms received the least amount of annual precipitation in 2020 (NOAA, 2021).

Biomass samples were collected to better understand the nutritional quality and quantity of the forages consumed by livestock during the duration of the study. According to the National Research Council (2007), non-lactating sheep require 7% crude protein for maintenance. When considering the results of the nutrient analysis conducted at all three farms, both Strike and 13 Mile farms were able to meet the crude protein requirements Black Cat farms however, had crude protein levels ranging from 1.5% - 4.9%, which are levels that do not meet the nutritional maintenance requirements of a non-lactating sheep (NRC, 2007). This may be due to the fact that sheep were grazing crop residues that were of lower forage quality such as tomato vines and pepper plants. It should be noted however that the sheep were also provided supplemental hay in addition to the crop residue.

By understanding the interaction between livestock grazing and soil nutrient cycles we can improve the knowledge base of how integrated livestock grazing impacts soil microbial

communities and plant growth, as microbes are essential to nutrient cycling and nutrient cycling is essential for plant growth (Wang et al., 2016; Bhowmik et al., 2017). Sheep grazing during fallow periods in wheat fields for weed control had no effect on NH4+ and NO3- levels when compared to tillage and herbicide weed management during a study conducted in SW Montana (Sainju et al., 2010). According to Wang et al., 2016, NH4+ and NO3- may increase with livestock grazing in grasslands. On the contrary, during the current study Nitrate-N decreased from 2018 to 2019 at Black Cat Farms. While some soil nutrients changed throughout the years of the study, no soil nutrients measured were affected by management type (grazed or un- grazed). Taking into consideration that each study site was tilled after grazing had occurred, tilling the soil may have made it difficult to observe any differences between treatments and only made it possible to detect differences between years.

While some research has explored the impacts that livestock grazing has on soil microbial diversity, little has been studied in terms of how livestock integrated cropping systems impacts soil microbial communities, specifically in horticulture systems (Yang et al., 2013; Chillo et al., 2017). In the current study, which focused on changes in soil microbial diversity in livestock integrated systems, while significant differences in soil microbial diversity were detected between farm locations, but no significant effects were detected for treatment, farm × treatment, or farm × year × treatment. Due to the size of the sampling areas (1-m^2), the sampling plots may have been too small to distinguish between the effects of the treatment and environmental effects on the soil microbial communities. Such environmental effects known to impact soil microbial communities may include climate, and other soil physical factors (Ishaq et al., 2017; Kaiser et al., 2016). Since soil microbial communities are affected by changes in soil and plant properties, it is understandable why the soil microbial

communities are clustered by farm location, as each farm location had different soil properties and crop species.

It has been shown that plant composition may positively correlate with microbial beta diversity of microbes even after removing environmental factors, as was reported in a study conducted in temperate grasslands across four continents (Prober et al., 2014). With this understanding, it is possible that the plant composition at each of the farm locations had a larger effect on soil microbial diversity than crop rotation or level of grazing. Another study in Eastern Australia indicated that any effects grazing had on soil microbial community diversity were also indirectly impacted by changes in plant and litter cover and soil pH and N levels, so it may also be difficult to detect any effects livestock grazing may have on soil microbial communities within the sampling sites as there are many other factors involved (Eldridge et al., 2020).

Along with environmental factors, soil microbial movement, and the size of the sampling areas, all the grazing exclosures were removed at the end of the grazing season to allow the landowners to till the study sites in preparation for seeding. The action of tilling may have also moved soil microbial communities around the study sites. As indicated in Sun et al., 2018, where tillage was seen to change the vertical distribution of soil microbial communities to become more homogenized when comparing various tillage practices to no-till. Whereas researchers indicated that microbial communities did differ among various soil depths that were sampled.

CHAPTER FOUR

CONCLUSION

Results from this study indicate that integrating livestock into small-scale vegetable farming systems in the Northern Great Plains does not have a negative impact on soil health measurements, including bulk density, soil nutrients and soil microbial communities. Further research utilizing larger sampling areas may assist in extracting out management and environmental factors that play a direct role in changes to soil microbial communities and could give a better understanding of the role livestock grazing plays on soil microbe communities. While no consistent differences in soil nutrients, bulk density and soil microbial diversity were found due to the treatments, grazing did not negatively impact any of the soil measurements among the years studied (2017-2020).

With the lack of research looking at the impacts of livestock integrated systems on small- scale vegetable farms, there is a need for a better understanding of the relationship between livestock grazing of farming systems and soil health. Results from this study demonstrates to farmers and livestock operators that the integrated livestock approach has no negative impacts to soil health indicators and has also become the starting point for further research into a little studied topic.

Table 1. Grazing dates, crop types, and number of livestock and area grazed for Black Cat, Strike and 13 Mile Farms (2017-2020)[1]

	Black Cat			Strike			13 Mile		
	2017	2018	2019	2017	2018	2019	2017	2018	2019
Crop type	Spelt residue	Tomatoes, purple potatoes, peppers, tomatillos & eggplant residue	Forage radish, buckwheat oats and peas cover crop	Alfalfa	Pea & oats cover crop	Fallow	Squash residue, mint & garlic	Hairy vetch & clover cover crop	Hairy vetch & clover cover crop, squash residue
Dates grazed	11/10/2017 - 04/15/2018	11/10/18-04/10/2019	12/20/2019 - 5/17/2020	08/07/2017 – 08/16/2017	06/25/2018 – 07/05/2018	06/25/2019 – 07/05/2019	09/26/2017 – 02/10/2018	12/15/2018 – 02/05/2019	11/10/2019-11/20/2019 + Over winter
Sample Date	8/15/2017	9/28/2018	5/10/2020	8/7/2017	7/15/2018	6/2/2019	9/26/2017	10/4/2018	5/8/2020
Number of head	30	30	30	60	22	22	45	45	75-100
Livestock type	Sheep (Tunis & Karakul)	Sheep (Tunis & Karakul)	Sheep (Tunis & Karakul)	Sheep (Targhee)	Sheep (Targhee)	Sheep (Targhee)	Sheep (Romney)	Sheep (Romney)	Sheep (Romney)
Size of grazed area (ha)	.4	1.2	1.2	2.4	.8	.8	1.2	1.2	1.2
AUMs[1]	18.7	6.0	6.0	0.3	0.4	0.4	2.6	1	5.2

[1] Animal Unit Months. Where 1 animal unit is equivalent to 454 kg animal

Table 2. Soil nutrient results (0-15 cm) for Black Cat Farm (2017-2020) from grazed and ungrazed treatments[1]

	2017		2018		2019		2020					
Variable	Grazed	Ungrazed	Grazed	Ungrazed	Grazed	Ungrazed	Grazed	Ungrazed	SE[2]	Treatment	Year	Treatment x Year
pH	7.87	7.85	7.95	7.88	7.83	7.83	7.87	7.83	1.14	0.54	0.28	0.48
OM[4], %	6.50	6.42	4.98	5.32	5.73	5.92	6.23	6.18	0.85	0.79	0.07	0.97
Salts, mmhos/cm	0.51	0.54	0.55	0.66	0.51	0.55	0.47	0.52	0.08	0.11	0.17	0.84
Nitrate-N, mg/kg	18.67	21.91	29.00	45.08	17.83	22.75	20.42	23.92	3.60	0.16	0.05	0.75
P-Olsen[5], mg/kg	10.50	10.50	10.33	12.50	9.33	11.83	15.83	15.33	1.74	0.58	0.20	0.92
K, kg/mg	216.83	209.17	235.00	212.00	289.33	276.83	309.33	309.17	0.32	0.81	0.20	0.89
Ca, kg/mg	5819	5943.50	5566.33	5803.67	5063.50	5136.50	4982.00	4874.50	12.55	0.53	<0.01	0.82
Mg, kg/mg	9.18	8.83	10.03	9.10	11.63	11.17	11.10	12.27	1.50	0.84	0.35	0.75
Na, kg/mg	23.17	25.00	30.83	38.00	27.83	27.70	23.70	23.00	0.05	0.48	0.01	0.78
Total N, %	0.33	0.33	0.29	0.32	0.28	0.29	0.32	0.31	0.04			
CEC[6], meq/100mg	32.75	32.32	31.75	32.92	29.60	29.75	29.03	28.75	4.47	0.73	0.34	0.88
S, mg/kg	14.00	14.17	16.83	17.83	11.50	11.33	9.17	8.50	1.86	0.93	<0.01	0.93
NH4, mg/kg	3.18	3.30	5.12	5.20	3.67	2.97	1.97	1.97	0.49	0.65	<0.01	0.68

[1]Grazed and ungrazed plots were randomly placed at each of the farms with six 1-m^{2}exclosures (UNGRAZE) and six 1-m^2 plots (GRAZE) in the grazed treatments.
[2]Pooled standard error of the means presented.
[3]*P* – value of grazed vs. ungrazed treatments.
[4]Organic matter
[5]Extractable phosphorus
[6]Cation exchange capacity

Table 3. Soil nutrient results (0-15 cm) for 13 Mile (2017-2020) from grazed and ungrazed treatments[1]

	2017		2018		2019		2020					
Variable	Grazed	Ungrazed	Grazed	Ungrazed	Grazed	Ungrazed	Grazed	Ungrazed	SE[2]	Treatment	Year	Treatment x Year
pH	7.15	7.22	7.53	7.45	7.35	7.08	7.27	7.02	1.15	0.13	0.95	1.00
OM[4], %	8.25	7.42	6.45	6.93	6.50	6.50	7.51	6.77	0.99	0.60	0.79	0.87
Salts, mmhos/cm	0.38	0.35	0.38	0.38	0.43	0.39	0.51	0.44	0.07	0.40	0.10	0.68
Nitrate-N, mg/kg	2.58	1.92	33.83	33.75	27.19	37.63	39.50	34.00	3.65	0.40	0.03	0.89
P-Olsen[5], mg/kg	19.00	16.67	18.17	18.67	25.25	25.63	33.00	21.83	1.40	0.95	0.94	0.57
K, mg/kg	505.83	521.17	492.17	453.17	536.17	507.33	420.83	595.33	0.57	0.62	0.94	0.58
Ca, mg/kg	5525.83	5434.17	5593.17	5577.50	5890.83	5789.17	6010.17	5649.00	12.31	0.41	0.36	0.90
Mg, mg/kg	407.83	421.83	418.50	421.83	411.83	431.70	440.70	414.83	1.52	0.88	0.97	0.84
Na, mg/kg	19.00	19.33	27.70	19.70	23.17	23.33	18.17	16.33	0.04	0.25	0.07	0.43
Total N, %	0.38	0.32	0.29	0.31	0.30	0.38	0.41	0.38	0.05	0.80	0.38	0.78
CEC[6], meq/100mg	21.78	22.00	21.87	21.28	21.02	19.63	20.12	19.57	4.89	0.51	0.37	0.95
S, mg/kg	12.00	14.00	11.00	12.17	10.33	11.50	13.33	10.83	2.05	0.79	<0.01	0.28
NH4, mg/kg	3.85	3.78	5.08	5.07	2.16	1.91	1.98	2.08	0.41	0.20	<0.01	0.62

[1]Grazed and ungrazed plots were randomly placed at each of the farms with six 1-m^2exclosures (UNGRAZE) and six 1-m^2 plots (GRAZE) in the grazed treatments.
[2]Pooled standard error of the means presented.
[3]*P* – value of grazed vs. ungrazed treatments.
[4]Organic matter
[5]Extractable phosphorus
[6]Cation exchange capacity

Table 4. Soil nutrient results (0-15 cm) for Strike Farms (2017-2020) from grazed and ungrazed treatments[1]

	2017		2018		2019		2020					
Variable	Grazed	Ungrazed	Grazed	Ungrazed	Grazed	Ungrazed	Grazed	Ungrazed	SE[2]	Treatments	Year	Treatments x Year
pH	7.98	7.88	7.97	7.90	7.95	7.87	7.82	7.93	1.05	0.20	0.11	0.62
OM[4], %	6.77	7.37	6.85	6.77	6.65	6.67	6.93	6.73	1.04	0.96	0.16	0.23
Salts, mmhos/cm	0.44	0.50	0.38	0.41	0.46	0.42	0.48	0.53	0.06	0.36	0.02	0.64
Nitrate-N, mg/kg	17.83	18.83	14.50	21.67	25.67	25.75	34.08	44.93	3.80	0.85	<0.01	0.46
P-Olsen[5], mg/kg	9.00	8.50	8.67	11.17	9.67	10.50	11.17	8.33	3.27	0.15	0.02	0.45
K, mg/kg	309.67	307.67	260.67	273.67	360.17	304.83	584.67	353.50	0.63	0.21	0.06	0.37
Ca, mg/kg	3613.50	3614.33	3640.00	3562.67	3420.33	3206.67	3133.67	3142.17	11.76	0.66	0.13	0.96
Mg, mg/kg	339.17	367.33	349.00	317.83	342.33	327.67	344.50	344.17	1.98	0.75	0.74	0.51
Na, mg/kg	23.50	21.00	24.50	26.33	20.50	18.50	16.83	18.00	0.06	0.85	0.03	0.82
Total N, %	0.37	0.38	0.35	0.35	0.38	0.38	0.41	0.38	0.05	0.35	<0.01	0.35
CEC[6], meq/100 mg	32.10	32.40	32.65	32.83	33.93	34.35	33.32	34.90	3.02	0.76	0.14	0.27
S, mg/kg	12.67	11.67	10.00	7.86	17.33	17.17	20.67	20.67	2.29	0.50	0.26	0.93
NH4, mg/kg	3.73	2.62	5.52	4.67	1.70	1.60	1.27	1.24	0.47	0.97	<0.01	1.00

[1]Grazed and ungrazed plots were randomly placed at each of the farms with six 1-m^2exclosures (UNGRAZE) and six 1-m^2 plots (GRAZE) in the grazed treatments.

[2]Pooled standard error of the means presented.

[3]*P* – value of grazed vs. ungrazed treatments.

[4]Organic matter
[5]Extractable phosphorus
[6]Cation exchange capacity

Table 5. Average biomass and forage composition (±1SD, n=3) for 13 Mile Farms (2017-2020).[1]

	2017		2018		2019		2020	
Variable	Pregraze	Grazed	Pregrazed	Grazed	Pregrazed	Grazed	Pregrazed	Grazed
ADF, % DM[2]	43.40	47.47	-	38.63	-	47.80	-	32.23
NDF, % DM[3]	50.40	55.13	-	47.40	-	53.57	-	38.83
CP, % DM[4]	8.33	6.98	-	12.00	-	10.62	-	9.37
DM, %[5]	53.11	90.20	-	34.53	-	77.85	-	62.43
Production, kg · ha^{-1}	941.74	443.13	-	444.83	-	369.19	-	300.67

[1] Three biomass samples were randomly collected using a 1-m^2 clipping frame ~x days before and ~y days after grazing.
[2] Acid Detergent Fiber
[3] Neutral Detergent Fiber
[4] Crude Protein
[5] Dry Matter

Table 6. Biomass and forage composition for Black Cat Farms (2017-2020)[1]

	2017		2018		2019		2020	
Variable	Pregraze	Grazed	Pregrazed	Grazed	Pregrazed	Grazed	Pregrazed	Grazed
ADF, % DM[2]	44.83	54.20	49.52	54.20	54.20	-	-	-
NDF, % DM[3]	65.17	75.98	70.57	75.97	75.97	-	-	-
CP, % DM[4]	4.92	2.97	1.58	1.20	2.62	-	-	-
DM, %[5]	96.86	97.59	53.88	47.32	73.94	-	-	-
Production, kg · ha^{-1}	941.65	444.95	444.83	284.13	369.07	-	-	-

[1] Three biomass samples were randomly collected using a 1-m^2 clipping frame before and after grazing.
[2]Acid Detergent Fiber
[3] Neutral Detergent Fiber
[4] Crude Protein
[5] Dry Matter

Table 7. Biomass and forage composition for Strike Farms (2017-2020)[1]

	2017		2018		2019		2020	
Variable	Pregraze	Grazed	Pregrazed	Grazed	Pregrazed	Grazed	Pregrazed	Grazed
ADF, % DM[2]	35.00	46.07	32.50	42.97	36.00	41.83	-	-
NDF, % DM[3]	51.43	66.23	49.03	56.13	47.87	61.50	-	-
CP, % DM[4]	13.48	7.80	16.58	10.60	12.79	9.65	-	-
DM, %[5]	72.92	96.68	21.27	31.26	50.00	40.98	-	-
Production, kg · ha^{-1}	544.71	192.91	899.62	823.13	1127.17	706.58	-	-

[1] Three biomass samples were randomly collected using a 1-m^2 clipping frame before and after grazing.
[2]Acid Detergent Fiber
[3] Neutral Detergent Fiber
[4] Crude Protein
[5] Dry Matter

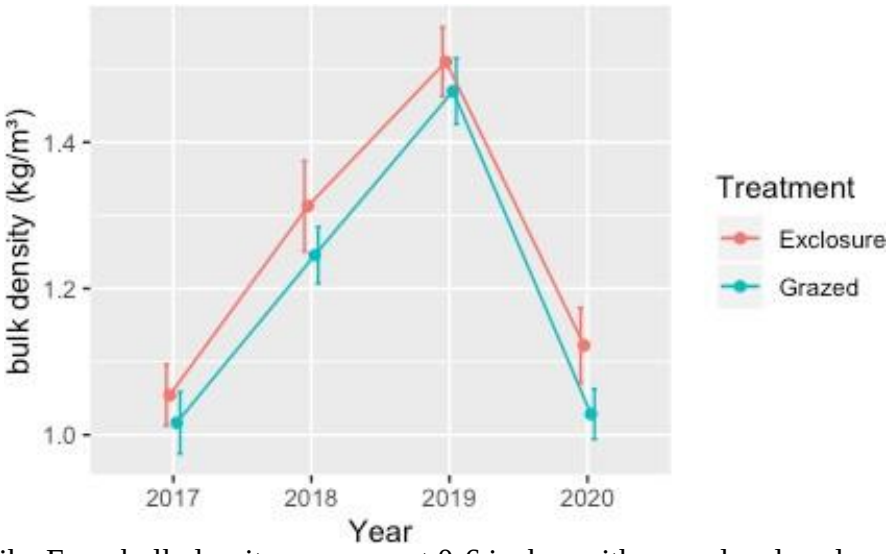

Figure 2. Strike Farm bulk density averages at 0-6 inches with grazed and exclosure (un-grazed) treatments

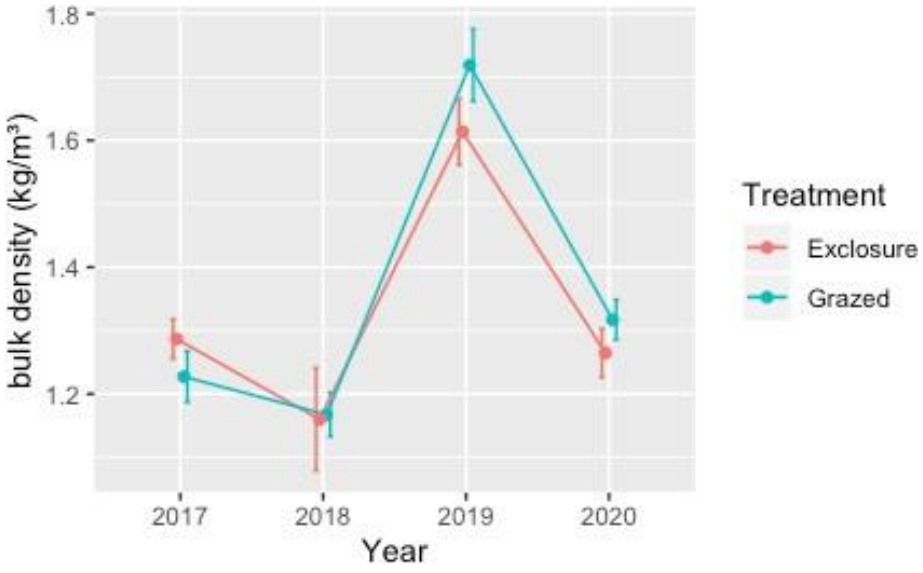

Figure 3. 13 Mile Lamb and Wool bulk density (g/cm^3) averages at 0-6 inches with grazed and exclosure (un-grazed) treatments

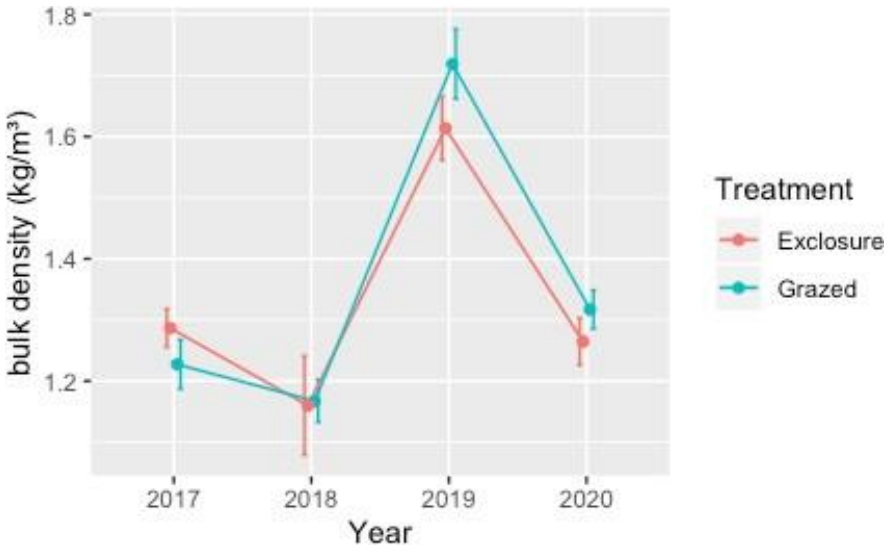

Figure 4. Black Cat Farm bulk density averages at 0-6 inches with grazed and exclosure (un-grazed) treatments

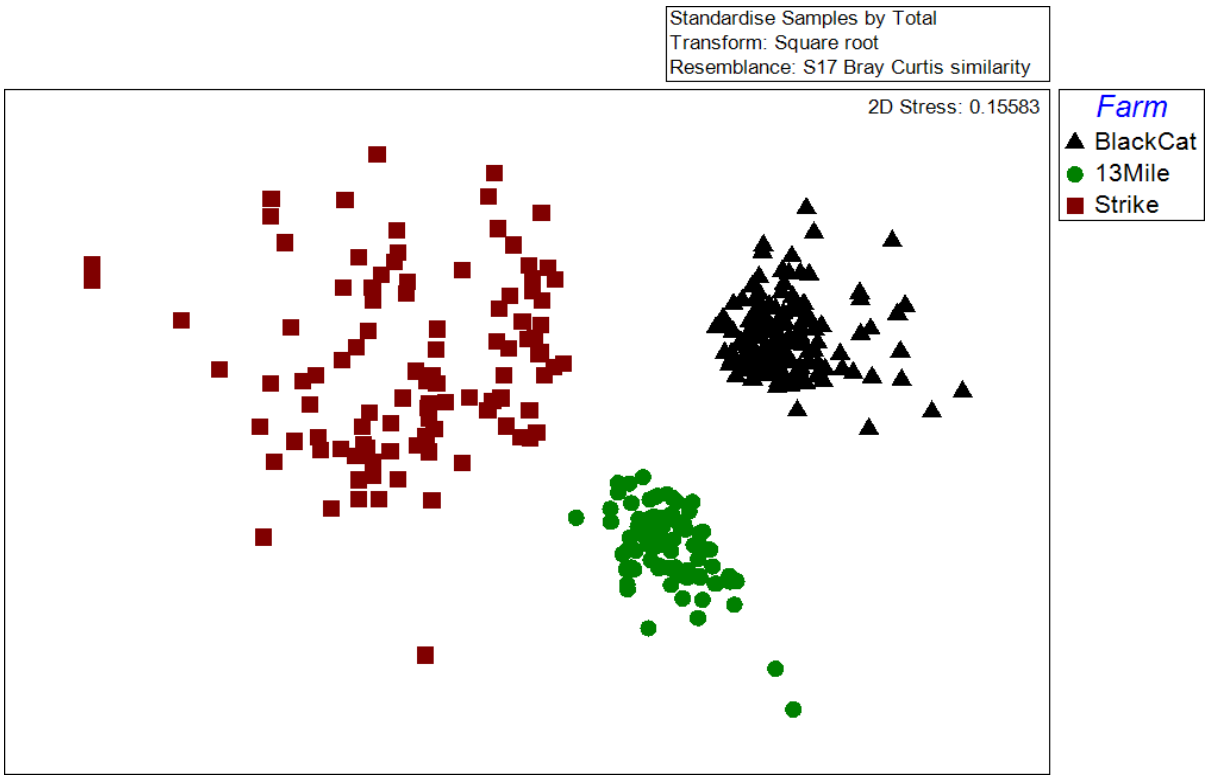

Figure 5. Bray-Curtis similarity index shows beta diversity of soil microbial communities among farms.

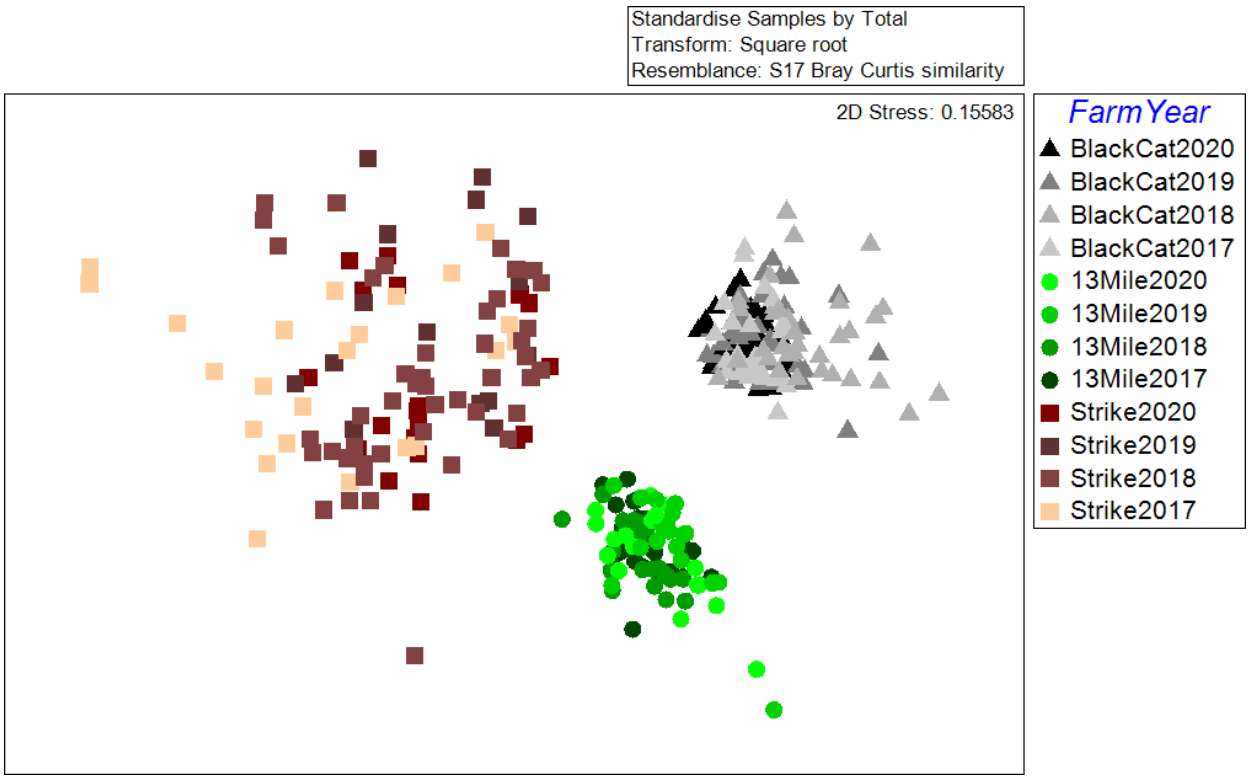

Figure 6. Bray-Curtis similarity index showing beta diversity of soil microbial communities comparing farm x year.

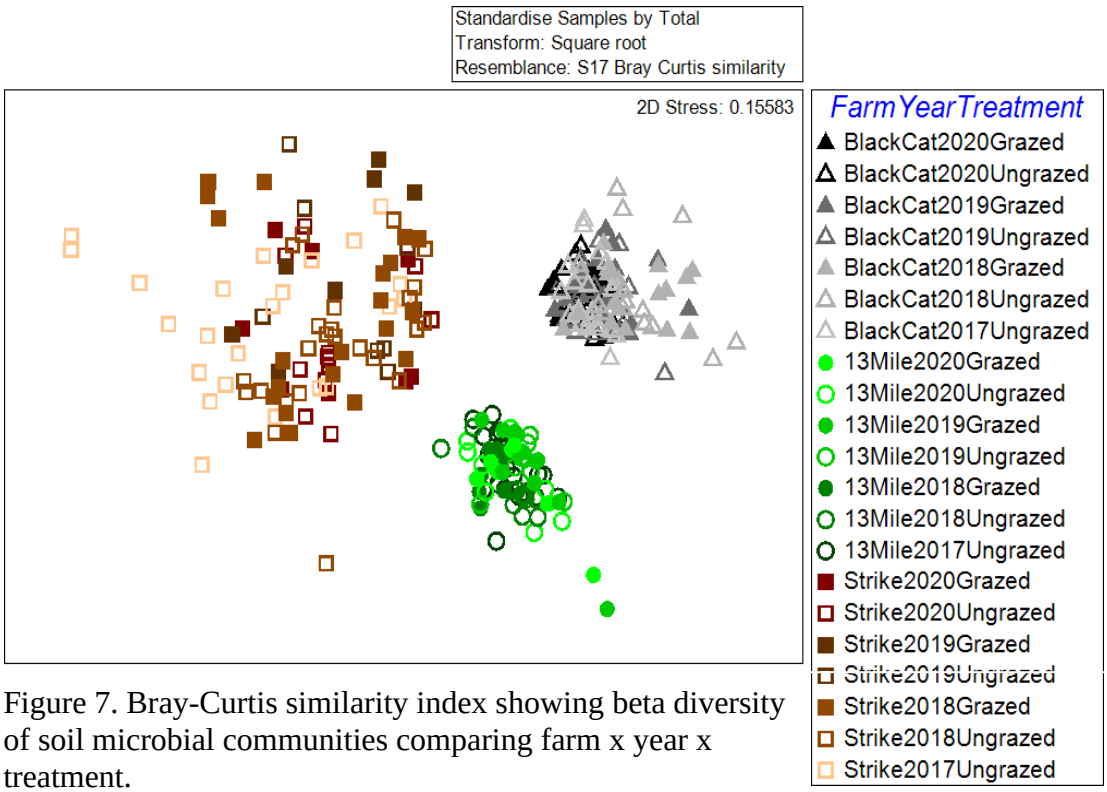

Figure 7. Bray-Curtis similarity index showing beta diversity of soil microbial communities comparing farm x year x treatment.

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