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EVALUATION OF A NOVEL APPROACH FOR ASSESSING
BIOLOGICAL ACTIVITY IN AGRICULTURAL SOILS

By

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EVALUATION OF A NOVEL APPROACH FOR ASSESSING
BIOLOGICAL ACTIVITY IN AGRICULTURAL SOILS

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University of Nebraska, 2021

Advisor: Amy Millmier Schmidt

Soil health is a key factor impacting soil resilience and fertility in crop production systems. Favorable soil physical and biological conditions facilitate plant nutrient absorption and nutrient cycling. Demonstrating to farmers the impacts and changes in soil biological activity under different soil management practices has been a challenge due to the limited availability of inexpensive tools for quantifying this component of soil health. The primary goal of this study was to present a simple and readily accessible tool for evaluating soil biological activity to promote the use of organic amendments in crop fields. Research plots were established in two studies to evaluate percent cotton fabric degradation using manual assessment and image analysis methods, and to demonstrate fabric degradation under organic soil amendment treatments in relation to established measures of soil biological health that included soil CO₂ respiration and soil arthropod analysis abundance and through soil biological quality index (QBS). To obtain QBS index, arthropods were classified to order level and a score from 1 to 20 was assigned based on their level of adaptation to soil living conditions. Treatments for study #1 included the application of beef feedlot manure (BM) and a control with no organic amendment (CON) during the fall to fallow soil. Image analysis to determine percent

degradation of fabric samples was initially made with Adobe Photoshop and compared to degradation estimated by manually counting degraded areas on the pieces of fabric. In study #2, treatments included swine slurry (SS), SS top-dressed with red cedar woodchips (SSW), and no treatment (CON) applied to land planted to corn during a summer growing season. Fabric degradation was assessed manually and with Photoshop and ImageJ. Across both studies, image analysis of fabric degradation with either software did not vary significantly from manual hand counting. In study #2, no difference in percent fabric degradation or rate of degradation were found among treatments and soil microbial respiration was also unaffected by the treatments. However, the SSW treatment yielded greater arthropod abundance than SS and CON treatments, resulting in a higher QBS for SSW. While this novel method for demonstrating soil biological activity requires additional refinement to improve automatization of the process and reduce sources of error, image analysis of fabric degradation is a promising tool that may be easily adapted to visually demonstrate soil biological activity under different soil management practices in an on-farm setting.

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CHAPTER 1: REVIEW OF THE LITERATURE

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1.1. Soil Health in Agricultural Systems

Soil is the combination of minerals, organic matter, liquid, and gases that occur on the surface of the Earth with the capability to grow land plants. Soil is one of the main substrates of life on earth, serving as a reservoir of water, nutrients, and gases that filter and process organic materials and contaminants and providing an important role in the carbon cycle. Soil and the cycles that occur in it are highly influenced by biological, climatic, geological, and topographical conditions. In agriculture, soils have a great capacity to sequester carbon (C). Carbon is strongly related to the biogeochemical cycles of nitrogen (N) and phosphorus (P). The interaction among these elements allows soil to sequester atmospheric C, which is an environmental concern related to climate change.

Soil C and N stocks play a key role in soil biogeochemical cycles and their availability depends on soil organic matter (SOM) concentration. As such, the quality of SOM largely determines the ratio of C and N concentrations in the soil. At the same time, SOM quality is impacted by the environmental conditions and time over which soil is exposed to such conditions. Optimal precipitation and temperature conditions along with soil N availability promote SOM turnover, resulting in an increased capacity of the soils to preserve C (MacDonald *et al.*, 2018). However, SOM quality and climate conditions are not the only factors affecting N and C dynamics in the soils. Physical properties of the soils have a major influence on N cycling as well. For example, Saha *et al.* (2010) reported increased SOC concentration, aggregate stability, moisture retention capacity, and infiltration rates, and reduced bulk density, with the addition of organic and inorganic

N, P, potassium (K), and lime after 5 years of application on corn fields. The reason for these changes in the soil environment are related to an improved capacity of plant roots to transport, mineralize, and exudate nutrients in soil pore spaces possible, due in part to symbiotic links with microorganisms.

1.2. Characteristics that Define Soil Health

1.2.1. Soil Physical Properties

To understand the dynamics that occur in a soil, it is important to first define the composition of a soil. Soil textural classes often inform the soil management strategies and are estimated in terms of size distribution of the primary particles of the soil (silt, clay, and loam) (Wang & Kroetsch, 2008). Under the right conditions, these particles form aggregates that can break down and disperse when changes occur in the soil, such as irrigation or organic matter degradation. In “healthy” soils, the resistance of aggregates to being broken down by rain, runoff, wind, tillage, or other processes is greater, thus reducing the degradation of soils (Hu *et al.*, 2019). Soil particle aggregation is defined as a basic unit of soil structure, in which physical forces hold the particles together. Aggregate stability, on the other hand, includes the binding agents, such as organic materials, iron, and aluminum oxides and clays, that hold particles together when the physical and environmental forces change around the particles (Lynch & Bragg, 1985). Although poor soil structural stability of soil is an increasing concern, organic matter addition may increase aggregate stability and reduce crusting and erosion problems (Abiven *et al.*, 2009).

Physical properties of soil create the conditions to facilitate plant absorption of nutrients, which occurs through mass flow and diffusion. More pore space in the soil

allows for water and nutrients to move easily towards the root by differences in water potential, soil hydraulic conductivity, and transpiration demand (Chapman *et al.*, 2012). However, the physical processes are not the only ones who play a role on plant nutrient uptake. Some bacteria in the soil produce hormones that stimulate nutrient uptake and root growth (Kraiser *et al.*, 2011). In general, soil particles and aggregation create the perfect biome for roots to interact with microorganisms. The spaces between soil aggregates hold hyphae, bacteria, plant roots and root hairs, and organic matter that interact with each other to form soil macroaggregates. At the same time, fecal matter produced by earthworm and microarthropods like mites, and collembola, contribute to the structure of the aggregates (Voroney & Heck, 2015), inputs on organic matter, and transformation of C and other nutrients in the soil.

Environmental conditions affect soil physical components. Often, wetting of the soil by rain or other inputs enhances the creation of pores and aggregates, while drying influences shrinkage of particles and strengthening of the soil. This is especially true in the presence of plant root exudates, which help “cement” soil particles together, creating space for nutrients, air, and water to circulate (Angers & Caron, 1998). Intensive use of soil can also modify soil physical properties, causing degradation that negatively influences plant growth (Biro *et al.*, 2011). The mobility of binding substances necessary to soil aggregation is also influenced by the smaller area between soil particles, called soil porosity or pore space. In healthy soils, these pore spaces are large and allow water retention, oxygen diffusion, and nutrient mobilization (Sasal *et al.*, 2006).

Soil compaction occurs slowly, and long-term effects are not always immediately clear to land managers. For example, wheel traffic on cropland can create changes in the

subsoil, rather than the top layers of the land, making it difficult to see and manage the long-term effects. These long-term effects are not improved by external environmental conditions like freezing and thawing or wetting and drying (Copas *et al.*, 2009). Soil compaction can contribute to larger volumes of surface runoff of nutrients and water, and major soil loss due to water erosion (Hanna & Al-Kaisi, 2009). Similar to soil aggregation, soil porosity is influenced by compaction due to traffic passes. Although total pore spaces may remain significantly high under tillage, the pore size is decreased (Pagliai & De Nobili, 1991), limiting the capability of the soil to transport water, oxygen, and nutrients.

Soil structural degradation is common on cropping lands in the United States, having a long history of agricultural production, especially in the Midwest. The effects of extensive agricultural production and soil modification can be observed in the major land stresses. In the Midwest, these include low SOM concentration and excessive nutrient leaching (U.S. Department of Agriculture, 2003). Paying close attention to the soil structure could be a strategy to preserve the integrity of agricultural soils. Some strategies to benefit soil aggregate stability and porosity include increasing the efficiency of machinery traffic in soils and tillage practices, as well as incorporating organic matter and cover crops.

1.2.2. Soil Chemical Properties

Fertile soils provide essential nutrients for crop growth and support microbial diversity and activity; this allows for degradation of plant residues and other organic inputs and sustains good soil structure (Maeder *et al.*, 2002). Traditionally, soil fertility has been associated with the nutrient content of the soils, rather than the biological and

physical soil properties. This approach is supported by the fact that plants require specific amounts of macro- and micronutrients for their optimal growth and production (Miransari, 2011). It is assumed that the nutrients absorbed by the plants during a cropping season need to be replaced after to make it available for future crops (Havlin, 2020). Managing land under this approach requires farmers to constantly test soil chemical properties to evaluate nutrient content. Soil tests are a great tool to observe the most recent state of the soils, as the results show the condition of the soils at the moment of sampling, making these analyses a good indicator for estimating P, K, and lime needs of the soil (Fernández & Hoef, 2009). But the capacity of soils to retain the applied nutrients relies heavily on the soil texture and the activity of microbial populations and carbon mineralization. Fine textured soils tend to hold more N and organic C than sandy soils (Hamarashid *et al.*, 2012). Organic matter and clay are important in soil nutrient cycles because their predominantly negative charge holds positively charged particles (e.g. hydrogen, aluminum, magnesium and potassium). Potentially toxic elements can be managed with the addition of soil organic amendments like manure, biochar, compost, and liming materials, by stabilizing elements and improving soil enzyme activity (Palansooriya *et al.*, 2020). So organic matter and clays play an important role in retaining and supplying plant nutrients and absorbing contaminants (Saidi, 2012).

Another important factor impacting nutrient availability is soil pH. This value impacts the ability of soil to retain and supply nutrients to plants and is closely related to cation exchange capacity (CEC). Both pH and CEC are a function of cationic hydrogen (H^+) concentration in the soil, which impacts the capacity of the soil to bind more cations. At a higher soil pH, H^+ concentration is lower and soils have a better capacity to bind

other cations, preventing leaching of cationic compounds. Conversely, higher H^+ concentration (low pH) limits the capacity for nutrients to bind to the soil. In this scenario, if the nutrients are not absorbed by the plants they can be easily lost through leaching or runoff (McCauley *et al.*, 2009). If soil pH is too high or too low, the efficacy of soil amendments, herbicides, or other chemical reactions may be affected. Some elements that can be toxic, like aluminum and manganese, are more available in acidic soils, but can also be remediated with management of pH and CEC.

Soil nutrients like N, P, and K are usually a result of the deposits of organic matter in the soil that have been transformed to plant-available forms. But the dynamics of these nutrients are highly affected by soil pH and microbial activity. Increased pH results in increased microbial biomass (Zhalnina *et al.*, 2014), which can be beneficial for overall soil health dynamics. Nutrient mineralization is also linked to microbial and biological activity. For example, the presence of certain types of Collembola may impact the mineralization of N, but it may also affect the presence of other compounds and microbes, which in turn, would alter the nutrient cycle in the soil (Bardett & Chan, 1999). Nitrification, for example, is an oxidation process mediated by autotrophic organisms to create their own energy, and as a result, produces various nitrogen oxides as intermediate species (NO_2 , NO , N_2O) (Kendall, 1998).

1.2.3. Soil Biological Properties

Nutrient absorption, pH, aggregate stability, and bulk density are all key drivers of soil biodiversity because they collectively create the conditions under which organisms can exist. The structure of soil microhabitats is changed drastically by mechanical operations, because perturbances can limit the space through which water and oxygen

move. But microorganisms also have a big role in creating these ecosystems. An important indicator of the functioning of these relationships is the organic matter consumption. Soils with lower abundance and diversity of microorganisms provide less consumption of organic matter (Domsch *et al.*, 1983), and are more susceptible to leaching nutrients and contaminants through the soils because they often lack aggregation provided by microbial activity. Soil microorganisms function differently in high or low pH environments, especially when transforming soil organic carbon. Managing the abiotic components of the soil (e.g., acidity, moisture, structure) can enhance plant production and provide with similar results as microbial activity on healthy soils (Malik *et al.*, 2018), however, including physiological attributes into land management strategies builds resilient soils on a long term.

Soil ecosystems are shaped by the living communities that inhabit them, and each one fulfills a different role in maintaining soil health dynamics. For example, numerous species of soil and rhizosphere microorganisms may produce organic acids that mobilize large pools of insoluble phosphorus, making it available for plants (Altomare & Tringovska, 2011). Understanding microbial ecology and population dynamics is crucial to ensure nutrient management that positively impacts plant growth. Microorganisms are known to solubilize P and shape soil structure around plant roots (Richardson, 2001) but the lack of knowledge on appropriate practices that include biological activity might be a factor preventing land managers from approaching soil health from this point of view.

Soil type has a big influence on how management practices affect microbial communities. Despite diversity of microorganisms remaining fairly unaltered by soil management practices, relative abundance of phyla and genera display changes in cases

of intensified agriculture in some soil types (Gömöryová *et al.*, 2020). Explaining this response is a complex process, usually approached using microbiological techniques to identify species. However, this technique is not the most accurate for all organisms, especially the ones not considered “fauna”. A more accurate approach is to classify species according to their functional role in the ecosystem or the adaptations to soil changes, and although some of these features are linked to morphology and taxonomical identity, it excludes inactive organisms in the soil.

Furthermore, each role played by different soil invertebrates in the dispersal and conversion of soil nutrients deserves consideration. Both meso and macrofauna are known for carrying microorganisms attached to their bodies or inside their guts that are later excreted and released to the soil. Earthworm skin, for example, produces exudates rich in labile C that stimulates microbial activities and accelerate the mineralization of soil organic matter (Briones, 2018). Microorganisms can also attract macro and meso fauna. Collembola – a group of arthropods that is very abundant in the soil – for example, are attracted to fungal odors which might enhance the dispersal of fungi. Bacterial communities found in fecal matter can also act as a way of intra-specific communication among some arthropod communities (Wada-Katsumata *et al.*, 2015). Earthworms are considered “soil engineers” because of their ability to transform soil structure and improve aeration, water infiltration, and water holding capacity. They also excrete nutritional substances that are utilized by organisms like Collembola. Collembola feed mostly on hyphae and fungi; thus, they are important facilitators of microbial succession during decomposition and are great indicators of soil health due to their sensitivity to soil degradation. Lastly, some of the smallest organisms – bacteria and fungi – are key drivers

of oxidation of organic carbon to carbon dioxide (CO₂) and mineralization of nutrients (Bispo *et al.*, 2009). This process is called soil respiration. Environmentally, this process is an important factor to moderate CO₂ emissions (Ryan & Law, 2005). Carbon dioxide fixed by plants is transferred into the soil. When soil is tilled or experiences other major changes in its structure, the CO₂ is released back into the atmosphere, contributing to important environmental issues like the overabundance of greenhouse gases (Schlesinger & Andrews, 2000). Soil respiration is an indicator of a balanced exchange of nutrients and SOM with microbial populations. When soil disturbance occurs, CO₂ emissions rise, indicating accelerated SOM decomposition due to the alteration of soil aggregates that protect SOM deposits (Gougoulas *et al.*, 2014).

Soil health and soil quality are both terms that consider the physical, chemical, and biological properties of soil as determinant factors in its functionality. Even though soil quality has historically been studied from a chemical property point of view, the Soil Society of America has defined it as “the capacity of a specific kind of soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality and support human health and nutrition”. This definition inevitably involves the action of chemical processes within the physical space and composition of the soil, and is driven by the biological actors that transform, transport, and consume nutrients and compounds in the soil (Karlen *et al.*, 1997). Under this concept, evaluating soil health should comprise multiple parameters and collaboration among different sciences. Measuring the soil response to a given practice only in terms of chemical, physical, or biological properties or crop yields does

not give enough information about the state of the soils. Managing soils based on these broader parameters is, therefore, expected to help achieve sustainable agriculture goals.

1.3. Nebraska Agriculture

Nebraska is nationally known for being one of the most important producers of agricultural products. With over 9 million acres of harvested corn for grain during 2017, valued at over \$7.6 billion, Nebraska is the third largest producer of grain corn, following Illinois (11 million acres) and Iowa (12 million acres) (USDA, 2019). It is also a major producer of forage and other important crops, including soybeans, hay and haylage, wheat, potatoes, sorghum, millet, sunflower, oats, beans, sugar beets, and peas (USDA - NASS, 2019).

Nebraska's landscape has changed during the last few decades, with crop diversification and farm sizes decreasing. Nebraska has experienced a general trend of fewer and larger farms that produce a less diverse portfolio of commodities. Despite these changes, the long-term effects of land use changes have not been perceived by most Nebraskans (Hiller *et al.*, 2009). Over the periods of 1974 to 2008, Nebraska experienced a 40% increase in agricultural land and 98% increase in yields, largely shaped by farm policies and programs (Lin & Huang, 2019). However, it also reflects the greater food production need of an increasing global population. As land use changes and production of livestock and crops intensifies, soil protection and sustainability become increasing concerns for this sector. Agricultural expansion puts pressure on biodiversity and natural ecosystems. It may reduce biodiversity, but in some cases, this may not affect functional composition, which has a notable effect on nutrient cycling, organic matter turnover, and soil carbon decomposition (Allan *et al.*, 2015). The effects of land change depend on the

management strategies of the soils. In Nebraska, cover crops, crop rotations, no-till, and manure application are some of the strategies used by landowners to maintain soil health (Oliveira *et al.*, 2019).

1.3.1. Nebraska Livestock Production

Animal production is an essential component of the Nebraska economy and cultural history, with the livestock industry constituting around 45% of all agriculture cash receipts (USDA-NASS, 2021). Nebraska is one of the leading livestock producers in the country, with over 6.8 million head of cattle and calves (USDA-NASS, 2021), comprising a competitive beef industry that is responsible for 17.7% of the country's cattle production (USDA ERS, 2021). The pork industry also represents an important economic source in the state, with 3.1 million head of pigs in 2013 and continued growth. The dairy industry has held fairly stable over the same period of time. The poultry industry in Nebraska experienced an increase of 13% from 2018 to 2019, with a total value of all chickens (layers and boilers) of \$42.5 million in 2019 (USDA-National Agricultural Statistics Service, 2020). Overall, the state's meat animal cash receipts are worth over \$10.4 billion, which constitutes about 12% of the country's cash receipts for this category (USDA ERS, 2020).

1.3.2. Manure Production in Nebraska

The relevance of livestock production for the economy in Nebraska presents with other challenges, mostly related to management of waste by-products from this industry. Beef cattle may produce roughly 26 kg of manure per day per animal unit (AU), grow-finish hogs and pigs 28.6 kg/AU-d, lactating dairy cows 36 kg/AU-d, and chickens between 27 and 36 kg/AU-d. All manure produced by confined livestock and poultry,

along with all process wastewater that is in contact with manure, must be managed to protect water quality. When managed incorrectly, manure can contribute P, N, and pathogenic organisms to surface water sources via runoff and erosion (Alegbeleye & Sant'Ana, 2020). On the other hand, when managed according to research-based best management practices (BMPs), manure is a locally available source of organic nutrients for cropland that can improve soil physical, chemical, and biological properties. Furthermore, using manure as an organic source of nutrients can help reduce commercial fertilizer inputs. In the U.S., about 15.8 million acres of cropland are fertilized with manure, equivalent to 5% of all the country's cropland. Challenges associated with manure utilization on cropland span social concerns – such as community perceptions relative to odor and water pollution – to logistical concerns, like transportation costs and variability of nutrients in different types of manure (USDA, 2009).

Encouraging crop farmers to value animal manure as a source of crop nutrients and soil quality improvement would benefit the economy of livestock producers by expanding the value of manure. Manure management may constitute an effective strategy to attain a circular economy model for animal producers (Toop *et al.*, 2017). Contrary to the traditional “linear economy”, based on a ‘take, make, dispose’ model of production, a circular economy aims to eliminate waste and pollution by recycling nutrients into the production chain (Ward *et al.*, 2016). This approach to agricultural waste utilization has proven to benefit communities by solving environmental issues caused by livestock production, contributing to the local economies through reduced expenses in crop fertilization and, in some cases, reducing dependence on non-renewable energies by creating bioenergy and biogas (Candido *et al.*, 2022; Åkerman, *et al.*, 2020).

Demonstrating the economic value of manure use on cropland could increase the adoption of the practice of utilizing manure in crop fertility planning (Waithaka *et al.*, 2007).

1.4. Manure and Soil Health

The use of animal manure as a fertilizer has been practiced for centuries. The extent of the effects of manure on soil depends on the initial state of the soil, manure characteristics, application methods, and environmental factors (Pahla *et al.*, 2013; van Es *et al.*, 2006). A common perception related to manure application is that manure increases SOM concentration regardless of the source of the manure. Increasing the concentrations of SOM and soil organic carbon (SOC) in a soil depends greatly on the source of manure, namely the species from which it originates and the presence or absence of bedding in the manure. In general, SOC is related to N mineralization due to the microbial activity in the soils that decompose organic matter using carbon as a source of energy. When the soil organisms consume carbon, SOC proportions are diminished due to microbial respiration (Rayne & Aula, 2020), which contributes to transforming organic N to plant-available N.

Manure nutrients also have an impact on crop quality. Miner *et al.*, (2020) demonstrated that the combination of manure N and inorganic N increases grain N concentration and manure alone can increase concentrations of P, K, and magnesium (Mg) in grain, which are important elements for animal nutrition. Besides the alteration of C and N chemical fractions, enzyme activity in soil has been increased by 26.8% and total fungi and bacteria concentrations also reflect higher ratios with the addition of dairy manure compared to inorganic fertilizer applied at planting (Ozlu *et al.*, 2019). Highly

erodible acidic soils have experienced improved yields, and increased pH, organic C, and total and available N, P, and K concentrations with the addition of manure (Zhong *et al.*, 2010). Further demonstrated by these researchers, the combination of manure and inorganic fertilizers is positively correlated to microbial functional diversity.

Long-term manure application can positively impact soil physical properties, like aggregate stability, available water holding capacity, and macro porosity (Iqbal *et al.*, 2014). Multiple studies demonstrate that repeatedly applying manure for multiple years, with the combination of other practices, like inorganic fertilizers, no-till, cover crops, and residue management, reduces soil bulk density and improves soil physical properties. Short-term benefits are more associated with chemical properties and crop yields (Romano *et al.*, 2017). Some negative impacts of manure application also exist, particularly elevated soil salinity and sodicity, especially from pig and poultry manure applications. Reduced soil structural stability and infiltration has been demonstrated on the days immediately following application (Goldberg *et al.*, 2020; Li-Xian *et al.*, 2007). Proper application of manure, according to best management practices and in compliance with regulatory requirements, can reduce environmental risks associated with manure application.

The state of Nebraska regulates the management of manure by operators of animal feeding operations and provides additional protocols for manure management on animal feeding operations in Title 130 (NDEQ). Briefly, permitted concentrated animal feeding operations (CAFOs) must develop and implement a nutrient management plan (NMP) with associated manure management reporting requirements on an annual basis. Application of manure must adhere to specified manure application setback distances

from surface water conveyances: 30.5 meters for large, or 10.7 m with a vegetated buffer of this width placed between the field and waterway; and 9.1 meters for small and medium animal feeding operations (AFOs). Stored manure, including stockpiles, need to be managed such that discharges of manure-contaminated runoff do not reach waters of the State. Inspections of permitted CAFOs are performed regularly by NDEE officers, and non-permitted operations can be designated as CAFOs and required to apply for a permit if manure is not properly managed. These regulations are promulgated by the state in accordance with regulations published by the U.S. Environmental Protection Agency (EPA) under the federal Clean Water Act (40 CFR 122, 2011) to protect surface water quality.

Nationwide, efforts to preserve and improve soil quality are aimed at reducing pollution transport to water from non-point sources like cropland. Federal institutions often create manuals to manage soils based on texture and class and create recommendations for land management based on erosion. However, soil quality assessment requires a holistic understanding of the soil components and how to interpret and address the characteristics of soil health. Soil quality and education must be aimed to provide a better understanding and awareness of soil resources as dynamic systems with inherent characteristics given by their geology, but also transformed and defined by biological, chemical, and physical properties that are sensitive to changes in land management (Karlen *et al.*, 2003). Achieving this level of soil literacy poses many challenges to the educational and scientific community to create tools that can be used by producers to monitor their soil health.

The barriers to implementing new methods for soil evaluation were addressed by Ditzler & Tugel (2002). Their approach included the implementation of soil quality evaluation cards that included a set of questions and indicators that reflect soil conditions and practices according to the farmers' and scientists' analytical perspectives of the described conditions. Additionally, they evaluated a soil quality test kit guide that included a description of tests performed on soil and practical interpretations of results relative to crop production. Despite the promising reviews given by the farmers who went through this study, neither of the two strategies have been fully adopted by farmers. Some of the concerns regarding the adoption include the time-consuming trainings to educate farmers and extension agents on the use of alternative soil quality tests.

Some environmental quality assessment methods can be adapted for use in crop production with relatively inexpensive tools. However, the interpretation of results remains a challenge. Earthworm and collembolan avoidance tests, for example, were used by Natal *et al.* (2009) to assess heavy metal contamination in areas near mines. Because of the sensitivity of soil arthropods to nutrient content in the soil and physical disturbance, studying soil arthropods has become a tool to monitor changes in soil properties that is relatively inexpensive and easy to interpret, depending on the depth of the identification of the specimens that are isolated.

More in-depth studies of soil organisms are available through commercial laboratories and educational institutions. Soil tests specialized in assessing soil biological quality are also available in some commercial laboratories. The Haney test is a popular method that provides a more thorough inspection of nutrient content in the soil than traditional soil tests. The Haney test includes a "Soil Health Calculation" that combines

different measurements of the soil's biological properties, including microbial respiration, C:N ratio, and its interaction with microbial biomass. A score from 0 to 50 is assigned to describe the overall state of the soil and an estimated value for the soil nutrients and the amount of nutrients available for the next year are provided (Sullivan, 2015). The cost of a Haney test, on average, is double the price of a routine soil analysis, making it less appealing to farmers. The applicability of the Haney test to determine economically optimum N rate has not always proved to be successful (Yost *et al.*, 2018). Managing N inputs on corn fields from a soil health approach indicated by a Haney test is more challenging than the traditional soil test approach because of the disconnection between soil biological properties from crop production.

Other tests, like the phospholipid fatty acid (PLFA) evaluation, distinguish bacteria, fungi, protozoa, nematodes, earthworms, and other important organisms in the soil in a more specific manner than the Haney test (Brinton, 2020). According to Frostegård *et al.* (2011), PLFA laboratory reports include the total mass of organisms belonging to each functional group within a soil sample and brief guide to interpret results. Some problems with the interpretation of PLFA relate to the complexity of the results provided by this test. The microbial communities identified by this test need further evaluation to determine the applicability of the results into the context. A first insight into the results allows the user to observe changes in microbial communities caused by an agricultural practice; however, determining the diversity of organisms requires further analysis. Additionally, a PLFA test analyzes degradation rates of organisms based on the respiration produced after killing the microorganisms in the soils. Results may be misleading because the process of killing the microorganisms of

interest may also kill others that are not intended to be studied. Furthermore, studies that aim to determine soil diversity based on PLFA are inaccurate, as this method does not reflect a good sample size to be evaluated on diversity and richness tests like Shannon or Evenness tests. RNA tests for microbial populations have also been used to determine ecosystem functions of microbial populations in the soil. The 16S rRNA test provides more detailed information than the PLFA tests, though both tools provide information about meaningful changes in bacterial communities (Orwin *et al.*, 2018).

The role of microbial populations and diversity is often analyzed under the assumption that organic practices provide more benefits to microbial populations, but the implementation of these tests under conventional farming practices with the addition of some organic fertilizers is a more challenging task. Genetic tests on microbial communities have also been implemented to observe changes in functional activity of soil organisms, mainly targeted to comparing organic management practices versus conventional. Results demonstrate that management practices rather than soil texture have a greater effect on functional gene abundance and diversity of microorganisms (Reeve *et al.*, 2010; Holland & Coleman, 1987).

The current methods for estimating microbial activity, richness, and diversity of soils under different management practices have provided scientists with valuable information about best practices to improve soil health. However, the implementation of these methods and indicators on a field setting by producers is a major challenge that still needs to be addressed. Although some soil tests like Haney or PLFA are offered through some commercial laboratories, more outreach opportunities are needed to provide land

managers with the tools to interpret the results correctly and consider soil biological properties as a priority in their management plans.

1.5. Connections Between Soil Health and Water Quality

Healthy soils have a role in preventing surface and ground water pollution by reducing the risks of leaching and sediment erosion that may carry nutrients, pesticides, sediments, salts, and trace elements (NRCS, 1993). Changes in land use often shape landscapes and alter the hydrologic properties of the soil. The capacity of the soil to retain excess water decreases when soils are poorly aggregated and impermeabilized as a consequence of reduced surface organic matter (OM). These conditions create higher risks of floods in extreme rainfall events and rapid movement of nutrients through soil profiles and across the surface into freshwater sources (Shortle & Abler, 2001).

1.5.1. Runoff and Water Quality

Phosphorus runoff is one of the main pollutant concerns associated with livestock manure due to its role in eutrophication of surface waters. The use of cover crops plays a key role in controlling excess P in the soil (Kleinman *et al.*, 2005). Despite the concerns of excess P on manure application fields, if managed correctly, manure can help restore degraded soils by increasing organic matter and the formation of aggregates, these characteristics stimulate C and N cycles through the increased microbial activity and accelerate crop growth, which in turn, might reduce P runoff from the application site (Ojeda *et al.*, 2003). To address the environmental concerns of P runoff, the Phosphorus Index is a risk assessment tool that evaluates land and soil characteristics to assign a rating to cropland based on potential for P losses to surface water. When the P index is very low to medium, manure application can be managed from a N-based rate (Wortman

et al., 2013). The main factors included in the Nebraska P-index are key for any nutrient runoff risk; ground cover, irrigation, manure credits, and dominant land form are some of the most important aspects to consider when managing nutrient runoff from agricultural land.

Excess of nitrate and P in surface waters commonly reduces concentrations of nutrients in the water by stimulating vegetative growth, however, when the vegetative biomass dies and decomposes dissolved oxygen in the water is depleted, resulting in lack of oxygen for aquatic flora and fauna (Wortman *et al.*, 2013). In eastern Nebraska eutrophication and algal blooms are mainly attributed to storm water draining from cropland, despite the lack of point waste sources such as waste-water effluents or runoff from feedlots (Hammer & Hergemader, 1973), the impact of a poorly managed nutrient program and soil structure increases the risks of nutrient spread into freshwater. Proper storing and processing techniques of manure destined for field application and efficient animal diets significantly reduces the impacts of P runoff in soils (Vadas *et al.*, 2004). Soil conservation practices that aim to preserve the soil structure and aggregate stability and the addition of organic materials has the potential to reduce the risk of nutrient runoff into water sources (Han *et al.*, 2018).

1.5.2. Nutrient Leaching

Similarly, protecting groundwater from high nitrate concentrations is critical in agricultural fields. Nitrate contamination generally occurs in irrigated areas with coarse textured soils, especially when the soil surface is within 15 m of the water table. Soil fertilization poses a threat of nutrient leaching in agricultural soils when application rates exceed plant demand. Jiao *et al.* (2004) report that soils receiving inorganic fertilizers had

70% more nitrate concentrations than soils fertilized with organic fertilizers; however, nutrient leaching is attributed to a combination of fertilization practices, tillage, and biological processes within the soil, regardless of the source of nutrients. Bender & van der Heijden (2014) reported that soil biological activity reduces soil leaching, while increasing crop yields because of the efficient transformation and consumption of nutrient by soil biota. Achieving such efficiency requires that land is managed under conservation practices like reduced tillage and crop rotation.

The awareness of soil conditions facilitates the process of determining the fate, transport, and microbiological transformations of applied fertilizers in the soil. Clay content, soil aggregation, microbial activity, and chemical content of soil impact plant nutrient uptake and movement of nutrients through the soil profile (Saka *et al.*, 2003).

1.5.3. Soil Erosion

Soil erosion refers to the gradual process of soil deterioration resulting from water or wind detachment and removal of soil particles. Soil erosion is a serious threat to food production because agricultural soils degrade 10 to 40 times faster than their capacity to recover. In agricultural land in the U.S., soil formation from parent material ranges from 0.5 to 1 t/ha/year (Pimentel & Burgess, 2013). While soil degradation is induced by wind or water, agricultural practices and land use greatly impact erosion (Hurni *et al.*, 2008). Soil erosion impacts local economies by reducing crop yields and land availability for crop production. Conservation practices, like no-till and cover crops, reduce the risk of erosion and runoff over time (Klik & Eitzinger, 2010). Water issues associated with soil erosion relate to sediment concentrations in runoff from eroded land (Schepers *et al.*, 1985). The lack of soil structure and high nutrient concentrations threaten crop

production by reducing soil fertility due to soil loss, and threaten water quality with the transport of sediment and nutrients to surface waters.

Farmers often choose commercial fertilizers over manure because of the variability and availability of nutrient content in manure. Although estimates for nutrient content can be found in literature, the variability due to animal diet, weather conditions, storage facilities or equipment deserves extra attention. Manure samples on the days before application can provide accurate information on the nutrient content (Lorimor *et al.*, 2004), which can be used to inform manure application rates.

Testing of cropland soils prior to manure and nutrient application is practiced by many crop producers to obtain information relevant to nutrient application to meet crop needs. Commonly, soil chemical analyses are recommended to quantify current supplies of nutrients from soil and to understand characteristics like pH and CEC. While soil chemical analyses can be informative to farmers, the frequency at which testing is performed can be limited by cost, as sampling is often performed by crop consultants or agronomists and samples must be sent to commercial laboratories for analysis (Pagaria & Kendra, 2011). Additionally, farmers commonly leave soil test result interpretation to their agronomist and only a portion of them incorporate the suggestions of their agronomist into their nutrient plan (Bruyn, 2019). Farmers' proficiency in addressing soil health issues is a determining factor in adopting different management practices and soil testing. Often, farmers do not perceive the benefits of obtaining soil tests (HongYun & ZengXu, 2011). The observation of soil biological properties through different methods could be an inexpensive tool to assess and monitor soil health changes that can be easily interpreted by farmers. In this thesis, a novel method for quantifying biological activity as

a measure of soil health is developed and used to assess the impacts on soil biology of manure and cedar mulch as organic soil amendments.

1.6. Objectives of the Study

- a. Evaluate a novel method for quantifying soil biological activity using cotton fabric squares to assess carbon degradation potential by soil microbes.
- b. Quantify the effects of organic soil amendments on soil biological activity using a quantitative method that demonstrates soil carbon decomposition rates in relation to soil respiration and arthropod abundance and diversity.

CHAPTER 2:
VALIDATION OF A NOVEL METHOD FOR ASSESSING
BIOLOGICAL ACTIVITY OF SOILS BASED ON
COTTON FABRIC DEGRADATION

Karla M. Melgar Velis, Amy Millmier Schmidt, and Mara Zelt

2.1. Abstract

Assessment of soil biological characteristics has traditionally been performed using specialized equipment in a laboratory setting to measure microbial activity. In an effort to motivate implementation of practices among agricultural land managers that are intended to improve soil health and resilience, a simplified and inexpensive in-field method for assessing soil biological activity is desirable. Outreach and extension educators have utilized carbon degradation demonstrations in field settings to demonstrate variations in soil biological activity induced by different management practices, but analytical methods to quantify those differences in degradation are lacking. In this study, pre-measured cotton fabric pieces were enclosed in non-biodegradable mesh bags and buried in soil to a depth of 5 cm under treatments of organic amendments and no amendment at two study sites. At site A, plots with and without beef feedlot manure applied to meet pre-season corn nitrogen needs were established in triplicate with twelve mesh bags containing pre-measured cotton fabric pieces buried in each plot to a depth of 5 cm. Bags were retrieved randomly from each plot every two weeks from September through November 2020. At site B, three treatments were randomly applied to 12 plots. Eight plots received swine manure slurry at a rate of 39,687 L/ha⁻¹ to meet corn nitrogen needs and four of these plots were top-dressed with cedar

woodchips at 22,417 kg/ha⁻¹ (10 tons/ac). The final four plots served as controls and received no amendments. In each plot, six mesh bags containing pre-measured cotton fabric pieces were buried to a depth of 5 cm. Bags were retrieved from each plot monthly from June through October 2021. Retrieved bags from both studies were carefully opened to reveal remaining fabric and percent of fabric degradation was quantified by three methods: manual quantification with an overlaid clear plastic grid, and image assessment using Photoshop and ImageJ software to differentiate areas of light (fabric) and dark (no fabric) pixels. Results demonstrate that image analysis with both Photoshop and ImageJ were effective at quantifying the percentage of fabric degradation with no significant difference in accuracy between the two methods or manual quantification ($p < 0.05$). Results suggest that the use of imaging analysis software may be an acceptable analytical tool for quantitatively assessing degradation of cotton fabric under varying soil conditions with little equipment, training, or cost.

Keywords: soil health, biological activity, carbon degradation, image editing software

2.2. Introduction

Enhancing the productivity and resilience of cropland soil requires an understanding of how the living components of soil contribute to agricultural productivity and environmental quality. Soil conservationists and educators continue to conduct outreach to improve knowledge and perceptions among cropland managers of the research-based practices demonstrated to positively impact soil health and advance the reliability and availability of comprehensive soil health assessments tools. Traditional soil testing and yield data facilitate the understanding of the role of plant nutrients in maximizing crop yield. But comparably fewer tangible methods exist to

incorporate understanding of soil physical properties, such as soil aggregate stability, sorptivity, and nutrient cycling capacity, and biological properties, such as soil arthropod abundance and diversity, soil respiration, and biological activity, into land management decision-making. One of the main limitations for assessing and valuing soil biological properties is the complexity and expense of methods to monitor these characteristics, especially with microbial analyses that require laboratory settings and highly trained scientists. Developing simple and accurate methods to observe how the biological characteristics of soil change under differing management practices may help increase the adoption of recommended soil health best management practices by farmers.

Soil organisms play a vital role in ensuring nutrient availability to crops in agricultural soils. The efficacy of organic fertilizers for crops is highly influenced by the ability of soil organisms to synthesize organic compounds and transform them into inorganic sources that can be utilized by the plants. The concept of soil organisms interacting to create a healthy and diverse soil is often described as the soil food web. Taxonomic groups within the soil food web are often aggregated by feeding guilds, which ultimately determines the roles of the organisms in the soil and their relevance on transforming nutrients and influencing soil structure. However, feeding habits of soil microorganisms requires a complex study of the soil communities, mainly because soil organisms have flexible eating habits that adapt to the availability of food sources (Scheu, 2002). Rather than studying a species-specific diet in the soil, it is important to observe functionality of species, especially when defining their relevance to the nutrient cycle.

Organic matter (OM) content in the soil is an explanatory factor when observing the dynamics in food web composition. One of the most important roles of soil microorganisms is the degradation of plant and animal matter. When these materials are processed, nutrients are released and made available for crops (Balamurugan *et al.*, 2011). Organic matter decomposition is a clear sign of microbial activity and is related to organism functionality. The functionality of microbial communities is affected by stressful events in their environment; for example, extreme hot weather can alter the functionality of a soil community. But diverse and rich soil communities are less affected by these events (Tardy *et al.*, 2014; Yuste *et al.*, 2014; Berg & Bengtsson, 2007).

The concept of organism functionality takes an important role in the process of organic matter decomposition because organisms have different mechanism to consume and transform organic matter. For example, fungi can transform organic nitrogen (N) into inorganic N during organic matter degradation. However, the enzymes that allow fungi species to transform organic matter have different mechanisms to access organic N entrapped in soil organic matter (SOM) (Nicolas *et al.*, 2019). Decomposers in the soil food web (bacteria and fungi) release enzymes into their environment that catalyze organic matter decomposition, which mineralize or partially mineralize N. At the same time, microbial feeding fauna like protozoa and nematodes help regulate microbe population while stimulating nutrient turnover by excreting excess N contained in the organisms on which they feed (Haynes, 2014). Additionally, soil arthropods are key influencers of nutrient mineralization, mainly by distributing organic matter into surrounding soils. For example, burrower arthropods help create tunnels and clumps of soil, creating soil aggregates. OM is stored in these aggregates, and is protected from

changes in the soil and available for consumption of other types of organisms (Schowalter, 2017).

Evaluating the role of soil organisms in the context of crop production is particularly important when organic amendments are being used. Organic fertilizers often enrich microbial populations in the soil and stimulate microbial activity (Jiangwei *et al.*, 2020), so observing the patterns in microbial activity in manure fertilized soils provides information that can help monitor the state of the soil and provide insight on future changes in structural composition driven by microorganisms. However, studying microbial communities often requires complex analysis, microbiological expertise, and specialized equipment or access to a commercial laboratory. Visual demonstrations of organic matter decomposition have been implemented to subjectively illustrate the impacts of land management strategies like tillage, cover crops, or manure application, on soil microbial activity, but a method for numerically quantifying carbon decomposition potential under different land management practices is lacking.

The similarity of cotton fabric to organic matter that “fuels” soil microorganisms allow for this material to be used in soil management experiments. Cotton fabric is composed mainly of cellulose with a smaller proportion of non-cellulosic components such as waxes, pectins, and proteins in the outermost layers of the fabric (Nevell & Zeronian, 1985). The exposure of this material to environmental factors and organisms deteriorates the composition of fabric, mainly by stretching C=O chains derived by microorganism consumption. Plant roots, mycorrhizal fungi, free living soil microbes and arthropods alter carbon decomposition by interacting with bacteria and enzymes (Moore *et al.*, 2015; Gupta *et al.*, 2012). Like cotton fabric, other organic materials, such as plant

residues and animal matter are mainly composed of cellulose (LaRowe & Van Cappellen, 2011). Degradation of these organic materials occurs in a fashion similar to cotton fabric; therefore, testing degradation processes with cotton fabric can simulate organic matter consumption and biological activity in soils. A rapid degradation rate of cotton fabric can be a good indicator of healthy and microbially-diverse soils.

Evaluating organic textiles degradation has been made traditionally under soil-laboratory conditions to evaluate durability of the fabric and resistance to environmental conditions, frequently with the goal to improve landfill management. For example, cotton fabric degradation rates were studied by Li *et al.*, (2010) under controlled laboratory conditions, testing carbon dioxide (CO₂) released under different treatments, including soil burial, composting and enzyme degradation. The samples were evaluated by weighing the fabric pieces over time and comparing results to microscopic images, as well as quantifying the evolution of CO₂ from the samples through the ASTM D 5988-03 method (United States Patent No. D 5988 – 03, N.D.). Soil laboratory conditions were also used to test cotton fabric resistance with the addition of fabric softeners using the ASTM 5988-12 standard for evaluating CO₂ generated during the process of fabric mineralization.

The “soil your undies” experiment gained popularity in recent years to demonstrate microbial activity in soil because of its visual impact on the users. The method involves burying cotton underwear in soil for a period of time and demonstrating the degradation of the fabric by soil microbes upon retrieval from the soil. Extension specialists have successfully used this technique to assess differences in tilled and no-till fields by recording initial and final weights of the fabric as the assessment method for

degradation, creating a measurable impact among farmers and extensionists of the short-term effects of changes in soil biological activity (Voth, 2016). In Australia, this method has been used as an indicator of optimal soil conditions for microbial activity and demonstration of this phenomenon served as a starting point to improve management and irrigation systems in cropland (Hughes, 2021). McDaniel (2017) recognized the importance of a simple, inexpensive biological activity indicator for extension agents and farmers, and the success of the burial of cotton fabric on demonstrations; however, an accurate, scientifically-robust, and applicable decomposition index still remains unexplored. Other organic materials such as tea bags have been used to estimate soil microbial activity (Van Os *et al.*, 2020), and weight measurements remain a useful technique to account for degradation. However, a primary challenge associated with this method is the over estimation of weight due to the accumulation of soil on the buried material.

Smith *et al.* (2021) used scanning electron microscope (SEM) images to understand the degradation behaviour of cotton fabric and discovered that some of the main interactions in fabric degradation were the actions of water absorption by the fabric creating optimal conditions for microorganism growth. Microscopic images are used in the microbiology field, typically, with the use of dyes to distinguish specific tissues from other materials and adjustment of image thresholds and histograms to measure areas of interest in their images (Ong *et al.*, 1996).

Image analysis can be adapted to different settings and can be an inexpensive and simple tool to demonstrate visual degradation of cotton fabric by soil microorganisms.

Image editing software that is widely available for different audiences and freely licensed software with basic operations may be useful to analyze cotton fabric degradation. Some examples include ImageJ, a software with the capacity to analyze particles, detect object edges, manipulate color, and determine areas and sizes (Abramoff *et al.*, 2004). Similarly, Adobe Photoshop offers tools like color threshold adjustment, draw selections on the image, edit colors, and estimate area sizes (Lahm *et al.*, 2010). The potential use of photo analysis tools could provide extension educators and farmers a valuable method to easily quantify cotton fabric degradation as a measure of soil microbial activity under different management techniques and environmental characteristics. This study evaluated ImageJ and Photoshop software applications for accuracy in determining percent of fabric degradation achieved with in-field burial of cotton fabric.

2.3. Materials and Methods

2.3.1. Fabric Sample Preparation

White, 100% cotton fabric cloths (Mainstays Flour Sack Kitchen Towels, Wal-Mart, USA) were cut into 29.21×29.84 cm (871.62 cm^2). One piece of fabric was placed flat inside a non-degradable mesh bag ($48 \text{ cm} \times 48 \text{ cm}$) (Phifer, Charcoal Fiberglass Screen, 116 mesh count per cm^2 , Tuscaloosa, AL, USA) that was then temperature sealed along all borders.

2.3.2. Study Sites

Two experiments were performed in field settings. Site A was at the University of Nebraska-Lincoln Rogers Memorial Farm located 18 km east of Lincoln, Nebraska, having silty clay loam soil. Six fallow plots were established to accommodate three replications of two treatments: beef feedlot manure applied at $22,417 \text{ kg/ha}$ (10 t/ac) and

control plots with no amendment (Figure 2.1) on a completely randomized design. Beef feedlot manure was obtained just prior to the plot establishment from a beef finishing feedlot at the University of Nebraska-Lincoln Eastern Nebraska Research and Extension Center near Mead, Nebraska. The manure was collected and stored in 20 L plastic buckets until it was applied. A subsample of the manure was obtained for chemical analysis at a commercial laboratory.

Site B was on a commercial crop farm near Julian, Nebraska having clay loam soil and planted to corn during the 2021 cropping season. Twelve plots were established on a completely randomized block design, representing four replicates of three treatments: control (CON), swine slurry (SS) applied to meet pre-season nitrogen needs of corn at a rate of 39,700 L/ha⁻¹, and swine slurry followed by top-dressing with cedar woodchips (SSW) hand applied to a rate of 22,500 kg/ha⁻¹ (Figure 2.2). Swine slurry was collected from a manure slurry storage on the property with a sample analyzed by a commercial laboratory to determine the appropriate manure application rate.

2.3.3. Treatment Applications and Sample Collection

At Site A, twelve bags were placed in each plot by hand excavating areas of soil approximately 30 x 30 cm to a depth of 5 cm. Each excavated area was sufficient to accommodate one fabric sample with the edges of the mesh bag left exposed for ease of retrieval. Bags were covered with 5 cm of soil or 5 cm of beef manure (Figure 2.3). One bag was randomly retrieved from each plot weekly from September through November 2020 for analysis.

At site B, swine slurry was surface applied on May 13, 2021. On May 27, 2021, five bags containing cotton fabric were placed between corn rows in each plot following

the procedure described for site A. On the same, woodchips were hand applied on the four SSW plots directly over the bags. One bag was retrieved randomly from each plot every two to three weeks from June to October of 2021.

2.3.4. Fabric Sample Collection

Upon retrieval, bags were transported to the University of Nebraska-Lincoln East Campus for analysis. For each fabric sample, soil was gently removed from the surface of the mesh bags and one layer of the mesh bags was carefully removed to expose the fabric sample within. Excess mesh material surrounding the remaining cotton fabric was cut away. When both layers of the mesh had remains of the cotton fabric, both pieces were maintained for analysis.

2.3.5. Degradation Estimation

A subset of the total fabric samples (72 total samples at site A, and 60 at site B) from each study site were randomly selected for degradation analysis (n=7 from site A; n=43 from site B).

Manual Quantification of Degradation

Manual evaluation of percent fabric degradation for each sample was performed by overlaying a clear plastic grid (Figure 2.4.), with primary graduations (darker lines) of 2.54 cm and secondary graduations (lighter lines) of 6.4 mm, on fabric samples and counting grid squares that were void of fabric. The total area void of fabric was divided by the original dimensions of the fabric sample and multiplied by 100 to yield a percentage of fabric degradation.

Photographs of the fabric were taken with an iPad A1701 (Apple Inc., Cupertino, CA, USA) mounted on a tripod and having a camera resolution of 12 MP. Fabric samples

were each placed in a premeasured area of 29.21×29.84 cm on a black surface and photographed for image analysis (Figure 2.5). Supplemental lighting was used to optimize photograph quality.

Adobe Photoshop Quantification of Degradation

Each photograph was edited using Adobe Photoshop 2020, (Adobe, San Jose, CA, USA). Briefly, the pre-marked area in which the fabric was placed during photograph capture was selected using the lasso polygonal tool and the number of pixels of the selected area obtained using the histogram tool was recorded as the initial area of the fabric to compare to the remaining pieces. Next, the areas of the fabric that were stained with soil were edited with the paint bucket tool or dodge tool to highlight the colors and imitate the original color of the fabric. The black background of the image was edited in locations where light caused a white reflection using the color burn tool or paint bucket to color these areas black. Following edits, a new selection was made using the color range of the picture, which selected the sections of the fabric remaining inside the 871.62 cm^2 rectangle. The fuzziness and range of the picture was adjusted to ensure a proper selection of the fabric to reduce noise caused by stains or blurriness. The quantity of pixels associated with remaining fabric pieces was recorded and subtracted from the initial area selection to determine the percentage of fabric degradation.

ImageJ Quantification of Degradation

Each photograph of fabric samples from Site B were analyzed with the free license program ImageJ (ImageJ with Java 1.8.0_172). Briefly, each image was opened in the program and initial fabric area (871.62 cm^2) was used to set a scale for measurement. The area of interest in the image was then selected with the “rectangle” tool and

duplicated for further processing. On the main menu, the image was converted to black and white with the 8-bit image selection tool. Brightness and contrast were adjusted as needed. The threshold of the image was adjusted and posteriorly measured using the “analyze” option. The “analyze particles” tools was used to provide an estimate of the area of remaining fabric and the percentage of degradation was estimated by subtracting this value from the initial area of the fabric piece.

2.3.6. Statistical Analysis

The degradation percentage obtained from each sample of cotton fabric was evaluated using R Studio. The differences in percent degradation of each sample provided by each of the three analysis methods were compared. Mean percent degradation by analysis methods were compared through an ANOVA test to identify significant differences among the area estimation methods with $\alpha=0.05$. Linear regression equations were developed to compare Photoshop and ImageJ estimations to hand count assessments.

2.4. Results

Percent degradation of selected samples from site A that were assessed by hand count and Photoshop are illustrated in Figure 2.6. While percent degradation estimated by each method agreed very well for 7 of 8 samples, the percent degradation determined with Photoshop for one sample exceeded the hand count value by 12.1%. Nevertheless, no statistical difference between mean percent degradation by method were found ($p<0.05$) (Table 2.1.).

Results for percent degradation of samples from site B that were determined by hand count, Photoshop, and ImageJ are illustrated in Figure 2.7. Again, percent

degradation estimated by each method agreed very well for the majority of samples. A maximum variability of 8.3% between Photoshop and hand counts was observed in one sample, and ImageJ estimated degradation to be 12.78% higher than hand count in one sample. As with site A results, no statistical differences in mean degradation of samples among the three methods were found ($p < 0.05$) (Table 2.2.).

Simple linear regression analysis was performed on data sets to further compare percent degradation assessed by the imaging methods to the percent degradation quantified by manual hand count. A line of best fit was generated by the least squares method for each data set. The model for Photoshop as a predictor of hand counts (Figure 2.8) had a slope of 0.99648, a standard error of 0.01604. The R^2 value of the best fit line for this data set was 0.995, indicating minimal variability in the percent degradation predicted by Photoshop and the percent degradation quantified by hand counting.

The line of best fit generated for percent degradation predicted by ImageJ compared to hand counts had a slope of 1.009165, a standard error of 0.014579, and an associated 95% confidence interval (0.98, 1.036). For this method, the R^2 value of the best fit line was 0.995, again indicating minimal variability in the percent degradation predicted by ImageJ and the percent degradation quantified by hand counting. Linear models for both image analysis methods had intercepts not statistically different from 0, thus the intercept was left out of both models. Based on R^2 values of at least 0.99 for each of the regression models, over 99% of the variation in degradation estimation by hand counting is accounted for using either of the image analysis applications.

2.5. Discussion

Hand counts were used as a reference to determine the accuracy of two photo analysis applications for quantifying percent fabric degradation. Percent degradation of fabric analyzed using Photoshop, ImageJ, or hand counts were not different ($p < 0.05$) in the sample sets analyzed. Although not statistically different, some minor variations in percent degradation by each of the methods in comparison to the manual hand count method were observed. While little potential for error exists in performing the degradation assessment using the hand count method, it is possible that an error in counting or recording data was the cause for these variations since such variations occurred for less than one percent of samples. Angle of observation by the person performing the manual hand count, lighting, and individual subjectivity may explain these differences. Spatial grid counting was initially developed for use with microscopic image sections, used mainly for estimating surface area, especially for arbitrary shapes. But “operator variance” has been identified as one of the main drivers of variability when using a grid method since different users can under- or over-estimate area by using their own criteria (Howard & Sandau, 1991). Establishing a standard method for accounting area is critical to minimizing variability among operators (Sugihara & May, 1990). Other concerns when using a grid method include the size of the grid gradations and the relative area, shape, and spatial arrangement of the component being measured. Although grid count is a simple and inexpensive approach, there are different grid shapes that need to be evaluated before selecting the most appropriate distribution of measuring points. Some common grid structures include rectangular grids, triangular grids, at symmetric locations or equally spaced dots (Hally, 1964). According to Terry & Chilingar, (1955) and Folk, (1951), preliminary stages for implementing the grid method should include a period of

trial of different grid arrangements and training of the users. Providing opportunities for inexperienced users to assess areas using irregular figures cut into a paper of a known area could allow users to improve their accuracy and allow the researcher to account for variability among users.

A more consistent estimation of percent degradation among assessment methods was observed for site B, potentially a result of a much larger sample size ($n=43$) compared to site A ($n=8$). A linear regression with both methods of estimation (ImageJ and Photoshop) compared to hand counts confirmed the accuracy of both methods in determining percent degradation.

Image based area estimation is a relatively simple tool that allows the user to obtain area measurements with accuracy in less time than manual hand counting. Some limitations for using image analysis in this way may still exist and are usually related to subjectivity. Sabliov *et al.* (2002) report some over estimation of surface area of three-dimensional objects by Photoshop due to high threshold values used in the photographs. Previous studies made on plant disease severity on leaves comparing human eye abilities and photographic analysis usually agree that human eye tends to overestimate disease severity (Liang & Greene, 2018), while photographic analysis generally provides more accuracy to determine infected areas on plant leaves (Kwak *et al.*, 2005). Additional studies demonstrate that photographic editing software provides more accurate results as the area of degradation increases (O'Neal, Landis, & Isaacs, 2002). Automated image processing techniques have been implemented in the past for estimating areas and separating colors on images. Some studies have incorporated the use of cellphone apps like BioLeaf to determine color areas in a picture with similar accuracy of data to ImageJ

(Ullah *et al.*, 2020); however, complex attenuation spectra is commonly found on image analysis, which makes it more difficult for software to return accurate results in terms of area and color separation. An effective technique to address this issue is to manually select and edit areas of interest on the image and edit the color hue, saturation, and luminosity or delimit boundaries on the picture (Lehr *et al.*, 1999).

Although image editing software is a simple and available tool for users, subjectivity is still a problem to be addressed, especially on irregular shapes and textures. Area selection by color segmentation is one of the fastest ways to select areas on a photograph; however, burying white cloths in soils leaves stains and fungal growth on the fabric that must be sorted out by the user's criteria to avoid it being mistaken for degraded areas in the picture. Using microscopic images can solve the problem of separating colors and shapes with the software; however, on bigger materials like the cotton fabric, increasing image resolution could help differentiate colors in the fabric (Zhang *et al.*, 2014).

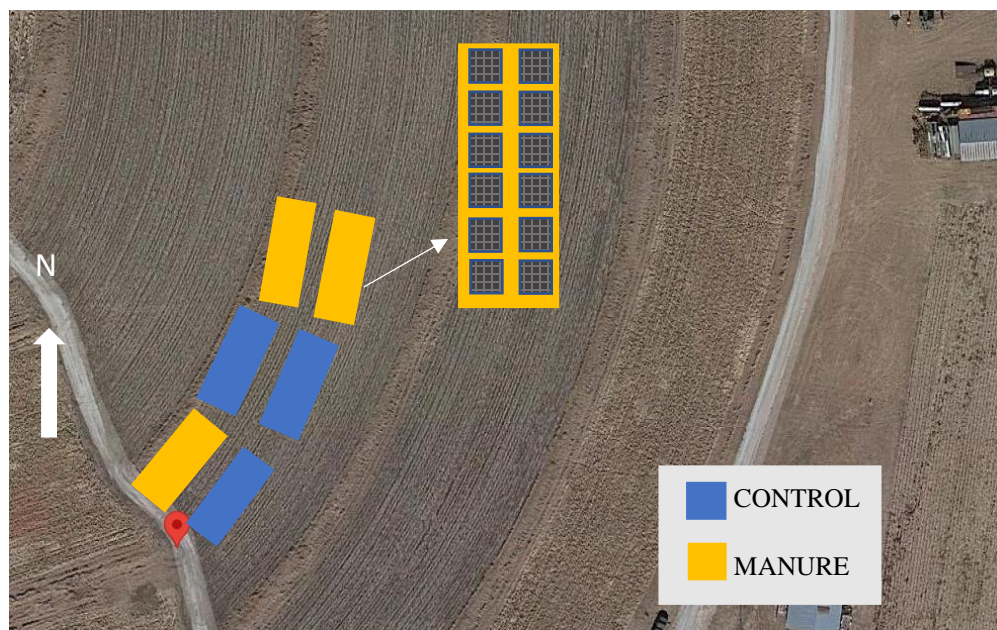
Tables and Figures

Figure 2.1. Study design at site A

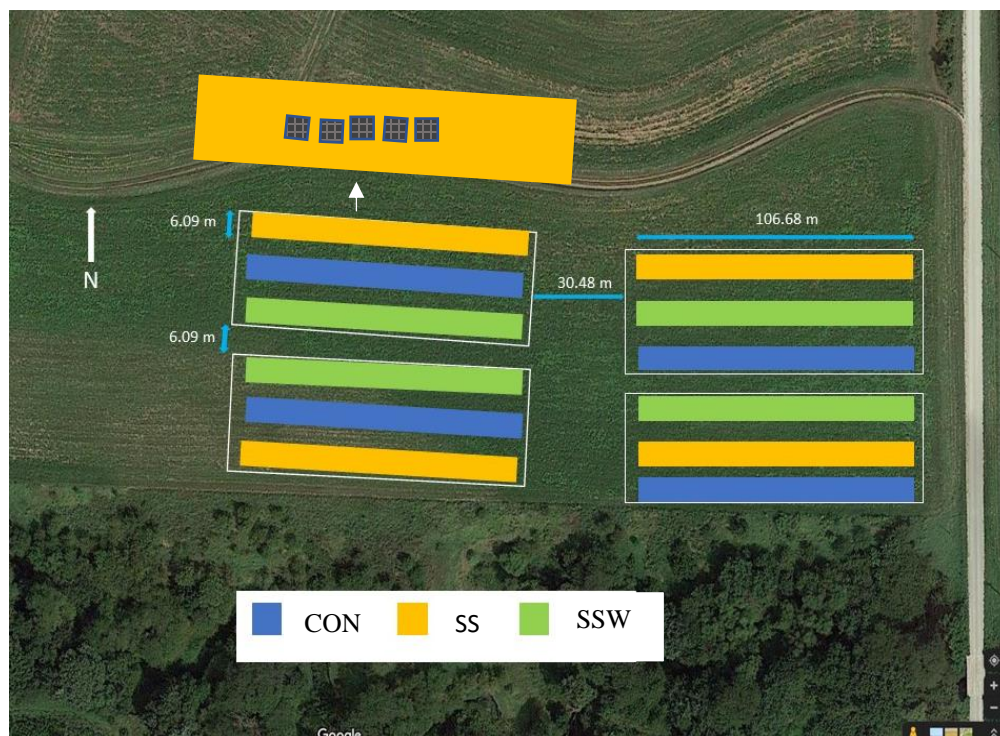


Figure 2.2. Study design at site B. CON=control, SS=swine slurry, SSW=swine slurry and woodchips



Figure 2.3. Fabric and mesh bag burial in research plots

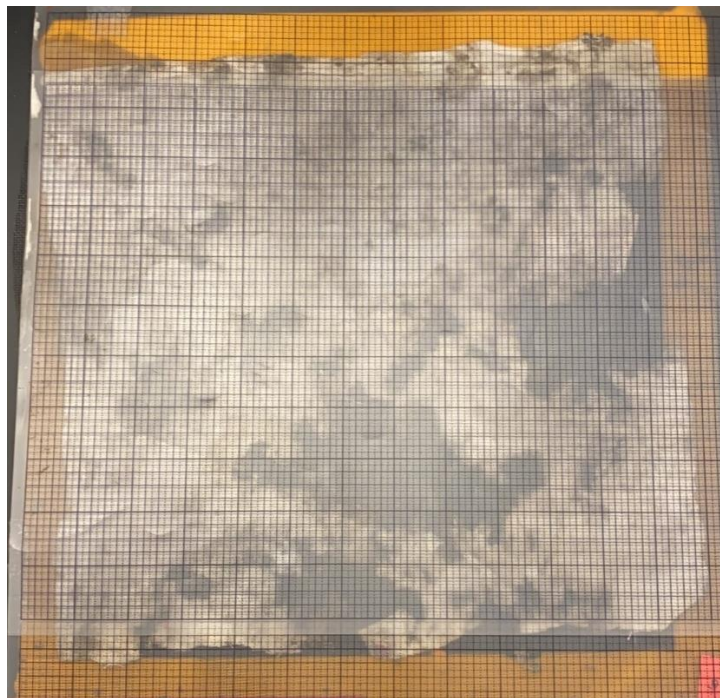


Figure 2.4. Grid overlayed on fabric sample for manual evaluation of percent fabric degradation

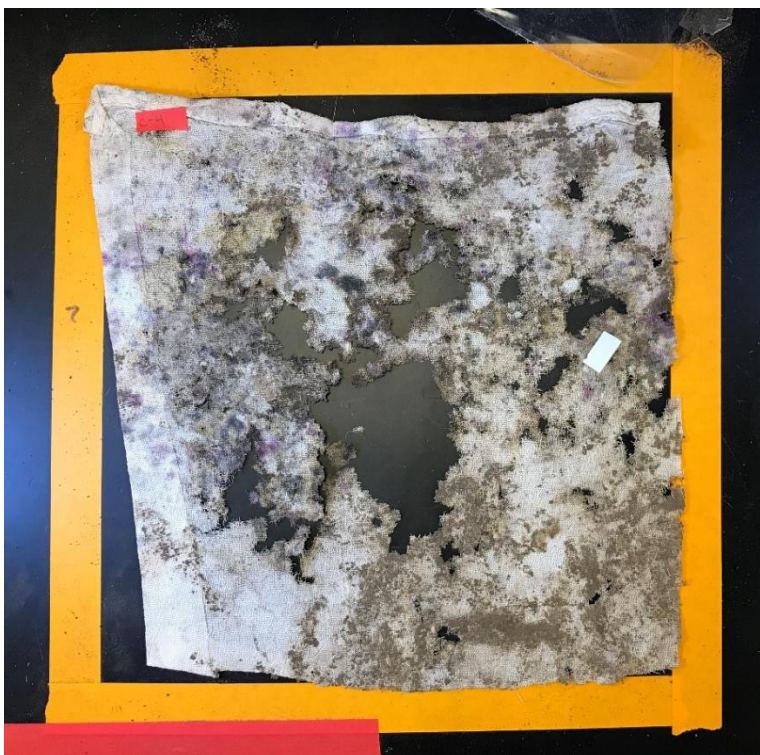


Figure 2.5. Fabric sample placement inside pre-measured area (29.21×29.84 cm) for photographing

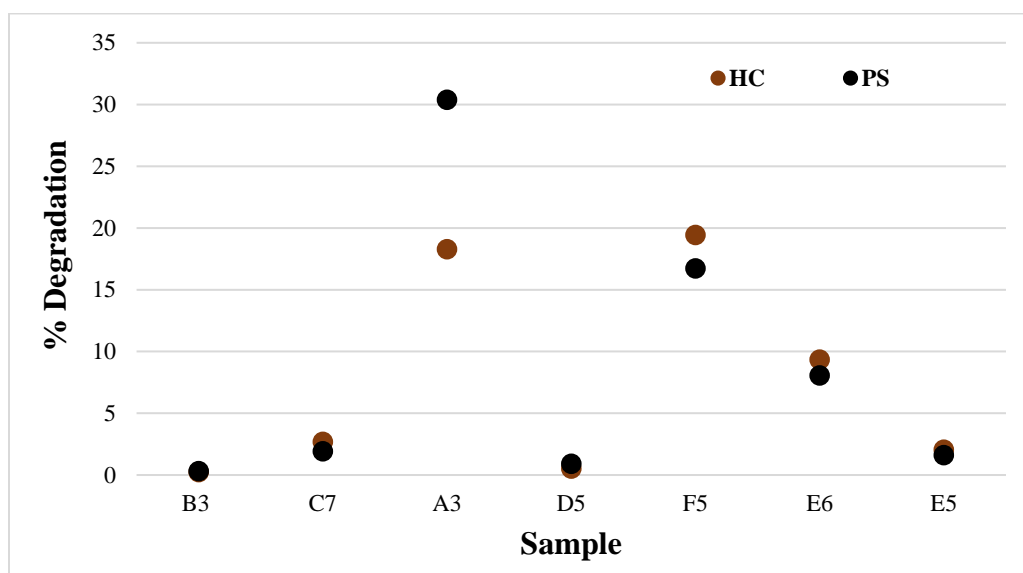


Figure 2.6. Percent degradation of selected fabric samples from site A.

HC=hand count, PS= Photoshop

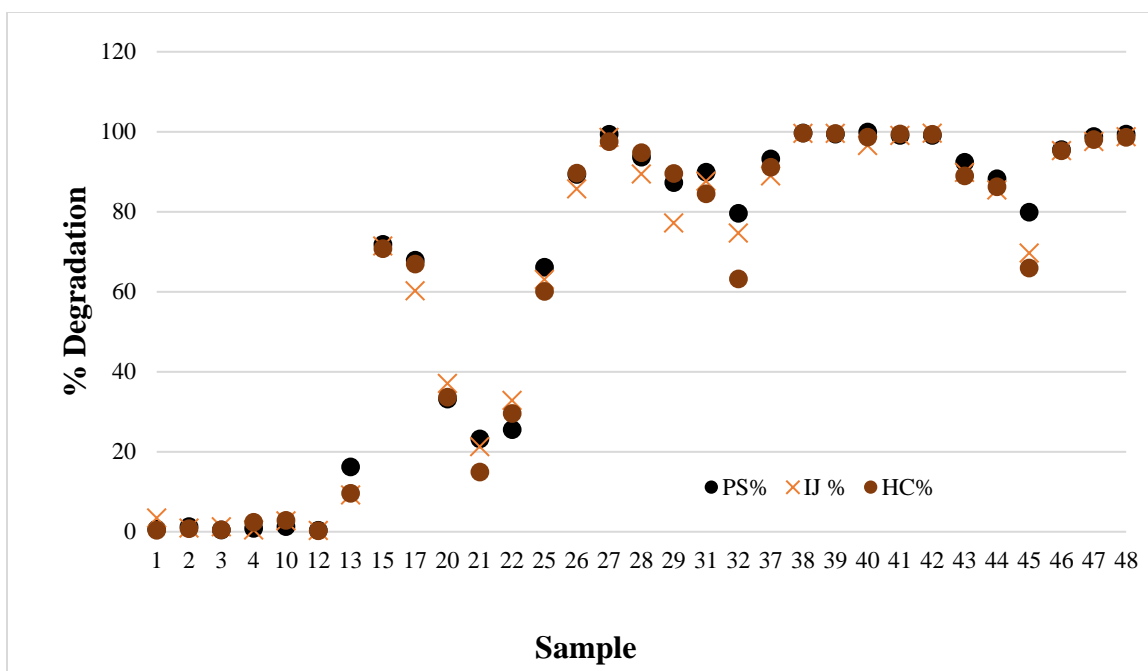


Figure 2.7. Percent degradation of selected fabric samples from site B.

HC= hand count, PS= Photoshop, IJ= ImageJ

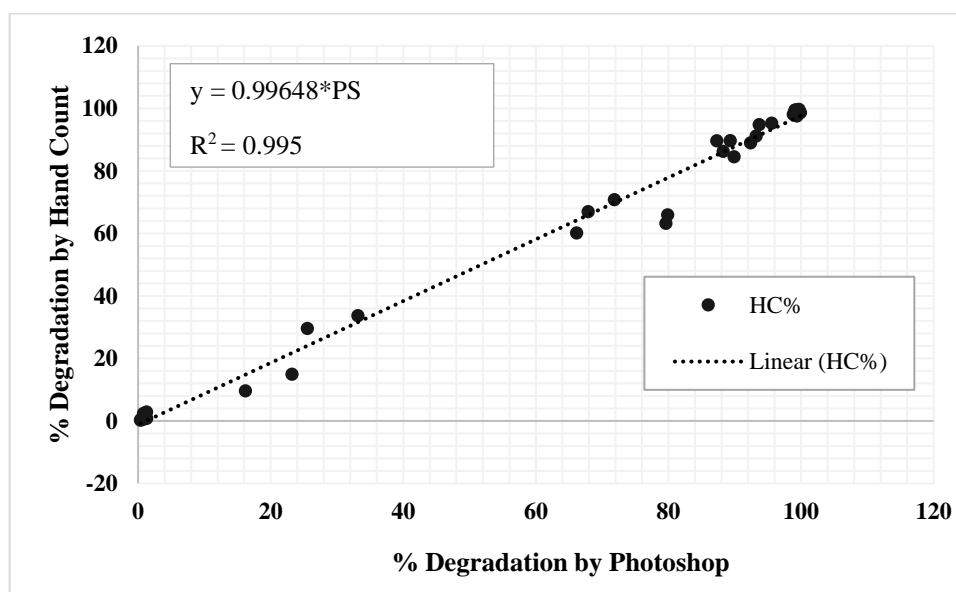


Figure 2.8. Linear regression model for degradation estimation via Photoshop relative to degradation value obtained by hand count

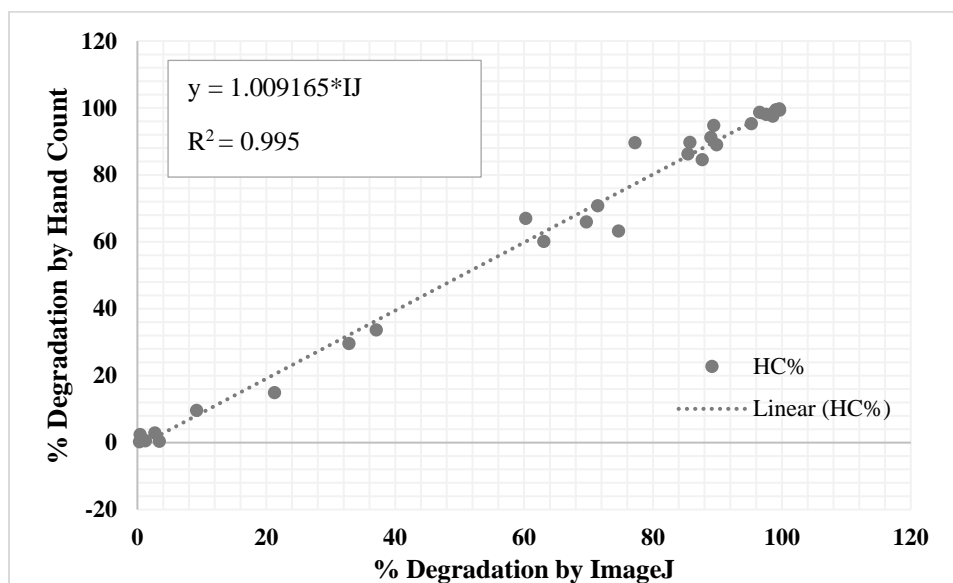


Figure 2.9. Linear regression model for degradation estimation via ImageJ relative to degradation value obtained by hand count

Table 2.1. Percent degradation of selected samples and mean degradation by assessment method for site A

Sample ID	% Degradation	
	Photoshop	Hand count
A3	30.37	18.28
F5	16.72	19.43
E6	8.05	9.34
C7	1.92	2.68
E5	1.60	2.03
D5	1.61	0.52
B3	0.31	0.23
Mean	7.66	6.63
Standard Error	0.000375	0.000375
F-value	0.039	
P-value	0.846	

No significant difference ($p < 0.05$) in percent degradation by assessment method was found.

Table 2.2. Percent degradation of selected samples and mean degradation by assessment method for site B

Record ID	PS%	IJ %	HC%	Record ID	PS%	IJ %	HC%
1	0.67	3.42	0.39	40	99.93	96.56	98.66
2	1.32	0.92	0.79	41	99.1	99.09	99.45
3	0.46	1.24	0.51	42	99.1	99.66	99.37
4	0.84	0.43	2.38	43	92.39	89.87	88.99
10	1.3	2.71	2.88	44	88.26	85.44	86.26
12	0.39	0.33	0.25	45	79.9	69.67	65.94
13	16.2	9.2	9.61	46	95.57	95.28	95.28
15	71.85	71.44	70.78	47	98.83	97.57	98.06
17	67.9	60.25	66.96	48	99.44	98.76	98.61
20	33.17	37.07	33.67	49	98.65	98.59	98.04
21	23.23	21.28	14.93	50	99.35	98.56	98.48
22	25.55	32.84	29.59	51	99.67	99.86	99.77
25	66.16	63.07	60.12	52	99.77	99.75	98.79
26	89.34	85.73	89.68	53	98.86	98.4	98.28
27	99.39	98.6	97.54	54	99.17	99.65	99.65
28	93.68	89.43	94.78	55	89.73	90.49	90.61
29	87.29	77.22	89.61	56	99.7	98.31	97.32
31	89.92	87.64	84.52	57	97.18	94.24	95.5
32	79.62	74.67	63.23	58	99.83	99.78	98.87
37	93.23	88.98	91.16	59	98.92	99.1	98.28
38	99.7	99.61	99.68	60	100	99.7	99.2
39	99.44	99.59	99.59	60	100	99.7	99.2
Mean					64.30	62.50	62.36
Method comparison p-value							
0.671							

PS%= Photoshop degradation percentage estimation, IJ%= ImageJ degradation percentage estimation, HC%= Hand count degradation estimation percentage. No significant differences were found among mean percent degradation by the three evaluation methods ($p < 0.05$).

CHAPTER 3:
ASSESSMENT OF MANURE AND CEDAR MULCH IMPACTS ON
CARBON DEGRADATION, ARTHROPOD BIODIVERSITY, AND
MICROBIAL RESPIRATION IN AGRICULTURAL SOILS

Karla M. Melgar Velis, Amy Millmier Schmidt, and Mara Zelt

Abstract

The benefits to plants and soil of applying organic nutrient amendments on crop fields have been demonstrated by several studies. Soil physical, chemical, and biological properties are positively impacted when organic amendments are used. Motivating crop farmers to utilize manure and other organic amendments to meet a portion of crop nutrient needs prior to importing inorganic fertilizer can help alleviate nutrient imbalances in regions of intensive livestock production. However, few studies have explored the short-term impacts of livestock manure and wood mulch application on soil microbial activity. The impacts of swine slurry (SS) and swine slurry top-dressed with cedar mulch (SSW) on carbon degradation, arthropod populations, and microbial respiration in soil were evaluated during a single growing season. Results demonstrate positive effects of combined applications of SSW, primarily on soil arthropod abundance and QBS. Total abundance of arthropods was higher under SSW treatments and Acari and Collembolans were the most abundant arthropods throughout the study. Soil carbon degradation potential and soil microbial respiration were not significantly affected by the application of treatments. No impact on soil nutrient concentrations was observed.

3.1. Introduction

The concept of a circular economy model focuses on minimizing waste and pollution by utilizing resources in a way that extends their useful life. While many businesses and industries adopt practices that support a circular economy model, agriculture has, in essence, demonstrated this model effectively for hundreds of years. Agricultural livestock and crop production mutually benefit from the use of livestock manure to fertilize crop fields and the subsequent production of crops to feed livestock. However, intensification and specialization of agricultural production systems separates livestock production from crop production, often limiting access by livestock farmers to sufficient land for manure nutrient assimilation. When crop production systems rely solely on inorganic fertilizer inputs to meet crop fertility needs, soil quality can degrade as soil resources are utilized but not replenished. Meeting a portion of crop nutrient needs with manure application helps balance nutrients on a regional basis and can improve soil physical, chemical, and biological properties. Demonstrating the benefits to crop producers of incorporating manure into crop fertility plans to maximize return on investment is critical to the sustainability of both crop and livestock production, particularly in regions of intensive livestock production.

Manure application at agronomic rates improves soil physical and chemical characteristics, with long term applications being responsible for reduced soil bulk density, increased soil organic matter (SOM) concentration, and increased corn biomass yield (Romano *et al.*, 2017). Changes in chemical and physical composition of soils are highly influenced by the stimulation of microbial communities and enzyme activity provoked by manure application. The soil rhizosphere is particularly influenced by the

addition of manure. Increased enzymatic and microbial activity, soil respiration rate, and plant nutrient uptake were demonstrated in the rhizosphere of barley supplemented with pig manure (Liang *et al.*, 2005). Manure application can be an important source of soil organic carbon, which contributes to efficient nutrient cycling in soil (Weyers *et al.*, 2018). Moreover, changes in soil physical properties following manure application can improve soil resistance to erosion, increase porosity, improve infiltration capacity (Arriaga & Lowery, 2003) increase hydraulic conductivity, water stable aggregation, and soil microbial activity, and decrease bulk density (Celik *et al.*, 2010; Guo *et al.*, 2016). But perhaps one of the most convincing approaches to demonstrating the positive impacts of organic amendments on soil is to observe the organisms living within the soil ecosystem.

In addition to an abundance of livestock manure in areas of intensive livestock production, Nebraska and neighboring states in the Great Plains of the United States have experienced environmental, ecological, economic, and social threats with the proliferation of eastern redcedar (*Juniperus virginiana*) trees. While these trees provide wind protection for homes and livestock, habitat for native species, and valuable wood products, their unmediated growth threatens forage production on grasslands, negatively impacting cattle production. Between 2005 and 2010, more than 10,000 hectares (25,000 acres) of Nebraska grasslands experienced new cedar growth that produced a 10% stocking density. As a result of negative impacts caused by cedar tree proliferation, multiple conservation agencies in Nebraska continue to promote sustainable and efficient management of eastern redcedar trees by land owners, along with funding investigations of innovative approaches to utilizing cedar tree waste.

Cedar tree mulch has the potential to be used in agriculture similar to commercial mulch on landscape to retain moisture, suppress weed growth (Kamara *et al.*, 2000), prevent erosion, reduce soil bulk density, improve infiltration, and reduce runoff risk (Omoro & Nair, 1993). The addition of cedar mulch to crop fields could benefit soil health and yields, depending on the application rate (Li R., 2020) and method of application (surface broadcast or incorporated) (Shirish *et al.*, 2013). The application of woodchips on crop fields can also reduce phosphorus (P) concentrations in soils, which could benefit cropland receiving high concentrations of P from some manure types (Li R., 2020). Soil microbiota can benefit from mulch application on crop fields, as well. Rosemeyer *et al.* (2000) evaluated microbial activity induced by mulch addition for its impact on plant disease suppression and found that some diseases might be controlled by the presence of a more abundant and diverse microbial community in mulched plots.

While traditional soil testing and yield data facilitate the understanding of the role of plant nutrients in maximizing crop yield, comparably fewer tangible methods exist to incorporate understanding of soil biological properties, such as soil arthropod abundance and diversity, soil respiration, and biological activity, into land management decision-making. Additionally, the impacts of manure application alone or in combination with woodchips on soil biological factors has received little attention. Arthropods are key organisms residing in the upper layers of soil that can be measured easily and inexpensively (Parisi *et al.*, 2005). Soil arthropods are highly sensitive to alterations in the soil environment (Sapkota *et al.*, 2012) making them an ideal indicator of soil biological status. In particular, arthropods that function as litter transformers contribute to the soil environment by ingesting and breaking down organic matter to forms that are

accessible to other soil organisms that then mineralize those nutrients into plant-available inorganic forms (Muturi *et al.*, 2011). Monitoring the diversity and abundance of soil arthropod communities can reveal the degree of activity within each trophic level of the soil food web. Using a system of classification called the QBS (“Qualità Biologica del Suolo” or biological quality of soil) index, an eco-morphological index (EMI) score is assigned following identification and quantification of arthropod groups. The score is based upon a number of arthropod characteristics, including level of pigmentation, appendage and visual apparatus development, and size, and collectively reflects the degree of soil adaptability of different arthropod taxa (Parisi *et al.*, 2005).

In addition to evaluating soil arthropod communities under various land management practices, visual demonstrations of organic matter decomposition in soil can illustrate changes in soil microbial activity. Organic cotton fabric is similar in structure to soil organic matter that soil microorganisms process as a food source. Composed mainly of cellulose (Nevell & Zeronian, 1985), cotton fabric closely resembles plant residues and other soil organic components that are also largely composed of cellulose (LaRowe & Van Cappellen, 2011). Because degradation of soil organic matter occurs in a fashion similar to cotton fabric, soil degradation processes can be visually demonstrated by monitoring the degradation of cotton fabric placed in the soil.

A third successful indicator of soil microbial activity is soil respiration, which is a measure of the production of carbon dioxide respired by soil organisms. While environmental conditions such as temperature, moisture content, nutrient content, and soil porosity significantly influence rates of soil respiration, so, too, does the availability of organic carbon in the soil which serves as a food source to heterotrophic organisms.

Given the abundance of both manure and woodchips in the Great Plains region, and a scarcity of data reporting the impacts of these organic soil amendments on multiple facets of soil biology, a study was undertaken to identify the impacts of manure and cedar mulch on soil biological activity. Cotton fabric degradation, arthropod diversity and abundance, and soil microbial respiration were measured as indicators of soil health in a southeast Nebraska cropping system.

3.2. Materials and Methods

3.2.1. *Site Description*

Research was conducted on a commercial crop and livestock farm located in Otoe County, Nebraska (Figure 3.1), 1.17 km north of Julian village (40°31'32.3"N 95°52'43.6"W). The predominant soils were Judson silt loam with 2-6% slopes and Nodaway-colo complex. The study was initiated in May of 2021 on a field planted to corn. Minimal tillage was conducted following corn harvest in October of 2020 and no irrigation was applied at this site. Average monthly temperatures and precipitation for this site were obtained from the nearest weather station Schubert Nebraska Weather Station) and are reported in Table 3.1.

3.2.2. *Fabric Sample Preparation*

Cotton fabric was utilized to assess soil microbial activity as a function of fabric degradation over time (Melgar *et al.*, 2021, unpublished data; see Chapter 2). White, 100% cotton fabric cloths (Mainstays Flour Sack Kitchen Towels, Wal-Mart, USA) were cut into 29.21 × 29.84 cm (871.62 cm²) pieces that were each placed flat inside non-degradable mesh bags (48 cm × 48 cm) with 116 mesh count per cm² (Phifer, Charcoal

Fiberglass Screen, Tuscaloosa, AL, USA). Mesh bags were then heat sealed along all borders and tagged for identification.

3.2.3. Plot Establishment and Treatment Applications

Twelve plots were delimited with dimensions of 6.09 m wide by 106.7 m long with a longitudinal buffer 6.09 m wide between plots (Figure 3.2.). Three treatments were randomly applied to plots in quadruplicate: control (CON) having no organic amendments, swine slurry (SS), and swine slurry followed by top-dressing with cedar woodchips (SSW) on a randomized complete block design. For SSW plots, a 3 m × 3 m area was delimited inside each plots following manure application to accommodate hand application of eastern red cedar woodchips.

Swine slurry obtained from the cooperating farmer's swine production system was applied using a Balzer 2600 manure vacuum tank equipped with a splash plate (Balzer, Inc., Mankato, MN, USA), on May 13, 2021 (one week ahead of corn planting) at a rate of 39,687 L-ha⁻¹ to meet pre-season nitrogen needs of corn. Manure application rate was calibrated using three 20-cm tall rain gauges positioned at the center of the applicator travel path and at 1 and 2.13 m each direction from center. Following application of manure, rain gauges were collected and depth of manure in each was recorded (Figure 3.3). The procedure was repeated three times during manure application to the study area and the mean application depth among all rain gauges was taken as the application rate for the study area.

Five mesh bags containing cotton fabric samples were placed between corn rows in each plot by hand excavating areas of soil approximately 30 × 30 cm to a depth of 5

cm. Each excavated area was sufficient to accommodate one fabric sample with the edges of the mesh bags left exposed for each of retrieval. Bags were covered with 5 cm of soil.

Woodchips generated from eastern red cedar tree management activities in north central Nebraska, approximately at 5.3 km northwest of Long Pine, NE were utilized in this study. The material was comprised of chipped bark, limbs, and trunks ranging from 1.5 cm to 13 cm in length, with smaller particles being predominant. Woodchips were hand applied to sub-plots within each of the four SSW plots at a rate of 21.52 Mg ha⁻¹ on May 27, 2021, following placement of fabric samples in plots. Samples of swine slurry and woodchips were collected at the time of application of each for nutrient analysis. Nutrient content and characteristics of woodchips and swine slurry are reported in Table 3.2.

3.2.4. Sample Collection

Carbon Degradation

One mesh bag was randomly retrieved from each plot on days 25, 54, 81, 99, and 128 with day 0 being the date of placement in the plots. The mesh bags were transported to the University of Nebraska-Lincoln upon collection. Soil was gently removed from the surface of the mesh bags prior to one layer of the mesh bags being carefully removed to expose the fabric sample within. Excess mesh material surrounding the remaining cotton fabric was cut away. When both layers of the mesh had remains of the cotton fabric, both pieces were maintained for analysis.

Photographs of the fabric samples were taken with an iPad A1701 (Apple Inc., Cupertino, CA, USA) mounted on a tripod and having a camera resolution of 12 MP.

Fabric samples were each placed in a premeasured area of 29.21×29.84 cm on a black surface. Supplemental lighting was used to optimize photograph quality.

Imaging analysis to determine percent fabric degradation was performed using Adobe Photoshop as described by Melgar *et al.*, 2021 (unpublished). Briefly, the pre-marked area in which the fabric was placed during photograph capture was selected using the lasso polygonal tool and the number of pixels of the selected area obtained using the histogram tool was recorded as the initial area of the fabric to compare to the remaining pieces. Next, the areas of the fabric that were stained with soil were edited with the paint bucket tool or dodge tool to highlight the colors and imitate the original color of the fabric. The black background of the image was edited in locations where light caused a white reflection using the color burn tool or paint bucket to color these areas black. Following edits, a new selection was made using the color range of the picture, which selected the sections of the fabric remaining inside the 871.62 cm^2 rectangle. The fuzziness and range of the picture was adjusted to ensure a proper selection of the fabric to reduce noise caused by stains or blurriness. The quantity of pixels associated with remaining fabric pieces was recorded and subtracted from the initial area selection to determine the percentage of fabric degradation.

Arthropod Collection and Classification

In accordance with the previously defined sampling schedule, approximately 15 soil samples were collected to a depth of 10 cm from each plot using a hand probe of with aw diameter of 3 cm (PN 001 JMC, Clements and Associates Inc., Newton, IA, USA). and composited by plot. The samples were weighed (wet weight) and placed into Berlese-Tullgren funnels following the procedures described by Parisi *et al.*, 2005. Based upon

the principle that arthropods and other soil organisms' dwell in a dark soil environment and will respond negatively to light and heat (MSU, 2006), a light placed above each funnel facilitated gradual heating and drying of the soil to induce downward migration of arthropods away from the heat source and through a mesh screen covering the outlet of each funnel that retains soil but allows the arthropods to pass through. A bottle containing 70% ethanol was placed at the outlet of each funnel to capture and preserve the arthropods for later analysis. Specimens were collected over a period of 7 days.

The QBS method of classification was used to assign EMI scores (Table 3.4) to each group of microarthropods (Parisi *et al.*, 2005). Briefly, specimens from each jar were observed through a Leica EZ4 educational stereomicroscope with 4.4:1 zoom (Leica Microsystems, Wetzlar, Germany) and manually identified and quantified. Arthropods were classified by Order, exclusive of collembolans, which were categorized by Family due to their substantial variation in soil adaptability characteristics. Members of the Order Coleoptera were individually assigned EMI scores based on specific attributes while collembolan and coleopteran EMI scores were assigned according to the methods of Parisi *et al.* (2005). QBS is reported as the sum of the highest EMI scores from each category of arthropod found by treatment on each sampling day. The abundance of arthropods from each edaphic microarthropod group was also recorded.

Soil moisture content was used as a covariate to explain soil QBS. Three levels of moisture content (0-3.3%, 3.4-4% and 4.1-5%) present in the collected samples were randomly selected to test differences in soil QBS.

Microbial Respiration

In accordance with the previously defined sampling schedule, soil samples were collected on May 27, 2020 (day 0) and at 25, 54, 81, 99 and 128 days thereafter using a 3.0 cm diameter PN 001 JMC hand probe (Clements and Associates Inc., Newton, IA, USA). Within each plot, approximately 20 cores were collected at random locations to a depth of 10 cm. Cores were composited by plot, placed in plastic bags (Ziploc Seal Top Bags, SC Johnson), and transported on ice to the University of Nebraska-Lincoln.

In the laboratory, samples were weighed to accommodate three replicate subsamples of 100 g of wet soil from each plot (Grant & Rochette, 1994) in glass jars. Samples were oven-dried at 65°C for at least 24 hours and weighed to obtain the dry weight. A volume of 20 mL of water was added to each sample and gently mixed to achieve 60% water-filled pore space (USDA NRCS). Test tubes containing 5 mL 1N NaOH were placed into each jar to collect CO₂ respired by the soil microbes. Lids were tightly secured to each jar placed in an incubator (Imperial III, Lab-Line) at 25°C for at least 24 hours. Three empty jar glass jars equipped with 5 mL tubes of NaOH were included to capture atmospheric CO₂ during the incubation period.

The tube containing the NaOH solution was removed and placed in a beaker to perform titration following the procedures described by Stotzky (1965) and RARC & JICA (2014). Briefly, two drops of phenolphthalein, which served as an indicator and changed the color of the solution to pink, and 5 ml of BaCl₂ were added to the test tube. A burette was used to slowly add 1N HCl to each test tube until the color changed to clear (endpoint). The amount of HCl added was recorded and the amount of CO₂ was calculated using the following formula:

$$CO_2 = (B - V) \times (NE)$$

Where:

V = volume (ml) of HCl needed to titrate the trap solution to endpoint.

B = volume (ml) of HCl needed to bring samples from the blank jars to the endpoint

N = normality of the acid (ml^{-1})

E = equivalent weight of C in CO_2 -C evolved from jars containing soil samples

Soil moisture content was determined as the difference from initial weight of samples and final weight of samples after being oven-dried at 65°C for a minimum of 4 hours.

Soil Chemical Properties

Soil samples were collected prior to the application of treatments to establish initial soil chemical properties and inform crop nutrient needs. Fifteen soil cores (0 to 10 and 10 to 20 cm depths) were collected at random locations in each plot using a 3.0 cm diameter PN 001 JMC hand probe (Clements and Associates Inc., Newton, IA, USA). Plant residues and other debris were cleared from the soil surface before taking each sample. Cores were composited within each plot according to depth and a single sample from each sampling depth within each plot was sent to a commercial laboratory for analysis of soil organic matter (SOM) concentration, pH (saturated paste, 1:1 soil to water ratio), cation exchange capacity (CEC), soil electrical conductivity (EC) (soluble salts, 1:1 soil to water ratio), nitrate nitrogen, organic nitrogen, available phosphorus, available sulfur, available potassium, available calcium, available magnesium, and available sodium.

3.2.5. *Statistical Analysis*

Statistical analysis was performed using SAS. Carbon fabric degradation was analyzed using a generalized linear mixed model (GLMM) with degradation following a beta distribution using the PROC GLIMMIX procedure. When appropriate, a Tukey test was conducted for multiple comparisons of means with 95% confidence level. Soil respiration, QBS index, EMI scores and soil moisture content were analyzed as a randomized complete block design (RCBD) with repeated measures. The PROC GLIMMIX procedure was used with plot as random effect and day as a split-plot factor. Soil chemical properties (N, P, K, CEC, EC, OM, Mg, Na, and pH) were analyzed as an RCBD using the PROC GLIMMIX procedure from SAS, with sampling depth as a split-plot factor with block as random effect.

3.3. **Results**

Carbon Fabric Degradation

Mean percent degradation of fabric by treatment and time since treatment application (Figure 3.4) reflect similar trends among treatments throughout the study period. Though not significantly different by treatment on any sampling day, percent of degradation was numerically greatest for the SS treatment on days 54 and 81. By day 128, little difference in percent degradation was observed among treatments, with all treatments resulting in nearly 100% degradation of samples.

Arthropod Abundance and Diversity

Select arthropod taxa population densities were analyzed for their response to soil amendment and time since treatment application (Table 3.5). The arthropod groups analyzed were selected based upon their relative abundance compared to other taxa

identified during the categorization process. Mean abundances in SSW plots of Acari (85.5), Araneae (65.23), Collembola (27) and Diplura (3), were significantly higher than CON and SS plots ($p < 0.05$). Furthermore, treatment by day interaction was significant for Acari and Diplura abundance. Differences between treatments are summarized in Table 3.6. Overall arthropod abundance was significantly higher for SSW, though no significant difference was observed between CON and SS.

Significant differences in QBS index were observed among treatments on days 54 and 99 after treatment application (Table 3.6). The SSW treatment had a higher QBS on both days (1350 and 141 for days 54 and 99, respectively) than SS (110 and 135 on days 54 and 99, respectively) and CON (97 and 105 on days 54 and 99, respectively). No significant differences were observed between CON and SS on any of the sampling days. The QBS index among all treatments was similar on day 0 (Figure 3.7), decreased by day 25, and then gradually increased throughout the remainder of the study, though not significantly. In comparing variability in QBS on each sampling day with mean monthly temperature and precipitation at the study site (Table 3.1), QBS appears to decrease between days 0 and 25 as mean air temperature increased from 16.66 to 25.09°C. Toward the end of the growing season, QBS index tended to increase for all treatments to levels similar to the beginning of the season. In October, which is when day 128 of sampling occurred, mean air temperature was 13.88°C, which may have contributed to a more favorable soil environment for arthropods. Mean soil moisture content by treatment and time since treatment application is reported in Table 3.7 and reveals that on days 54 and 99, when the QBS was different ($p < 0.05$) for SSW than SS and CON, soil moisture was significantly greater for SSW (4.16%) than for SS (3.92%) and CON (3.75%). Likewise,

differences in soil moisture content by treatment follow a similar trend on day 99, with mean soil moisture contents of 5.54, 4.38, and 4.77% for SSW, SS, and CON, respectively.

A significant soil moisture by QBS index interaction was observed (Table 3.8). Based on mean soil moisture contents among treatments throughout the study, analysis of QBS among treatments was conducted at three soil moisture content ranges: 3.3 to 4.0, 4.1 to 5.0, and 5.1% and greater. While no differences in QBS are observed at soil moisture content between 3.3 and 4.9%, QBS index among treatments was significantly different at soil moisture contents of 5% and greater, (Table 3.8).

Microbial Respiration

No significant differences ($p < 0.05$) were found among mean CO₂ respiration by treatment in the 24 h following sample collection (Table 3.9). However, treatment by day interaction was significant, with a lower mean CO₂ respiration for CON at day 0.

3.4. Discussion

Cotton Fabric Degradation

Monitoring the rate at which cotton fabric degrades in soil, or simply the total amount of cotton fabric degradation achieved during a period of time, is often used to demonstrate differences in microbial activity among soil types and/or among land management practices. Because the composition of cotton fabric resembles the composition of organic matter in soil, the fabric provides a very visible indication of carbon degradation potential in a soil. Although fabric degradation was not affected by treatment in this study, different theories have been proposed to explain how microbial activity relates to organic matter decomposition and nutrient mineralization in soils.

Trichoderma enzymes are usually responsible for cellulosic degradation and are present in almost all soil types (Vinale *et al.*, n.d.). But the relative importance of microbial action in decomposition is still to be determined because organic matter decomposition is also influenced by climatic and environmental factors like remoistening of dried soils, mechanized soil perturbances, and soil compaction (Van Veen & Kuikman, 1990).

The role of fertilization in stimulating microbial decomposition is also debated. An experiment performed by Sanyal *et al.*, (2021) demonstrated that carbon degradation in soil was subject to P and K concentrations in the soil and less dependent on N suppression. However, high N rates (above 90 kg/ha⁻¹) were very limiting for microbial activity, which was reflected in reduced fabric degradation rates among nutrient amended soils.

Carbon decomposition performed by soil microorganisms is subject to substrate additions to the soil. Depending on the quality of substrate (C/N ratio or labile substance of organic matter added), the microorganisms either degrade these organic compounds or decompose SOM to meet their nutrient needs (Shahbaz *et al.*, 2017). If enough nutrients are available, it is possible that degradation rates of soil amendments occur more slowly than degradation of existing SOM, though environmental factors may still be the predominant driver of carbon degradation. Condrón *et al.*, (2010) suggests that inorganic nitrogen fertilization reduces microbial biomass, but that microbial decomposition of organic matter is tied to the carbon to nutrient balance. When carbon and nutrient content meet the microbial demand, higher rates of decomposition are present. A limiting amount of nutrients results in soil C storage and low degradation, while low C application rates and high nutrient content produce more rapid C degradation. Contrarily, other studies

suggest that nutrient addition, specifically N, does not have a direct negative effect on microbial activity but could be linked to disturbance to decomposer organisms by blocking the production of certain enzymes that decompose and mineralize organic matter (Fog, 1988). Deeper soil layers with poor nutrient content responded positively to readily available nutrients, increasing organic matter degradation, presumably by providing organisms with energy to synthesize degradation enzymes (Wild *et al.*, 2014). With no significant changes in N, P, or K content among treatments in this study, it is possible that decomposition rate remained similar among treatments due to stable microbial activity throughout the 0 to 10 cm layer of the soil.

QBS Index and Arthropod Diversity

In this study, QBS index was significantly greater on day 0 and then declined on day 25 and increased over the remainder of the study (Figure 3.4). One potential explanation for this response in microarthropod communities might be linked to soil moisture content at the time of each sample collection (Table 3.7). Arthropods tend to migrate away from heat and drying in soil to more favorable conditions (cooler and wetter environment). On days when soil moisture content was higher, QBS differed significantly among treatments, while no differences among treatments were evident during periods of low soil moisture content. Supporting this theory, a higher QBS value is present on day 0 when mean soil moisture content was greater than 4.63% across all treatments. On day 54, a more variable moisture content of the soil was observed, with SSW, SS and CON having moisture contents of 4.16, 3.92, and 3.75, respectively. On this same day, QBS was also significantly greater for SSW (QBS=1350) compared to SS (110) and CON (97). Similarly, on day 99 the mean moisture content for the SSW

treatment (5.54%) was greater than for SS (4.38%) and CON (4.77%; $p < 0.05$). These QBS indices results are consistent with those for arthropod abundance, where significantly greater mean abundance in the SSW treatment also correlated with significantly greater mean soil moisture content in that treatment. It is reasonable to suggest that soil moisture content and nutrient availability in the soil independently or interdependently influence QBS index and arthropod diversity.

QBS index can be adjusted to different species of soil fauna. Fusaro *et al.* (2018) classified earthworms using the EMI and QBS approach and found that endogenic (moderately high soil adapted species) were more abundant under organic systems than conventional farming systems at a 10- to 15-cm depth in soil. Other studies reporting arthropod diversity and QBS index presented a greater abundance of mites, collembola, and acari in conventionally managed plots than in long- and short-term organic plots (Mantoni C. *et al.*, 2021; Gkisakis *et al.*, 2014). However, QBS indices on younger organic plots were greater than those on plots managed organically for a longer period of time (Simoni, *et al.*, 2013). Furthermore, the same study suggests that arthropods that are sensitive to soil disturbance, such as oribatids, were present in a lower abundance in conventional fields, indicating that the presence or absence of arthropod groups may not be limited by the source of nutrients in the soil alone. In support of this theory, Mantoni *et al.* (2021) found that arthropod diversity and richness and QBS index was mainly affected by mechanical alterations in crop fields (tillage practices), but also suggested that arthropod communities are influenced by inorganic sources of fertilizers.

Additionally, the effects of slurry application method (broadcast, injection, or no application) on micro arthropod communities in agricultural fields was studied in

Nebraska (Schuster *et al.*, 2015). Similar to other studies, arthropod abundance was lesser in plots on which slurry was injected, suggesting that the mechanical perturbation of soil caused by implement tines cutting through the soil to apply slurry below the soil surface negatively affected some arthropod populations. Plots receiving slurry by broadcast application – which involves spraying manure onto the soil surface – had greater abundance and diversity of arthropods. Injection is often a preferred application method for nutrient rich slurry manure to minimize ammonia volatilization and phosphorus runoff risks from manured soils. Although characteristics of manure applied to plots was the same for both application methods in the study by (Schuster *et al.*, (2015). Schuster *et al.* (2015), examination of soil samples revealed a greater OM concentration on broadcast application and control plots versus injected slurry application, potentially being the reason for a greater collembolan population on the broadcast plots. In line with this theory, it is possible that plots with SSW had a higher QBS index due to added fresh OM from slurry and woodchip applications.

Abundance of the taxonomic groups found in the plots was also recorded (Table 3.9). The most abundant group of species found was Acari, with a total of 4,137 specimens found throughout the study. Acari (mites) were readily identified in every sample, with a significantly greater mean abundance in SSW (85.5) than SS (65.25) and CON (56.75). Throughout the study, the abundance of acari was consistently greater for the SSW treatment than the SS or CON. Forty-seven percent of these specimens were found on SSW plots with a large proportion of Acari found on day 0 (486 specimens) day 54 (417 specimens). The abundance of arthropods on day 0 might reflect established field conditions or soil moisture content. Additionally, arthropod abundance, in general, was

lower 25 days after treatment application. The diminished abundance of arthropod specimens on this date might support the theory of soil disturbance impacting arthropods, especially considering that an increase in abundance occurred in subsequent sample events until lesser fluctuations were reported over the final two sampling events, on days 99 and 128.

Acari populations are related to fungi presence, which is sensitive to phosphorus concentrations. A large fraction of soil nutrients available to plants is a result of microbial-grazing and nutrient release by fauna, to which mites contribute. Fertilizer application, either from organic or mineral sources, suppresses fungi, thus reducing food sources for acari (Cao *et al.*, 2011). Agricultural soils, regardless of the management system, experience frequent changes, especially in annual crops like corn and soybeans and these conditions favor organisms with short life cycles and dispersal (Minor & Cianciolo, 2007). The function of certain types of acari in the soil could be beneficial if there is heterogeneity of species in the sampled plots. Certain orders of Acari, like Oribatida, have species that are susceptible to land changes like cultivation, crop rotation, and pesticide application, which inhibits its growth in agricultural systems (Behan-Pelletier V. M., 2002). Acari are particularly important for soil health because of their role in organic matter decomposition.

The second most commonly identified order of arthropods across all sampling dates was Collembola. A total of 3,945 specimens were found and the majority of those specimens were assigned an EMI score of 20 points. The total number of these high-EMI score specimens found in SSW plots was 1,758. For the SS treatment, 1,290 collembolas with an EMI score of 6 were found, and 897 collembolas with a score of 8 were identified

in CON plots. In this study, low soil moisture content in certain sampling days reflected a decrease in collembola biomass. In a non-irrigated fields like this study site, it is possible that dry periods affect diversity and richness of collembola (Xu *et al.*, 2012). A higher moisture content in the soil, especially on SSW plots, driven by the presence of woodchips on the surface may have diminished the effects of dry periods of time, at least partially, resulting in more abundance of well-adapted collembolans. Soil compaction and increased bulk density is also a limiting factor for collembola populations (Larsen *et al.*, 2004), although no differences were found in bulk density among treatments.

The presence of collembola in the soil is important for plant litter decomposition and formation of soil microstructure. Likewise, host protozoa and nematodes that are used as food by other predators and some species feed on plant pathogens, bacteria and fungi, which are all key aspects of soil health (Rusek, 1998).

The ratio between collembola and acari has been used as a soil health indicator in previous studies, with a greater abundance of acari compared to collembola suggesting good soil quality and habitat stability (Santorufio *et al.*, 2012; Menta *et al.*, 2008). In this study, a greater ratio of acari:collembola was observed in the SSW treatment (1.12:1) than the CON (1.06:1) or SS (0.93:1) treatments, again supporting the assertion that a combination of manure slurry and woodchips produces a more favorable environment for these important arthropod species than manure application alone. In agreement with soil QBS, SSW plots had a higher mean abundance of Collembolans throughout the study (27) compared to CON (11.25) and SS (2.25). Although, abundance on SSW plots was consistently higher on each sampling day, SS and CON plots varied in terms of mean

abundance, on days 0, 54, 99 and 128, SS plots demonstrated a higher abundance of collembolans compared to CON plots.

Diplura are a group of arthropods highly sensitive to changes in the soil. They are commonly found in native forest regions and used as an indicator of low soil disturbance. Additionally, Diplura thrive in high moisture environments (Palacios-Vargas & Garcia-Gomez, 2014). Consistent with the findings in this project, the mean abundance of Diplura in SSW plots compared to SS and CON plots was numerically much greater on day 99, the sampling day with the highest soil moisture content. Similar to Diplura, Pauropoda and Symphyla are indicators of soil disturbance. Although commonly found in woods and forest regions, some species are less sensitive to human intervention and can be found in agricultural fields. High bulk density and compaction have a negative effect on these species, but in general, they play a key role in organic matter decomposition (Voigtlander *et al.*, 2016).

Diplura, along with Protura, play a critical role in decomposition processes in the soil, feeding on dead Acari. However, Protura communities display a more complex dynamic within the soil ecosystem. The density of this group can be found from hundreds to thousands of specimens per m². Although they are known to feed on fungal hyphae and concentrate around this source of food, little is known about other drivers of Protura community densities (Galli *et al.*, 2020). On average, Protura abundance was greatest in SSW plots, indicating that swine manure and woodchips provided favorable soil conditions for this group of arthropods. The presence of a greater abundance of Protura on SSW plots is consistent with a significantly greater abundance of Acari under the

same treatment, suggesting that Protura respond to increased availability of Acari as a food source.

Mean abundance of symphyla was variable throughout the study, but generally quite low. These organisms are highly sensitivity to stress and generally are not abundant in soil that has experienced mechanical or chemical perturbations, so their relative absence in the study site plots is not unsurprising. Because they feed on plant roots and present as a major crop pest if their population is not controlled by other organisms, their numerically lower abundance in SS and SSW treatments compared to CON may support the theory that their population is controlled better in soils with greater organism diversity.

Thysanoptera were rarely identified in samples throughout this study, only being found on two sampling dates and at very low abundances. Commonly known as thrips, these arthropods are a plant pest that damage plant surfaces, so low abundance of them is favorable, but did not appear to be impacted by treatments.

Arthropods that are higher on the food chain with lower EMI scores, such as Hymenoptera and Coleoptera, play critical roles in fragmentation and transportation of organic matter into deeper soil layers (Roy *et al.*, 2018). The role of Araneae in the soil ecosystem as predators is important for regulating arthropod populations and are related to soil surface conditions (De Morais Pereira *et al.*, 2021), spiders are geeneralist predators with the capability of fullfilling a wide variety of ecological niches by praying on a wide variety of arthropod species (Daniel, 2021). A significantly greater ($p < 0.05$) mean abundance of Araneae was found in the SSW treatment (65.23) over the entire study

period than in the SS treatment (46.48), which was significantly greater ($p < 0.05$) than the abundance found in the CON treatment (35.25). This suggests that either the presence of woodchips on the soil surface created a favorable environment for these arthropods or the abundance of other orders of arthropods that are commonly a food source for Araneae were more abundant under this treatment.

Diptera are commonly found on the surface of soil. They are highly adapted to soil disturbance, being present in a wide range of habitats from climax forests to agroecosystems. However, Diptera larvae are more sensitive to disturbances and play a key role in plant litter decomposition (Frouz, 1999). While adult Diptera are flying arthropods, hence their low presence in soil samples, Diptera larvae are soil-dwelling and were found consistently in the three treatments, consistent with the lack of selectivity of these organisms to undisturbed habitats.

Research on the dynamics of arthropod and earthworm populations relative to carbon decomposition revealed that pig manure application did not directly influence collembola and acari populations, but served as a food source for earthworms, which increased arthropod populations by excreting compounds that are consumed by collembola and acari (Monroy *et al.*, 2011). Mulch application can alter the soil composition by increasing habitat complexity. Oribati mites and collembola specimens were more abundant in agricultural soils amended with organic mulch, with species population also being significantly impacted by the state of the mulch (fresh or partially decomposed) (Manwaring *et al.*, 2018).

Other arthropod populations also play an important role in soil health and are good indicators of soil disturbance because of their sensitivity to physical changes in the

soil, adaptation, and resilience to change (Menta & Remelli, 2020). Many research studies show the importance of diversity of arthropods in the soil. The presence of certain types of microarthropods influence the communities of others, which are more closely influenced by fertilization sources, like manure, agricultural practices, and crops (Weil & Kroontje, 1979; Rowen & Tooker, 2019). The QBS index allows for a comparison of overall soil health based upon the characteristics of soil arthropods that are present (Kerrouche *et al.*, 2018). The susceptibility of some arthropod species to changes in agricultural practices allows for observing changes in soil biological status that may be less apparent using chemical or physical analyses because of the response time required for these changes to be measurable.

Furthermore, although QBS index can provide an insight into soil quality based on the presence of certain groups of organisms, shifts in species richness may be overlooked under this assessment because changes in soil fauna community often occur in species abundance (Yan *et al.*, 2012). So, while QBS index is a good indicator of soil quality, monitoring species abundance may give more in-depth information about the state of soil organisms communities and monitor the dynamics within the soil but is a more time-consuming activity compared to QBS assessment. Furthermore, this study demonstrated similar tendencies in QBS values and arthropod abundance. Overall, higher QBS index and higher arthropod abundance was found on SSW treatments. In agreement with these results, Purvis & Curry, (1984) found that pre-plant manure application on crop fields has a positive response on arthropod communities. However, SS plots did not benefit from applications as much as SSW. The addition of cedar woodchips clearly enhances some soil properties that are positive for arthropod communities in general.

Studies performed on organic mulch utilization demonstrate that, Diptera, in particular, are more abundant in woody mulches while other species of Hemiptera, like aphids, thrips, and whiteflies benefit from the absence of mulch because they feed on small plants that are suppressed by mulch. Furthermore, Collembola and Hymenoptera do not seem to be affected by mulch (Gill *et al.*, 2011). Higher abundance of Acari, Collembola, and Hymenoptera were found on sites with forage crop residues compared to bare soils (Menta *et al.*, 2020). The combination of woodchip mulch and swine slurry may create better conditions for more arthropod communities to thrive.

The impact of treatment applications on arthropod abundance and QBS trends need further assessment. Higher QBS indices on day 0 (day of woodchip application) may be driven by moisture content of the soil rather than the application of treatments. Swine slurry was applied to the corresponding plots 2 weeks prior to the first day of sampling, so it is possible that the effect of treatment application is overshadowed by soil moisture content. While some arthropod groups benefit from N and P applications, CON plots had a higher abundance and QBS at day 0 than some of the following days, which supports the relevance of soil moisture content over nutrient concentrations. Furthermore, arthropod abundance on the days following swine slurry application deserves special attention. Herbivore arthropods respond positively to N and P fertilization (Bishop *et al.*, 2010), but the presence of detritivore or predator arthropods may be influenced by the presence of other conditions, such as enzymes and fungi induced by plant litter (Mukhopadhyay *et al.*, 2014).

Table 3.4 reports the mean counts of various arthropod groups by treatment collected during this study and provides insight about arthropod abundance in the soil.

However, further analysis is required to make claims about treatment effects on the abundance of arthropods in groups. A typical model for this type of data can be performed as a general linear model (GLM) for count data. Additional biological and biodiversity indices could be performed if arthropods were identified in species.

Microbial Respiration

No significant differences were found in microbial production of CO₂ between treatments. Factors like temperature or C and N inputs tend to increase CO₂ emissions, limiting C stocks in following periods (Lai *et al.*, 2017). The addition of manure and other organic sources of nutrients can replenish C stocks, as opposed to mineral fertilizers. But microbial biomass carbon also responds to other management practices, like soil disturbance by tilling, planting, or residue incorporation (Chakraborty *et al.*, 2011). Nutrient management strategies are particularly important for microbial respiration. N availability, regardless of the source (mineral or organic), suppresses microbial respiration (Ramirez *et al.*, 2010).

Soil physical characteristics, like aggregate stability, may limit the responses of soil respiration by creating barriers around microbial communities that prevent the movement of water, oxygen and organic matter through the soil aggregate. Additionally, some authors believe that the outer regions of soil aggregates are most active, while microbes located in the interior of the aggregate are limited by the lack of access to nutrients (Goebel *et al.*, 2009). Soil CO₂ also tends to decrease with depth, total organic carbon, dissolved organic carbon and soil microbial carbon (Fang & Moncrieff, 2005). Microbial respiration is also susceptible to soil pH and salinity (Yang *et al.*, 2020), which were not significantly different among treatments during the experiment. So the response

in microbial respiration to treatment application is consistent with the lack of response to added amendments.

A high soil respiration rate over short periods of time is an indicator of rapid degradation and disturbance in the soils. C pools are taken out of the soil in form of gas and expelled to the atmosphere. On the other hand, microbial biomass is a positive indicator of soil health. Microorganisms are in charge of transforming nutrients and benefit from plant root interaction, soil structure, water retention and porosity (Johansson *et al.*, 2004; Habig & Swanepoel, 2015). Soil CO₂ emission observed in the research plots demonstrate that manure and woodchip application have no negative effects on microbial biomass, because the application of treatment does not produce more CO₂ emission in the soil. C obtained from the amendments is most likely kept in the soil or used by plants, contributing to nutrient cycling.

Soil Moisture

Significant differences in moisture content of soil samples were found between CON and SSW but not between CON and SS or SS and SSW (Table 3.9). These values were consistent with QBS values by treatment. QBS index in each treatment was higher on days with higher moisture, which could explain the presence of more arthropods in certain sampling events, like day 0 and 128 with the highest moisture content recorded (mean of 4.65% and 4.56% moisture content among all treatments). Analysis when soil moisture level is 3.30%, 4.0% and 5.0% indicate that at a higher moisture content, the differences of QBS indices among treatments increase (Table 3.10).

Soil Chemical Properties

Soil chemical analysis results obtained in the fall of each cropping year after harvest are represented in Table 3.9. Changes in soil physical properties driven by fertilization management changes usually a longer period. According to Chaudhari *et al.* (2013) some of the chemical properties of the soil are strongly bonded to changes in soil bulk density, higher concentrations of CaCO_3 and EC are strongly correlated to an increase in bulk density. Long term application of high rates of manure is not sustainable for agricultural fields, especially in non-irrigated land (Hao & Chang, Does Long-Term Heavy Cattle Manure Application Increase Salinity of a Clay Loam Soil in Semi-Arid Southern Alberta, 2003). However, EC was not severely affected by swine slurry application, in general, the top 0-10 cm of soil had an average EC content of 0.14 mmhos/cm. The USSL Staff (1954) describes saline soils as those that have an EC of over 4 mmhos/cm. Pig slurry in general, is rich in salts and EC; however, contents vary in different stages of the production cycle. Finishing pigs generate slurry with higher content of salts, possibly due to a dietary intake of salts (Moral *et al.*, 2008). Swine slurry in this study contained an average of 4.65 mmhos/cm, which is not a concerning amount of salts to add to the soil. Although a significant increase was observed after 2 years of treatment, it would take several applications to reach threatening concentrations of salts in this crop field.

Additionally, soil EC is related to soil texture and available water capacity (USDA NRCS, 1998). Clay and loam soils, in general, have a higher EC than sandy soils that simultaneously increases with water content in the soils (Faruque *et al.*, 2006). Soil EC under the application of other organic wastes and manure is often increased by the

inputs of nutrients and salts contained in the amendments. However, EC values usually stay below damaging ranges (Lopes do Carmo *et al.*, 2016).

Similarly, cation exchange capacity at the Julian research site was not significantly affected by the application of swine slurry, nevertheless, the 10-20 cm layer of soil had a significantly higher amount of CEC (17.8 me/100g) compared to the top 0-10 cm of soil (15.4 me/100g). Under both scenarios CEC on this type of soil allows for fall application of treatment, a higher CEC relates to a higher buffer capacity, reducing the risk of soil acidification and nutrient leaching to lower layers of soil. Changes in soil CEC are mostly related to the natural processes that occur within the soil, rather than the application of soil organic amendments (Gao & Chang, 1996) However, solid cattle manure has proved to increase CEC after long term application, as opposed to pig slurry, which showed no difference in CEC after 10 years of study. Ndayegamiye & Côté, (1989) and Bernal *et al.*, (1992) conducted a study to evaluate changes in soil physical and chemical properties with the addition of pig slurry on different types of soils, the results of this study match to the results obtained at the Julian research site: clay soils are less prone to changes in CEC with the addition of pig or swine slurry, cation exchange capacity generally increases with the addition of pig slurry on soils with low clay content.

No difference was found between treatments and control plots but organic matter content was consistently higher in the top 0-10 cm of the soil. Although pig slurry has the potential to increase soil organic matter, the formation of stable organic matter is not an immediate process, but is linked to enzyme action, microbial activity, and substrate degradation. Sollins *et al.*, (1996); Kononova, (1961). Mineralization of organic carbon added to the soils occurs due to microorganism action, (Hernández *et al.*, 2007). Manure

and mulch application have potentially a bigger impact on the lighter fraction of soil organic matter, which refers to free and occluded organic C within aggregates and is sensitive to changes in land management practices but is also a good indicator of long-term impacts on SOM (Yagi *et al.*, 2012). Mulch addition on soil has similar responses, especially with materials such as straw or woodchips, because mulch takes longer to degrade, changes in SOM might take longer than with the addition of more readily available source of nutrients, like compost or manures (Ceccanti *et al.*, 2007). Wood addition on agricultural soils have proven to increase microbial activity, therefore, contributing to SOM formation mulch (Lloyd *et al.*, 2002), additionally, Li, *et al.*, (2019) found that the addition of woodchips incorporated into the soil increased water storage capacity and the consistent improvement of this characteristic for 5 years, mainly due to the slow decomposition of coarse woodchips in the soil.

Along with microbial activity, N content in the soil helps stabilize and transform carbon from organic sources so SOM formation, so supplying the soil with enough nutrients with the addition of carbon sources would result in enhanced organic matter formation (Moran *et al.*, 2005). The addition of non-organic fertilizers can also lead to SOM formation, but it usually corresponds to the lighter fraction of SOM, as opposed to manure addition, which predominantly forms humus, which is linked to soil fertility (Nardi *et al.*, 2004).

The existence of a higher SOM on the top layer of the soil could also be attributed to the incorporation of corn residues into the soil and the decomposition of it in the top layer. A light tillage is performed after harvest in the research site, altering the top layer composition of soil and addition of carbon-rich materials which has a positive impact on

microbial communities that build SOM (Fang *et al.*, 2007). Pig manure contains an average of 67% OM, the availability of organic matter for plant use would depend on the storage time of manure and state of stabilization of chemical components (Moral *et al.*, 2005).

Nitrate nitrogen was not significantly different with swine slurry application or cedar woodchips. In cropland with manure application, especially swine or pig slurry, N losses and water quality are a big concern, inadequate application of N results in a significant portion of N being leached from the crop root zone and contaminate groundwaters. N content from inorganic fertilizers is readily available for plants and if managed properly, N leaching is less concerning, however, manure N is slowly mineralized and the accumulative effects of N concentration may become a problem years after the first application, (Angle *et al.*, 1993). Pig manure nutrient concentration varies on the type of storage system and application method, around 60% of organic nitrogen is mineralized from pig slurry, depending on the handling conditions, and another portion of the manure is expected to be mineralized or leached during the following years of application (Chastain *et al.*, 1998). Other authors suggest that N losses in pig manures are around 23-24% plus 23-33% losses during storage (Petersen *et al.*, 1998). Manure nutrient content is also subject to the season of the year when is sampled, and most likely when it is applied according to Kowalski *et al.*, (2013), pig slurries sampled in Poland maintained an average N content of 5.70 ppm during the summer and fall season, but values decrease towards the winter. Low manure application rates (150 kg N ha⁻¹ y⁻¹) are often not a concern for nitrate contamination of water. Long & Sun, (2012) observed that nitrate levels remained the same after 8 years of pig slurry application, high

rates of pig slurry ($600 \text{ kg N ha}^{-1} \text{ y}^{-1}$) did increase nitrate concentration in water but stabilized towards 4 years of application and remained constant for the following 8 years of study. Following these trends nitrate concentration in the research site in Julian at a low application rate do not seem to represent a threat to water sources or deeper layers of the soil, although nitrate movement withing deeper layers of the soil profile need to be assessed. High rates of pig manure application were also studied by Wang *et al.*, (2017) and reported increase immobilization rates of nitrates, accumulated N in the soil and correlated with nitrous oxide (N_2O) emissions.

Soil phosphorus concentration was not significantly impacted by treatments and did not vary significantly between depths or years, remaining constant during the whole study. Swine slurry phosphorus was most likely absorbed by the plants for its utilization and is possible that an extra source of phosphorus is added to supply the corn needs, although corn yield was not limited by phosphorus deficit. The combination of pig manure along with chemical fertilizers has showed positive effects in increasing P availability and alleviating soil P immobilization (Song *et al.*, 2011). In other studies, soil test P did not increase after incorporation of P-rich organic amendments like plant residues because soil microbial biomass competed with plants for available P of soils (Guo *et al.*, 2009). No significant difference was found in S content between treatments or between the two depths. A large portion of soil sulfur is protected by soil aggregation, preserving the stability of it from outside changes in the soil aggregate. S mineralization and turnover rates are correlated to microbial activity, which often times results in S immobilization, additional process occur in the S cycle before its available for plants (Eriksen J. , 2009). Soil S has low efficiency in terms or mineralization, and often only

5% of total soil S is found in the form of sulfates, which are available for plant uptake (Scherer, 2009).

3.5. Conclusions

The application of swine slurry with woodchips has a positive effect on soil quality biological index and arthropod abundance. The application of red cedar woodchips seemed to provide with a good habitat for soil arthropods, which in the future may increase microbial activity and soil aggregation through decomposition of organic matter and binding. Soil moisture content remained higher with surface woodchips application, which influenced QBS and soil arthropod abundance. Abundance of some orders of arthropods related to soil health like acari and collembola, was also higher in plots receiving woodchips, indicating the possibility of increased soil quality with woodchip applications in combination of swine slurry. Microbial respiration and carbon degradation was not affected by treatment application. Further studies should consider other factors that might affect soil arthropod communities and carbon degradation like soil type, manure sources and changes over medium and long-term periods of manure application.

3.6. Acknowledgements

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Figure 3.1. Research site location in Southeast Nebraska



Figure 3.2. Plot size and treatment arrangement at study site; CON=control, SS=swine slurry, SSW=swine slurry and cedar woodchips.



Figure 3.3. Swine slurry application calibration in field

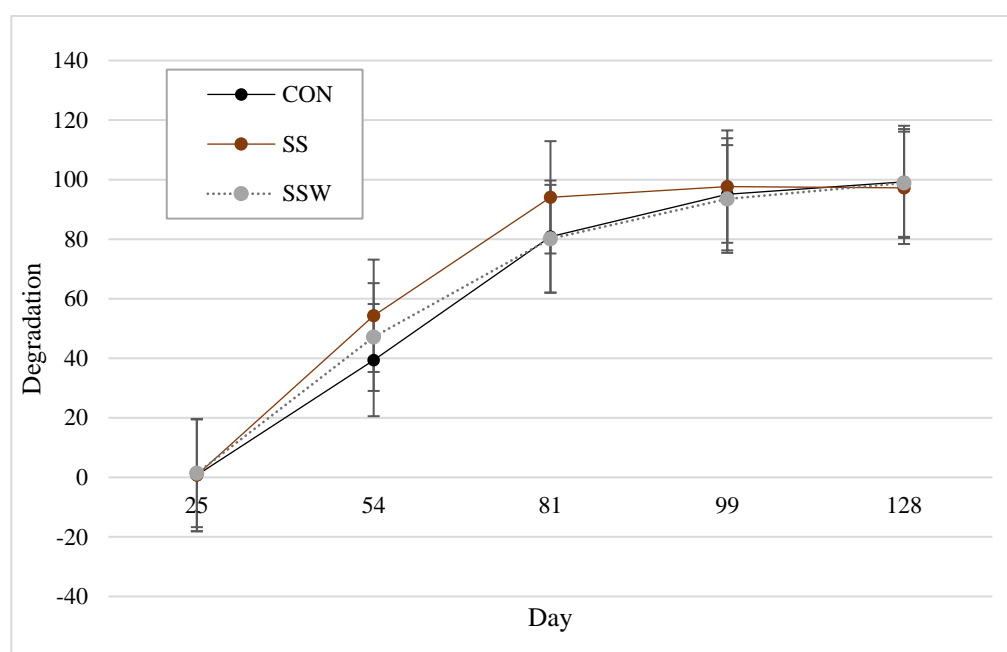


Figure 3.4. Mean percent fabric degradation by treatment and days since treatment application; CON=control, SS=swine slurry, SSW=swine slurry and woodchips; bars represent standard error of means. Absence of superscripts indicates no significant differences among treatments ($p>0.05$).

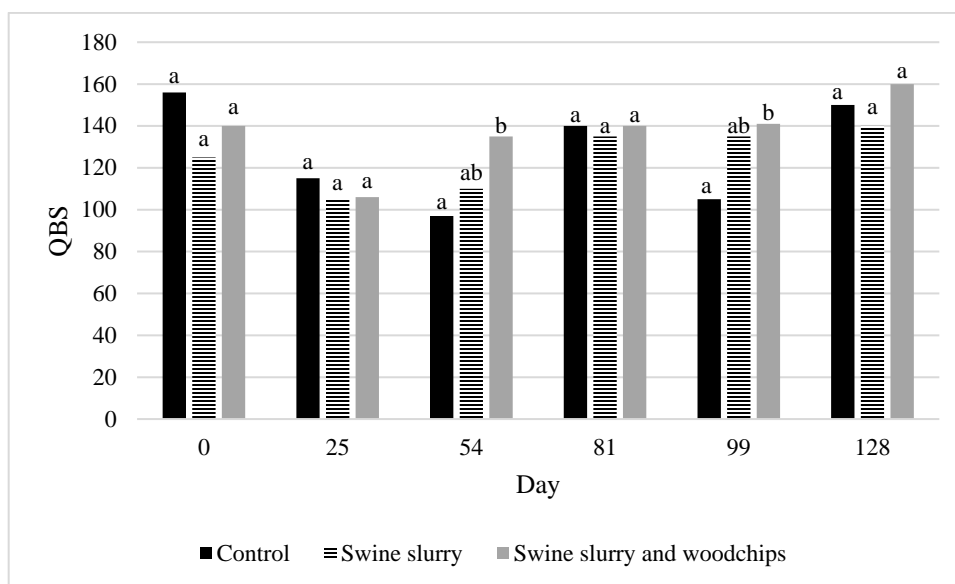


Figure 3.5. QBS by treatment and days since treatment application; CON=control, SS=swine slurry, SSW=swine slurry; different letters within sampling day indicate significant differences ($p<0.05$).

Table 3.1. Average monthly temperature and precipitation at study site during 2021

	T (°C)	PP (mm)
January	13.88	31.75
February	-8.53	20.06
March	8.23	132.84
April	11.52	44.20
May	16.66	64.77
June	25.09	113.28
July	24.90	37.85
August	25.50	90.68
September	21.69	18.28
October	13.88	102.62
November	-	12.45
December	-	-

T=temperature; PP=precipitation; data retrieved from Weather Underground (<https://www.wunderground.com/>), collected from Shubert Nebraska Area Weather Station.

Table 3.2. Chemical properties of applied treatments

	Swine Slurry	Woodchips
Dry matter (%)	0.41	71.29
Moisture (%)	-	28.71
pH	7.7	5.75
EC (mmho/cm)	2.98	3.45
Ash (ppm)	-	9.0
Total-C (ppm)	-	46.69
Organic-C (ppm)	-	52.78
C:N Ratio	-	83.8
Organic-N (ppm)	455.17	0.63
NH ₄ ⁺ -N (ppm)	241.8	0.007
P (ppm)	982.66	0.17
K (ppm)	300.4	0.45
S (ppm)	126.56	0.06
Ca (ppm)	590.9	1.38
Mg (ppm)	178.63	0.13
Na (ppm)	91.5	0.015

Note: Analysis results “As received”. EC=electrical conductivity, Total-C=total carbon, Organic-C=Organic carbon, Total C:N=total carbon-to-nitrogen ratio, Organic-N=organic nitrogen, NH₄-N=ammonium nitrogen, P=phosphorous (% P₂O₅), K=potassium (% K₂O), SO₄-S =sulfate-sulfur, Ca=calcium, Mg=magnesium, Na=sodium

Table 3.3. EMI scoring range by arthropod group

Group	EMI Score
Protura	20
Diplura	20
Collembola	1-20
Microcroryphia	10
Zyngentomata	10
Dermaptera	1
Orthoptera	1-20
Embioptera	10
Blattaria	5
Psocoptera	1
Hemiptera	1-10
Thysanoptera	1
Coleoptera	1-20
Hymenoptera	1-5
Diptera (larvae)	10
Other holometabolous insects (larvae)	10
Other holometabolous insects (adult)	1
Acari	20
Araneae	1-5
Opiliones	10
Palpigradi	20
Pseudoscorpiones	20
Isopoda	10
Chilopoda	10-20
Diplopoda	10-20
Pauropoda	20
Symphyla	20

A simplified scheme to calculate collembolan's EMI

Character	EMI score
(1) Clearly epigeous forms: middle to large size, complex pigmentation present, long, well-developed appendages, well developed visual apparatus (eye spot and eyes)	1
(2) Epigeous forms not related with grass, shrubs or trees well-developed appendages (possible), well-developed setae or protective cover of scales, well-developed visual apparatus	2
(3) Small size—though not necessarily—forms, usually limited to litter, with modest pigmentation, average length of appendages, developed visual apparatus	4
(4) Hemi-edaphic forms with visual apparatus still developed, not elongated appendages, cuticle with pigmentation	6
(5) Hemi-edaphic forms with reduced number of ommatidia, scarcely developed appendages, often short or absent furca, pigmentation present	8
(6) Eu-edaphic forms with no pigmentation, reduction or absence of ommatidia, furca present—but reduced	10
(7) Clearly eu-edaphic forms: no pigmentation, absent furca, short appendages, presence of typical structures such as pseudo-oculi, developed postantennal organs (character not necessarily present), apomorphic sensorial structures	20

EMI group scores as described by Parisi *et al.* (2005)

Table 3.4. Mean arthropod specimen abundance and QBS index as affected by treatment and time since treatment application

Treatment	QBS Score	Protura	Diplura*	Paupoda	Symphyla	Acari*	Collembola *	Hymenoptera	Araneae*	Chilopoda	Diplopoda	Blattaria	Psocoptera	Thysanoptera	Diptera	Pseudoscorpiones	Orthoptera	Hemiptera	Coleoptera	Total
Mean abundance and QBS																				
CON	127.17 ^a	3.12	1.12 ^a	0.62	5.87	56.75 ^a	11.25 ^a	4.87	35.25 ^a	1.5	1.86	0	0.37	0.12	5	0.25	0.12	0	3.25	7.30 ^a
SS	125 ^{ab}	4.12	1.87 ^{ab}	1.75	0.75	65.25 ^{ab}	2.25 ^{ab}	3.12	46.48 ^b	3.12	2.12	0.12	0	0.5	4.87	0	0	0.12	7	7.97 ^a
SSW	137 ^b	7.87	3 ^b	7.12	1.25	85.5 ^b	27 ^b	15.3 7	65.23 ^c	1.75	3.37	0	0.5	0	4.5	0	0.12	0.12	5.37	12.67 ^b
Day 0																				
CON	156 ^a	2.25	6 ^a	0	0.75	88.5	54 ^a	1.5	27	0	2.2	0	0.75	0	3	1.5	0	0	4.5	8.25
SS	125 ^a	1.5	9 ^a	3	0	118.5	78.7 ^{ab}	0	26.2	0	0.75	0.75	0	0	3.75	0	0	0	4.5	9.93
SSW	140 ^a	4.5	26.25 ^b	0.75	1.5	121.5	97.5 ^b	0.75	57.7	0	0	0	0	0	6	0	0.75	0	3	12.27
Day 25																				
CON	115 ^a	3.75	0.75	0	0	12.75 ^a	9 ^a	2.25	3.7 ^a	0	0	0	0	0	9	0	0.75	0	1.5	1.2
SS	105 ^a	0.75	2.25	0	0	11.25 ^a	4.5 ^{ab}	0.75	6 ^b	0	0	0	0	3	0	0	0	0	9	1.05
SSW	106 ^a	2.25	2.25	0	0	49.5 ^b	14.2 ^b	0	27 ^a	0	3	0	0	0	6	0	0	0	12	1.7

Treatment	QBS Score	Protura	Diplura*	Paupoda	Symphyla	Acari*	Collembola *	Hymenoptera	Araneae	Chilopoda	Diplopoda	Blattaria	Psocoptera	Thysanoptera	Diptera	Pseudoscorpiones	Orthoptera	Hemiptera	Coleoptera	Total
Day 54																				
CON	115 ^a	3	1.5 ^a	0	0	16.5 ^a	18.7 ^a	3.75	18 ^a	0	0.75	0	1.5	0.75	1.5	0	0	0	2.25	1
SS	110 ^a _b	6	1.5 ^a	3	0	31.5 ^a	36.75 ^a	0	15.75 ^a	0	2.25	0	0	0	7.5	0	0	0	16.5	2.45
SSW	135 ^b	6.75	3.75 ^b	5.25	0	104.5 ^b	74.75 ^b	1.5	53.25 ^b	0	0.75	0	0	0	0.75	0	0	0	7.5	3.1
Day 81																				
CON	140 ^a	3	3.75 ^a	3	30.75	24.0	11.25	4.5	8.25 ^a	0	0	0	0	0	3.75	0	0	0	6.75	5.43
SS	135 ^a	6	0.75 ^b	1.5	3	19.5	2.25	1.5	13.5 ^a	0	2.25	0	0	0	3.75	0	0	0	3.75	2.72
SSW	140 ^a	4.5	4.5 ^a	0	0.75	32.25	27	17.25	36 ^b	0	10.5	0	0	0	6	0	0	0	6	6.51
Day 99																				
CON	105 ^a	0	3.75 ^a	0	3	39.75 ^a	75 ^a	12	40.5	3.75	0	0	0	0	0	0	0	0	4.5	4.25
SS	135 ^a _b	3.75	8.25 ^a	0.75	0.75	63 ^{ab}	106.5 ^{ab}	12	72.75	10.5	3	0	0	0	6	0	0	0.75	7.5	7.85
SSW	141 ^b	3.75	13.5 ^b	36	3.75	83.25 ^b	140.25 ^b	65.25	72.75	5.25	0	0	3	0	1.5	0	0	0.75	2.25	12.95
Day 128																				
CON	150 ^a	6.75	0.75 ^a	0.75	0.75	57.75	56.25 ^a	5.25	38.25	5.25	8.25	0	0	0	12.75	0	0	0	0	8.48
SS	140 ^a	6.75	0.75 ^a	2.25	0.75	57.75	70.5 ^b	4.5	40.5	8.25	4.5	0	0	0	8.25	0	0	0	0.75	8.39
SSW	160 ^a	5.25	5.25 ^b	0.75	1.5	102.75	86.25 ^b	7.5	65.25	5.25	6	0	0	0	6.75	0	0	0	1.5	12.65

Mean arthropod abundance by day. Arthropod groups marked with * were analyzed for significant differences in abundance among treatments and day. Values with same superscripts within an arthropod group and sampling day are not significantly different ($p>0.05$); CON=control, SS=swine slurry, SSW=swine slurry and woodchips.

Table 3.6. QBS index by treatment and sampling day

Treatment	Day						Mean QBS
	0	25	54	81	99	128	
Control	156	115	97 ^a	140	105 ^a	150	127.17 ^a
Swine slurry	125	106	110 ^{ab}	135	135 ^b	140	125 ^{ab}
Swine slurry and woodchips	140	105	1350 ^b	160	141 ^b	160	137 ^b

QBS values having the same superscript within each sampling day are not significantly different.

Absence of subscript represent no significant difference between treatments on that day ($p \geq 0.05$).

CON=control, SS=swine slurry, SSW=swine slurry and woodchips.

Table 3.7. Treatment and day effects and associated p-values

Effect		P-value
Treatment mean		0.0053
CON	SS	0.0848
CON	SSW	0.0368
SS	SSW	0.7328
Day		0.0011
Simple Effect	Treatment	P-value
Day 0	CON vs SS	0.9918
	CON vs SSW	0.9537
	SS vs SSW	0.9094
Day 25	CON vs SS	0.4631
	CON vs SSW	0.7108
	SS vs SSW	0.9148
Day 54	CON vs SS	0.4561
	CON vs SSW	0.0021
	SS vs SSW	0.0531
Day 81	CON vs SS	0.9736
	CON vs SSW	0.6329
	SS vs SSW	0.4967
Day 99	CON vs SS	0.4912
	CON vs SSW	0.0103
	SS vs SSW	0.1510
	CON vs SS	0.3952
	CON vs SSW	0.1566
	SS vs SSW	0.8407
Treatment* day		0.3739

Table 3.8. Mean soil moisture content by treatment and time since treatment application

Treatment	Moisture %					
	Day 0	Day 25	Day 54	Day 81	Day 99	Day 128
CON	4.65	3.43	3.75 ^a	3.95	4.77 ^{ab}	4.31
SS	4.63	3.42	3.92 ^{ab}	3.71	4.38 ^a	4.10
SSW	4.68	3.72	4.16 ^b	4.27	5.54 ^b	4.63
Effect	p-value					
Mositure level	0.47					
Moisture*treatment	0.05					

CON=control, SS=swine slurry, SSW=swine slurry and woodchips; values within columns having the same superscript are not significantly different ($p>0.05$).

Table 3.9. Differences in QBS index by treatment at different soil moisture content ranges.

Moisture %	Treatment	p-value
<3.3	CON vs SS	0.16
	CON vs SSW	0.24
	SS vs SSW	0.99
3.4-4.0	CON vs SS	0.08
	CON vs SSW	0.03
	SS vs SSW	0.73
4.1-5.0	CON vs SS	<0.0001
	CON vs SSW	<0.0001
	SS vs SSW	<0.0001

CON=control, SS=swine slurry, SSW=swine slurry and woodchips; (p-values are shown for each comparison between treatments at different moisture content ranges)

Table 3.10. Mean microbial CO₂ respiration by treatment and time since treatment application

Day	CON	SS	SSW
0	21.3 ^a	40.8 ^b	36.9 ^b
25	26.1	20.9	25.7
54	28.5	23.4	19.8
81	39.6	34.8	27.9
99	12.0	13.2	18.0
128	10.8	16.2	13.8
Simple effect	P-value		
Day	<0.001		
Treatment	0.73		
Treatment *Day	0.01		

CON=control, SS=swine slurry, SSW=swine slurry and woodchips. Different superscripts within a row indicate differences in treatment x time interaction (p<0.05). Absence of superscripts indicates no significant difference among treatments (p>0.05).

Table 3.11. Initial soil chemical properties at study site

Depth	OM	CEC	pH	EC	NO ₃ -N	P	K	SO ₄ -S	Ca	Mg	Na
(cm)	(%)	(meq/100g)		(mmho cm ⁻¹)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)
0-10	3.69	16.08	6.08	0.18	24.7	18.08	272.42	8.26	1865.50	226.92	13.75
10-20	3.26	17.18	5.72	0.75	30.55	14.92	194.8	9.33	1810.83	235.92	14.75

OM=organic matter, CEC=cation exchange capacity, EC=electrical conductivity, NO₃-N=nitrate-nitrogen, P=phosphorous, K=potassium, SO₄-S=sulfate-sulfur, Ca=calcium, Mg=magnesium, Na=sodium

Table 3.12. Mean soil chemical properties by treatment and sampling depth in October of 2021

Depth	Treatment	pH	EC (mmhos/cm)	OM (%)	Nitrate-N (ppm)	K (ppm)	P (ppm)	S (ppm)	Ca (ppm)	Mg (ppm)	Na (ppm)	CEC (meq/100 g)
0-10	CON	5.9 ^a	0.14 ^a	4.07 ^{ab}	11.72 ^a	222.5	17.7 ^{ab}	5.9 ^b	1837 ^a	231 ^a	4 ^c	15.6 ^{ab}
	SS	5.9 ^a	0.16 ^b	3.8 ^{bc}	10.27 ^a	240	26.7 ^{ab}	6.5 ^a	1956 ^a	213.7 ^a	9.7 ^{ab}	15.3 ^{ab}
	SSW	6.1 ^b	0.12 ^b	4.5	6.5 ^a	222.2	19.7 ^{ab}	6.1 ^{ab}	1941 ^a	260 ^a	7 ^{bc}	16.6 ^{ab}
10-20	CON	5.4 ^b	0.09 ^b	3.3 ^{ecd}	5.07 ^b	147.75	10.25 ^b	6.6 ^a	1776 ^b	236 ^a	8.7 ^{abc}	18.5 ^a
	SS	5.6 ^{bc}	0.10 ^{ab}	3.2 ^{ed}	4.35 ^b	151.5	11.25 ^b	6.6 ^a	1788 ^b	228 ^a	7.5 ^{abc}	17 ^{ab}
	SSW	5.4 ^b	0.09 ^b	3.5 ^{ecd}	3.82 ^b	121.75	16.5 ^{ab}	6.8 ^a	1719 ^b	229 ^a	7.2 ^{abc}	18 ^{ab}

CON=control, SS=swine slurry, SSW=swine slurry and woodchips; values for each property having the same superscript within each sampling depth are not significantly different ($p>0.05$). values with no subscript in the same property are not significantly different from each other.

CHAPTER 4: SUMMARY AND RECOMMENDATIONS

Karla Melgar Velis

4.1. Key Findings

- Cotton fabric degradation and photographic editing software are a suitable tool to measure biological activity in the soils through carbon degradation. The accuracy of this tool is comparable to manual area estimation on a shorter period.
Automatization of the process needs further exploration but provides reliable and clear information for extension educators and farmers to compare agricultural practices and their impact on soil biological properties.
- Soil quality, determined by arthropod ecomorphological index was improved by the addition of swine slurry and woodchips on corn fields. Arthropod abundance was also improved by this treatment. Arthropod groups related to soil health were particularly benefited from the combination of swine slurry and woodchips. Other biological properties were not affected by the application of swine slurry or woodchips.

4.2. Summary

This project aimed to evaluate soil health, focusing on inexpensive, practical, and accurate tools to observe biological activity with the use of locally available organic amendments.

The primary objectives of this work were to:

- 1) Assess the accuracy and reliability of photographic editing software to evaluate soil health through carbon decomposition as an inexpensive tool for measuring soil microbial activity.
- 2) Determine the short-term effects of swine slurry application and its combination with cedar woodchips on biological soil health variables for corn production in Nebraska and soil chemical properties of the soil.

Chapter 2 of this thesis addressed objective number one. The evaluation of different methods to measure carbon degradation under different soil amendments initiated with a small-scale experiment comparing cotton fabric degradation with the use of cattle manure as a soil amendment and control plots with no fertilization. Fabric degradation was estimated using Adobe photoshop and compared to hand counts by different university personnel. The initial comparison between methods showed a wide variability on area measurements, however, subsequent fabric analysis became more consistent. A second experiment was performed using swine slurry and the combination of swine slurry with woodchips as a soil amendment on top of the cotton fabric. The pieces of fabric were retrieved from the field each 3-4 weeks in the summer of 2021. The pieces of fabric were removed from the mesh bags, photographed and edited to enhance contrast between remaining fabric and a dark background. The degradation percentage was estimated using the same method as the first experiment, ImageJ, a free license image analysis software, was also used in the comparison of methods along with hand count. Results for the three methods were similar, indicating that the three methods may

give and accurate estimation of degradation. Future work should be oriented towards the automatization of the process, since previous editing of the images is necessary to obtain an accurate area of remaining fabric, however, the method is replicable and relatively quick and can be used to test degradation rates under different management practices like no-till, cover crops or organic amendments.

Chapter 3 of this thesis addressed objective number 2. The evaluation of soil quality under the three aspects of soil health, with a major focus on biological activity and its impacts on soil nutrient content, and physical properties.

On-farm research was performed in a commercial farm in southeast Nebraska to be divided in plots to hold swine slurry, swine slurry and woodchips and control plots with no amendments. The plots were located on silt loam soils with no irrigation. Initially, soil samples were taken before the application of treatments to observe soil chemical properties like N, SOM, P, K, EC and CEC. After harvest, subsequent samples were taken and analyzed at a commercial lab. Five pieces of fabric were buried on each plot and retrieved from the soil ever 3-4 weeks. Additional soil samples taken during the summer were arthropod extraction and soil respiration, which were analyzed at a University of Nebraska-Lincoln laboratory. Soil biological properties evaluated demonstrated no short-term changes with swine slurry and woodchip applications. Fabric degradation was not different between treatments, but it did increase over time, soil respiration remained uniform through all of the sampling events, and lastly soil arthropod diversity and soil quality index were higher on plots receiving swine slurry and woodchips. Soil nutrient concentrations were not affected by the application of swine

slurry or woodchips. However, nitrates, pH and SOM were higher on the top layer of all were higher on the top layer of the soil.

Literature suggests that most changes in soil properties with the application of organic amendments occur over longer periods of time, therefore more years of study are needed to observe changes in all aspects of soil health. Additionally, the combination of organic amendments with inorganic fertilizers may be needed to supply plant nutrients while the mineralization of organic nutrients takes place. Some studies suggest that plant nutrient availability may be suppressed by the consumption of it by soil microorganisms. Over time, changes in soil structure and aggregation driven by microbial activity should yield in improved soil fertility. Swine slurry and red cedar woodchips proved to be a reliable source of nutrients when they are locally available and applied at agronomic rates.

4.3. Recommendations for Future Research and Extension Programming

- Fabric degradation evaluation using photographic editing software's was a relatively simple tool to estimate carbon degradation. Other common practices like weighing of samples before and after retrieval of the soils introduce can be compared to degradation percentage of photo editing software's to determine the accuracy of estimations.
- Photo editing software was a relatively quick tool to determine fabric degradation in time, however, other tools and programs should be explored to improve the automatization of the process, especially in delimiting-stained pieces of fabric versus degraded portions in the pictures. Other techniques could

include utilizing different color fabric or patterns, lighting arrangements when taking the pictures and the use of higher resolution cameras.

- Multiple soil types could be tested and compared in terms of fabric degradation. Literature suggests that microbe accessibility to nutrients and oxygen is limited by soil texture and aggregation. In order to determine the interaction of soil type with amendments and soil biology. Initial studies on fabric degradation at the university farm demonstrated a significantly different rate of degradation between manure and no manure plots, but these changes were not present at the Julian research site. It is possible that soil texture, management, crop stage and time of the year influence degradation. These factors should be compared under different crop fields to estimate the relevance of each factor on carbon degradation and microbial activity.
- Nutrient balance should be made to compare the performance of organic amendments like manure and woodchips to inorganic fertilizers.

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