

**EVALUATION OF SOIL HEALTH INDICATORS IN THE RED RIVER VALLEY OF
MANITOBA**

BY

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Abstract

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The Comprehensive Assessment of Soil Health (CASH) uses multiple biological, chemical, and physical soil indicators to assess soil health. These indicators were originally selected because they were responsive to management in the northeastern United States. The objective of this research was to determine if the indicators found in the CASH would be responsive to agronomic management on humic vertisols in the Red River Valley of Manitoba. To test this, 14 soil health indicators were used to assess the effect of three different treatments from the National Centre for Livestock and the Environment long-term nutrient management study. In this experiment, the crop history treatment contained an annual crop history or a perennial crop history treatment. The perennial crops had been terminated the growing season prior to soil sample collection. The second treatment was fertility history containing an unfertilized control, a synthetic fertilizer, liquid pig manure applied at the nitrogen rate, and solid dairy manure applied at the nitrogen rate. The fertility treatment had ceased five years prior to sampling. The third treatment was soil management, including soil building management or conventional management. The soil management treatment started the year of sampling for this study. Seven of the 14 soil health indicators tested were responsive to a fertility history treatment. These indicators were total organic carbon, active carbon, autoclave citrate extractable soil protein, potentially mineralizable nitrogen, soil test potassium, Olsen phosphorus, and soil test zinc. Within the soil fertility treatment, the soil with a history of solid dairy manure was the best at increasing soil health

indicator values. Four of the soil health indicators were responsive to the cropping system history treatment. These indicators were potentially mineralizable nitrogen, soil respiration, wet aggregate stability, and soil test potassium. Soil with a history of perennial crops increased three of the responsive indicators compared with soil with a history of annual crops. While soil with a history of perennial crops decreased soil test potassium relative to soil with a history of annual crops. None of the soil health indicators were responsive to the land management treatment. However, this was likely due to there only being one year of treatment effect. Future research will need to focus on developing scoring functions that help interpret soil health indicator values.

1.0 Introduction

Soil health is a relatively new concept in modern agriculture that focuses on the overall soil ecosystem. The official definition of soil health according to the USDA is “the continued capacity of soil to function as a vital living ecosystem that sustains plants, animals, and humans” (USDA, 2020). The paradigm of soil health is in contrast to the conventional view of soil, which focuses on the chemical and physical soil properties that affect crop productivity. This conventional view of soil has led to the development of both agronomic and environmental issues. Wind erosion, water erosion, soil compaction, and soil crusting are some examples of the agronomic issues that have arisen from conventional practices. They can be found in the Red River Valley of Manitoba at this present time, water erosion (Figure 1.1), soil crusting (Figure 1.2), and wind erosion (Figure 1.3). These agronomic issues have a negative economic impact. Soil erosion is estimated to cost Canadian farmers \$3.1 billion a year (Arnason, 2019) while soil compaction is estimated to cost \$583 million over 2 years for farmers in North Dakota and Minnesota (Daigh et al. 2020).

In addition to the agronomic issues, environmental issues are in part a consequence of conventional management. Issues such as climate change and eutrophication threaten food security and the quality of our environment. Climate change is predicted to increase the frequency of severe weather events such as flooding or drought (Ellen Gray, 2019), and eutrophication is already a problem that decreases the water quality of Lake Winnipeg (Schindler, Hecky & McCullough, 2012). Overall, the conventional view of soil has created issues that can negatively impact Manitobans' standard of living, deteriorate Manitoba's natural resources, and threaten food security. Improving soil health will help mitigate these issues, build

resiliency into our agricultural systems (Karlen and Rice, 2015), and lower the environmental impact of modern agriculture (FAO. 2020).

An important step towards improving soil health is being able to measure it. Since the 1990's soil scientists have been developing ways to measure soil health using soil health indicators (Gregorich et al., 1994; Doran & Jones, 1996; Drinkwater et al., 1996). A soil health indicator is a metric that attempts to measure an aspect of soil health (Karlen, Stott & Mikha, 2021). There are two criteria for a good soil health indicator. The first criterion is that a soil health indicator must be responsive to management, and the second criterion is that a soil health indicator must be relevant to crop production (Andrews, Karlen and Cambardella, 2004). Once measured, these indicators can be interpreted to determine if the test result is good or bad. These interpreted results from a set of soil health indicators can then be integrated to produce an overall soil health score. A well-known and researched soil health test is the Cornell soil health test (CSHT), also known as the comprehensive assessment of soil health (CASH) (Moebius-Clune et al., 2016). The CASH test evaluates soil health using indicators that fall into one of three categories: physical, chemical, or biological.

The CASH soil health test was developed by Cornell University and has been used to assess the health of soils found in New York state and the northeast United States. The validation of CASH as an effective soil health test in New York state sparked the interest of researchers from outside the state. The out of state researchers have found that the CASH is not an effective soil health test for other parts of the United States (Roper et al., 2017). This is because of the need for the indicator scoring functions to be calibrated to the local soil type and growing conditions. Congreves et al., (2015) used the CASH framework and calibrated it for soils in southern Ontario. In response to the shortcomings of the CASH, the researchers at Cornell

University created variations of the soil health indicators scoring functions that change depending on the region and the soil type (Fine, van Es & Schindelbeck, 2017). This trend towards the calibration of soil health indicators has continued with soil in Saskatchewan (Wu & Congreves, 2022).

This need for region specific soil health testing is also echoed by the demand from farmers whose interest in soil health is growing. Evidence of this demand for soil health information and testing is found in the popularity of soil health articles from prairie farm media outlets such as Grain News (Lovell, 2020), The Western Producer (Briere, 2020), and The Manitoban Cooperator (Wichens, 2019). With an increasing awareness of soil health by farmers and agronomists, it is important that soil health can be measured effectively so that management decisions can be made with confidence. Therefore, it is important to evaluate CASH as a potential tool for evaluating soil health in Manitoba.

To properly evaluate CASH, it must be tested on soils that are under management that is known to improve soil health in the other parts of the world. Crop livestock integration is one of the ways that agriculture can improve soil health and become more sustainable (Martin et al., 2016). Sustainability is a part of the definition of healthy soil and crop livestock integration is the fifth principle of improving soil health outlined by the USDA (Fuhrer, 2020). The National Center for Livestock and the Environment (NCLE) is a University of Manitoba based research group that focuses on understanding the connections between integrated crop-livestock systems and the environment. The NCLE long-term nutrient management study is a long-term study that investigates nutrient availability from manure. The NCLE long term nutrient management study was established in 2007 at the University of Manitoba's Glenlea Research Station in the Red River Valley of Manitoba.

To date, there have been three phases in the NCLE study. The first phase investigated the availability of nitrogen and phosphorus from a range of fertility treatments in both annual and perennial cropping systems. The fertility treatments included liquid pig manure, solid dairy manure, composted pig manure, as well as a synthetic fertilized treatment, and an unfertilized control. The second phase investigated the draw down of phosphorus after being built up in the manure treatments, as well as nitrous oxide emissions from annual and perennial systems. The third and current phase of the study is investigating how conventional soil management practices compare with a set of soil health building management practices.

The main objective of this study is to determine if the soil health indicators from the CASH test are responsive to a range of agronomic management practices on the humic vertisol in the Red River Valley of Manitoba. The 13-year history with contrasting fertility and crop rotation treatments in the NCLE long term study makes it an ideal research location for evaluating the CASH in Manitoba. There are multiple hypotheses that were tested in this study:

1. The chemical soil health indicators: zinc, iron, manganese, magnesium, potassium, and phosphorus are greater in the liquid pig manure and solid dairy manure when compared with the unfertilized control.
2. The biological soil health indicators: total organic carbon, active carbon, potentially mineralizable nitrogen, soil respiration, and soil protein will be higher in the solid dairy manure and liquid pig manure compared to the unfertilized control and synthetic fertilizer treatment.
3. The biological soil health indicators: total organic carbon, active carbon, soil respiration, and PMN will be higher in the perennial crop history treatment compared to the annual crop history treatment.

4. The physical soil health indicators: wet aggregate stability and available water holding capacity will be higher in the perennial crop history compared to the annual history treatment.
5. The physical soil health indicators: wet aggregate stability and available water holding capacity will be higher in the solid dairy manure and liquid pig manure treatments compared to the unfertilized control and synthetic fertilizer treatments.



Figure 1.1. Evidence of water erosion at the National Center for Livestock and the Environment long term nutrient management study in Glenlea MB. May 2022.



Figure 1.2. Evidence of soil crusting at the National Center for Livestock and the Environment long-term nutrient management study in Glenlea MB. May 2022.



Figure 1.3. Evidence of soil wind erosion in the rural municipality of Springfield Manitoba. March 2021.

2.0 Literature Review

Soil is the foundation of a productive society. For over a century, soil has been intensively farmed, leading to its degradation. The Dust Bowl of the 1930s is an example of this degradation, where tillage and fallow caused severe wind erosion, resulting in the economic distress of rural farming communities (Belshaw, 2016). From the hardships of the Dust Bowl, the conservation movement arose leading to the formation of organizations such as the Prairie Farm Rehabilitation Administration (PFRA) (National Farmers Union, 2024), Ducks Unlimited Canada (Ducks Unlimited Canada, 2024), and the National Conservation Reclamation Service (NRCS, 2024). Currently, there are still soil-related issues that agriculture faces, such as erosion, compaction, salinity, acidification, drought, and flooding (Senate of Canada, 2024). These issues decrease crop yields, resulting in a negative economic impact (Badreldin & Lobb, 2023; Province of Manitoba, 2021). Improving soil health will be essential for resilient agricultural systems and ensure that soil remains productive.

2.1 History of Soil Health

The concept of soil health has evolved from earlier concepts, such as soil quality and soil productivity. The awareness of the importance of maintaining soil productivity has been documented in ancient civilizations (Ilski & Kotłowska, 2024) as well as by scientists in the 1800's who were studying prairie soils in North America (Lawes & Gilbert, 1885). The first mention of the term soil health in academic writing was in 1910 (Brevik, 2018). The use of the term soil health has increased in recent decades. For context, there were 10 articles in the Soil Science Society of America (SSSA) journal that mentioned the term soil health in the 1980's and

from 2010 to 2018, there were 141 SSSA journal articles that mentioned the term soil health (Brevik, 2018).

Interest in the concept of soil health has not been limited to academia. The Soil Conservation Council of Canada (SCCC) is a not-for-profit group in Canada founded in 1987 with a focus on soil conservation. More recently, the mission of the organization has expanded to include soil health. In the United States, the promotion and research of soil health is supported by United States Department of Agriculture (USDA, 2024) and the NRCS, which created a Soil Health Division in 2014. In addition to government and research groups, farm media has also taken an interest in soil health. In 2020, Grain News published an article titled “The Five Principles of Soil Health” which described the general principles of building a healthy soil (Lovell, 2020). A 2021 article published in the Manitoba Cooperator titled “Soil Health a Moving Target” talked about the difficulties in assessing soil health and the work needed to improve soil health (Leathers, 2021). Trends in these organizations, the use of the term soil health in the academic literature, and publications in farm media illustrate the increased interest of scientists, agronomists, governments, and farmers in soil health.

2.1.1 Soil Health in Canada

There is a long history of soil health research in Canada and on the Canadian prairies. Lawes & Gilbert (1885) studied prairie soils from Selkirk, Winnipeg, Niverville, and Brandon. They were trying to understand what made these soils naturally fertile and ultimately concluded that the fertility was from the high levels of soil organic matter. Lawes & Gilbert (1885) noted that these high levels of soil organic matter had built up over years in the undisturbed natural prairie landscape. They expressed concern early on about the sustainability of the productivity of these

soils due to observed declines in soil organic matter that were attributed to the widespread use of fallow and tillage. Lawes & Gilbert (1885) also commented on the importance of applying manure as a strategy to maintain soil productivity.

The modern soil health movement in Canada evolved from within soil conservation. The PFRA Prairie Farm Rehabilitation Administration (PFRA) was created in 1935 in response to severe drought and wind erosion on the prairie provinces during the 1930's. The efforts of the PFRA focused on reducing soil erosion and improving water management (Agriculture and Agri-Food Canada, 2010). After the drought of the dust bowl ended and the conclusion of the second world war, much of the soil science research on the Canadian prairies was focused on soil fertility and how to effectively use fertilizer (Alexander & Robertson, 1968; Cairns et al., 1967; MacLean, 1962; Sheppard & Racz, 1984).

Interest in soil health in Canada regained momentum 50 years later when a report from the Canadian Senate was published in 1984. This senate report, titled "*Soil at Risk Canada's Eroding Future*" focused on the degradation of soil in Canada and addressed regional concerns. Although the report did not make use of the term soil health, much of the concerns brought forth by the report directly relate to soil health. For example, the report cited that prairie soils at the time were declining in organic matter, which negatively impacted the natural fertility and water-holding capacity of soil. The report also mentioned that tillage increased the susceptibility of soil to wind and water erosion and that practices like conservation tillage could address the problem (Senate Committee on Agriculture, 1987).

In the 1990's with renewed interest in soil health and an increase in research funding, a number of scientists studied soil health on the Canadian prairies. For example, Canadian

researchers Gregorich et al. (1994) examined soil quality using a minimum dataset of soil quality indicators that included soil texture, organic matter, pH, bulk density, and rooting depth. Research by Janzen et al. (1998) found that soil carbon was increased by reducing tillage, adding organic amendments, and including perennial vegetation. A study by Angers & Giroux (1996) found that crop residues contributed a larger portion of carbon to macro aggregates than micro aggregates.

Interest in soil health continued, and in 1995, a report titled “*The Health of our Soils: Toward Sustainable Agriculture in Canada*” was published as a follow up to the 1984 Senate report. In the 1995 report, soil health was defined as “The soil’s fitness to support crop growth without becoming degraded or otherwise harming the environment.” This report was a significant milestone for soil health research in Canada and compiled research from 27 sites across Canada. The report highlighted the importance of the 15-30% drop in organic matter when agricultural soils were compared to undisturbed prairie soils. The report summarized that increased soil organic matter led to increases in other important soil characteristics, like soil water holding capacity and soil structure. This report identified the practices that can be used to help improve the health of Canadian soils as well as future research needs for Canadian soils. The report lists the following conservation practices as practices that help improve soil health: crop rotation, conservation tillage, erosion control, permanent cover, reduced fallow, organic recycling, integrated pest management, and water management (Acton & Gregorich 1995).

Soil health research in Canada has continued into the 21st century. A study from southwest Ontario found that active carbon was a sensitive soil health indicator (Hargreaves et al., 2019). A study from Saskatchewan found that a legume cover crop could improve soil aggregate stability when little nitrogen fertilizer was added (Stainsby et al., 2020a). A soil health

scoring framework was developed for Saskatchewan by Wu & Congreves (2022). In addition to this research, soil health remains a priority for politicians. In 2024, a new report was released from the Canadian Senate titled “ Critical ground: why soil health is essential to Canada’s economic, environmental, human and social health” (Senate of Canada, 2024). This latest Senate report emphasizes the continued importance of soil health and makes multiple recommendations to the federal and provincial governments on how to improve soil health with a focus on extension and education.

2.2 Soil Health Tests

When evaluating soil health, a range of measurements are used to assess physical, chemical, and biological soil properties. These metrics are also known as soil health indicators. Soil health is assessed using combinations of these indicators to calculate an index (Moebius-Clune et al., 2016). In North America, the Cornell Soil Health Test and the Haney test are two commercial tests that are growing in popularity. The Cornell soil health test (CSHT), now known as the Comprehensive Assessment of Soil Health (CASH), was developed by Cornell University in Ithica, New York (Idowu et al., 2009). The CASH uses 13 soil health indicators which are indexed to produce a soil health score depending upon the soil textural class and geographic region. The CASH test uses the following indicators: total organic carbon (TOC), active carbon, autoclave citrate extractable (ACE) soil protein index, potentially mineralizable nitrogen (PMN), soil respiration, available water holding capacity (AWHC), surface hardness, subsurface hardness, wet aggregate stability (WAS), extractible phosphorus, extractible potassium, micronutrient elements, iron, zinc, manganese, and magnesium (Fe, Zn, Mn, Mg), and soil pH. One issue with using CASH in Manitoba is that it is calibrated for soil in the northeastern and

midwestern US and not for Manitoban or Canadian soil (Moebius-Clune et al., 2016). There is a need to calibrate CASH to local soils on the prairies, this research has already started in Saskatchewan (Wu & Congreves, 2022) and in Ontario (Congreves et al., 2015).

The Haney soil health test was developed by Dr. Rick Haney of the USDA -Agriculture Research Service (ARS) in Temple Texas. The Haney test assesses soil health using chemical and biological soil health indicators. The Haney test uses the following to assess soil health: Slotiva CO₂, organic N:P, organic C:N, inorganic N and P, organic N, % microbially active carbon, and organic carbon. These indicators are interpreted and then integrated to produce a soil health score ranging from 1 to 50. A major difference between the Haney test and the CASH is that the Haney test does not include any physical soil health indicators (Ohio State University Extension, 2019)

2.2.1 Soil Health Indicators

Soil health indicators are the individual metrics that are used to evaluate soil health. There are two main criteria for a soil health indicator. First, it must be responsive to changes in management (Doran & Jones, 1996). Being responsive to changes in management means that the soil health indicator is dynamic and can be improved or degraded. A soil metric that is unresponsive to management would provide little utility when evaluating how management impacts soil health. The second criterion for a soil health indicator is relevance to crop production. If the indicator does not influence the capacity of soil to produce a crop, it has limited agricultural utility (Moebius-Clune et al., 2016).

There are many potential soil health indicators however, not all of them can or should be used. The collection of soil health indicators used in a soil health test is known as the minimum dataset (Andrews et al., 2004). The purpose of a minimum data set is to maximize the

information gained about soil while minimizing the number of indicators tested. A test using the minimum number of indicators lowers cost and would potentially increase the adoption of soil health testing. Once a minimum dataset of indicators has been chosen, scoring functions must be developed.

2.2.2 Soil Health Scoring

Raw soil health indicator values alone are not very insightful, as they need to be interpreted using soil health scoring functions. These functions are equations developed from a calibration dataset and are used to rate soil health indicator values on a unitless scale. Calibration datasets are a collection of soil health indicator values from the soils with a range of known soil properties. Properly calibrating the scoring functions ensures that they are accurate, as soil health indicator values will vary with geographic location and texture (Moebius-Clune et al., 2016). The scoring functions fall into one of three categories: higher is better, lower is better, or optimum is best. An example of an indicator for each scoring function would be ACE soil protein, where higher is better, surface hardness, where lower is better, and soil pH, where an optimum is best (Moebius-Clune et al., 2016). As soil properties differ geographically, there is a need to calibrate soil health tests for the soils of a region, such as Manitoba or the Canadian Prairies.

2.3 Comprehensive Assessment of Soil Health

The goal of CASH is to assess the health of agricultural soils in a comprehensive manner (Idowu et al., 2009). To achieve this, CASH evaluates the physical, biological, and chemical properties of soil using 13 different soil health indicators. The following sections will review how each soil health indicator in CASH is measured and how indicators meet the two criteria of being relevant to crop production and responsive to management.

2.3.1 Total Organic Carbon

Total organic carbon is a keystone soil health indicator because TOC plays many roles in soil that are agronomically and environmentally important. Increasing TOC can improve soil aggregation (Six et al., 2004), decrease bulk density (Périé & Ouimet, 2008), increase cation exchange capacity (Solly et al., 2020), and increase biological activity in the soil (Krzic et al., 2021). The importance of TOC to soil function and its responsiveness to management makes it a good soil health indicator (Andrews et al., 2004). There is a substantial amount of literature that supports TOC as a soil health indicator.

Several long-term experiments in Canada have quantified the impact of soil management on TOC. VandenBygaart et al. (2010) summarized the impact of management on soil organic carbon across 27 long-term agroecosystem experiments (LTAE) in Canada. Seven LTAE experiments with perennial cropping systems increased TOC on average by 9 Mg C ha⁻¹ after 17 years. For six of the LTAE in the western prairie provinces, the removal of fallow from the rotation increased TOC by 5.2 Mg C ha⁻¹. Research from Lethbridge, Alberta found that 80 years of a continuous wheat rotation decreased TOC by 17% in the top 15 cm of soil, whereas the wheat-fallow rotation decreased TOC by 23% (Monreal & Janzen, 1993). A meta-analysis by VandenBygaart et al. (2003) evaluated the impact of management on SOC across Canada. The meta-analysis found that the conversion of native prairie to agricultural land led to a decrease in SOC by 24%. The study also found that no-till, compared to conventional tillage, increased SOC stock by 2.9 Mg C ha⁻¹. A meta-analysis by Angers & Eriksen-Hamel (2008) evaluated the impact of tillage on soil organic carbon from 23 studies. The meta-analysis found that soil under no-till contained on average 4.9 Mg ha⁻¹ more organic carbon than soil under full inversion tillage.

2.3.2 Active Carbon

There are multiple fractions of carbon in soil that have different properties (Krzic et al., 2021). Active carbon is a measure of the fraction of soil organic carbon that is easily oxidized by soil microorganisms as a source of energy (Moebius-Clune et al., 2016). The difference between active carbon and TOC is that active carbon is a more labile form of soil carbon, making it more sensitive to management (Blair et al., 1995). Because active carbon is more labile than TOC, it detects the effects of management faster (Weil et al., 2003). Active carbon has been found to be responsive to management in the United States. The calibration dataset of soil used to develop the active carbon method included silt loam soils from North Dakota in the Northern Great Plains and was able to detect differences between soils with long term conventional and no-till management history (Weil et al., 2003).

A study on a silt loam soil in Oregon by Awale et al. (2017) found that active carbon was more sensitive to management than TOC. The researchers evaluated the effects of multiple tillage treatments on soil carbon pools and found that active carbon was able to detect differences between treatments better than TOC. Total organic carbon in a grass pasture was 26% greater than no-till and 33% greater than plow tillage. For active carbon, the grass pasture treatment had active carbon levels that were 4% greater than the no-till and 10% greater than the plow tillage. In addition, active carbon was also able to detect that no-till was 6% greater than plow tillage.

Research by Morrow et al. (2016) evaluated the effect of tillage and crop rotation on active carbon across five research stations in Idaho, Oregon, and Washington. All of these research sites had silt loam soils and mean annual precipitation ranging from 200-663 mm. The study found that no-till increased active carbon by 17-68% when compared to conventional tillage. The study found that active carbon was strongly correlated with SOC ($r=0.93$) and total nitrogen ($r=0.93$).

2.3.3 ACE Soil Protein

A portion of the organic nitrogen pool in soil is composed of proteins (Jan et al., 2009). During mineralization, these proteins are depolymerized by soil microorganisms, and the subsequent amino acids become ammonium which can enter the inorganic nitrogen pool in soil. Thus, measuring ACE soil protein can give insight into how much nitrogen may become available to crops throughout the growing season (Hurisso et al., 2018). Subsequently, managing soil protein can make nitrogen management more efficient, which is important environmentally and economically.

The ACE soil protein extraction was originally developed by (Wright & Upadhyaya, 1998) to measure glomalin in soil. Glomalin was believed to be a glycoprotein produced by arbuscular mycorrhizal fungi that had many claimed soil and plant health benefits (Irving et al., 2021). However, subsequent studies found that the ACE soil protein extraction method extracts more than just glomalin and includes a wider array of soil proteins that can withstand the 121 °C temperature that is part of the extraction method (Hurisso et al., 2018).

The ACE soil protein extraction has been found to be responsive to management. Research from Manitoba with clay loam soils found that ACE soil protein was responsive to the tillage treatment when measured in the fall (Crittenden et al., 2024). There was an ACE soil protein increase by 19-10% for the cultivator tillage and raised bed tillage treatments when compared to the vertical tillage or deep tillage treatments. A meta-analysis of 302 soil health studies over the past 40 years from various soil types from across the United States found that the conversion of a full inversion tillage system to a no-till system significantly increased ACE soil protein (Márcio R. Nunes et al., 2020). This responsiveness of ACE soil protein makes it a good soil health indicator.

2.3.4 Potentially Mineralizable Nitrogen

Potentially mineralizable nitrogen is a soil health indicator that measures the release of ammonium from soil during an anaerobic incubation (USDA NRCS, 2014). Similar to soil protein, the goal of potentially mineralizable nitrogen is to estimate the organic nitrogen that may become available throughout the growing season. The difference between PMN and ACE soil protein is that ACE soil protein measures the organic nitrogen pool, whereas PMN measures the ability of soil organisms to mineralize organic nitrogen. This process depends on the soil microorganisms as well as the quantity of organic nitrogen in the soil (USDA NRCS, 2014).

There are multiple methods to measure potentially mineralizable nitrogen. These methods vary in incubation length, redox state, temperature, and moisture content (Carter & Gregorich, 2007; Drinkwater et al., 1996). The CASH uses an anaerobic seven-day incubation to measure potentially mineralizable nitrogen (Moebius-Clune et al., 2016). The benefit of anaerobic incubation is that it protects the ammonium produced from nitrification (Drinkwater et al., 1996). Previous research has found that PMN is responsive to management (Diederich et al., 2019). A study by Diederich et al. (2019) in Arlington, Wisconsin, on a silt loam soil found that a rotational grazing cropping system had approximately 54% more PMN than a continuous corn crop system.

2.3.5 Soil Respiration

Soil respiration measures the activity of soil microbial communities by quantifying the amount of carbon dioxide that is released over a defined period of time. Soil temperature and moisture content are environmental factors that affect soil respiration, while organic residues and microbial populations are biological factors that impact soil respiration (USDA NRCS, 2009).

Understanding the activity of soil microbes is important as they perform and influence many soil processes that impact crop growth, such as cycling nutrients, decomposing crop residues, and building soil structure. Because of the importance of soil microbes to soil function and the responsiveness of soil respiration to agronomic management practices, it is included as a soil health indicator in CASH (Moebius-Clune et al., 2016).

There are multiple examples in the literature of agronomic management practices impacting soil respiration. A study on a loam soil from Ottawa, Ontario, found that a solid dairy manure treatment increased soil respiration by 200-300% (Rochette & Gregorich, 1998) when compared to a conventional fertilizer treatment with 200 kg N ha⁻¹ and an unfertilized control. The authors of the study noted that temperature was a large factor in predicting respiration (Rochette & Gregorich, 1998). A second study on a loamy soil from Michigan found that a perennial polyculture increased soil respiration (Sprunger et al., 2020). After 5 years, the perennial polyculture increased soil respiration by 88 and 23% when compared to an annual monoculture and a perennial monoculture, respectively.

2.3.6 Available Water Holding Capacity

Available water holding capacity (AWHC) is a physical soil health indicator that is defined as the water held by the soil between osmotic potentials of field capacity -10 kPa, and permanent wilting point -1500kpa (Moebius-Clune et al., 2016). Available water holding capacity represents the water that a soil can supply to crops (Krzic et al., 2021). Available water holding capacity is a soil health indicator because AWHC is a factor influencing crop productivity, and AWHC can be affected through soil management.

Research by Moebius et al. (2007) investigated the responsiveness of physical soil health indicators to management at five long-term experiments that spanned three sites in New York State. They found that AWHC was highly sensitive to tillage treatments in long-term experiments. In the no-till treatment, AWHC was 15% higher than in the plow-till treatment. The research by Moebius et al. (2007) concluded that AWHC was a responsive indicator and should be included in a soil health test. This is supported by other research, both Andrews & Carroll (2001) and Doran & Parkin (1994) found that AWHC was a responsive soil health indicator and included it in their minimum dataset for measuring soil health.

2.3.7 Wet Aggregate Stability

Wet aggregate stability (WAS) is a physical soil health indicator included in CASH. Wet aggregate stability is defined as the ability of soil aggregates to withstand the destructive forces from water (Kemper & Rosenau, 1986). Wet aggregate stability is included as a soil health indicator in CASH because soil aggregates improve water infiltration and soil aeration (Kemper & Rosenau, 1986), which are both processes important for crop growth. Wet aggregate stability is also responsive to soil management. The same research by Moebius et al. (2007) that studied AWHC also studied WAS and found that wet aggregate stability was 170% higher in the no-till treatment compared to the plow-till treatment. The use of WAS as a soil health indicator is also supported by research from the Canadian prairies. Research by Stainsby & Entz (2022) found that aggregate stability for the 2-6.3mm size class was ten times greater in a perennial grassland than in an annual conventional cropping system. Research in Saskatchewan by Stainsby et al. (2020b) found that a black medic cover crop increased aggregate stability by 21%.

2.3.8 Surface and Sub-surface Hardness

Surface hardness is a soil health indicator in CASH that measures the penetration resistance of the soil (Moebius-Clune et al., 2016). Surface hardness is a way to measure compaction in the field. With less compaction, plant emergence is improved, and plant roots have increased access to nutrients and water (Krzic et al., 2021). The importance of penetration resistance to crop growth has been noted by researchers looking for potential soil health indicators (Karlen et al., 2001). Moebius et al. (2007) evaluated penetration resistance and found that laboratory-based surface penetration resistance was not a good measurement of soil health. Thus, it was recommended that field penetration resistance measurements be used. Surface hardness has also been found to be responsive on clay soil in both Manitoba (Martino & Shaykewich, 1994) and Saskatchewan (Grant & Lafond, 1993).

Sub-surface hardness is an extension of surface hardness (Moebius-Clune et al., 2016). It is the measurement of penetration resistance to a depth greater than 15 cm. The benefit of measuring penetration resistance to a depth greater than 15 cm is that it will give you an idea of deep soil compaction. This deep compaction can impede root growth, reduce crop yield, and limit the movement of water through the soil profile (McKenzie H. Ross, 2010).

2.3.9 Potassium

Potassium is a macro-nutrient essential to multiple metabolic and physiological processes in crops, deficiency can decrease yield (Krzic et al., 2021). Potassium fertility is largely affected by a soil's parent material, but it is responsive to fertilization with potash or manure (Manitoba Agriculture, 2007). The importance of potassium to crop growth and yield, along with the

responsiveness of potassium to management, makes it a good soil health indicator for many soil types (Moebius-Clune et al., 2016).

Research from southern Manitoba found that potassium fertilization could increase wheat yields at low soil test levels (Elvanek, 1970). The study evaluated five different soils from southern Manitoba that had a range of potassium fertility levels. The study found that potassium chloride fertilization at a rate of 15 lb ac⁻¹ K₂O increased wheat yields by 40% in a soil with a soil test potassium level of 48 ppm. Soils with higher potassium levels were not responsive to potassium fertilization.

2.3.10 Phosphorus

Phosphorus is a macro-nutrient that is used as a soil health indicator. Phosphorus is a good soil health indicator because it is a critical nutrient for crop growth (Krzic et al., 2021) and is responsive to management (Dodd & Mallarino, 2005). There have been multiple studies on the effect of phosphorus fertility on crop yield (Grant et al., 2001 ; Hou et al., 2020). Research by Bailey et al. (1977) found that phosphorus application increased yields in southwestern Manitoba. The study investigated the effect of multiple phosphorous application rates for a wheat-flax rotation. The study contained four research sites. Two of the research sites were on a clay loam near Goodlands Manitoba, and two were on a clay loam near Griswold Manitoba. In the study, multiple rates of superphosphate were broadcast (0, 100, 200, 400 kg ha⁻¹). The 100 kg ha⁻¹ rate was the most consistent and efficient at increasing yields compared to the unfertilized control. In 1966, the 100 kg ha⁻¹ rate increased yield by 32% compared to the unfertilized control, whereas the 400 kg ha⁻¹ rate increased yield by 17% compared to the 100 kg ha⁻¹ treatment.

Research on a clay loam from Brandon Manitoba found that a banded phosphorus application of 22 kg ha⁻¹ increased canola seed yield in three years compared to an unfertilized control (Grant et al., 2009). The increases were 46% in 1999, 14% in 2000, and 16% in 2001. The study also evaluated the response of phosphorous application and tillage and found that there was no response.

2.3.11 Micronutrients

Micronutrients are essential to crop growth but are required in much smaller quantities than macronutrients. The increases in yield from high-yielding varieties, along with the use of synthetic fertilizer, can lead to a drawdown of micronutrients in soil (Shiwakoti et al., 2019). The micronutrient levels for Zn, Mn, Mg, and Fe are used as soil health indicators in CASH. In CASH these micronutrients are combined into one indicator through the use of sub-scores for each micronutrient. Because micronutrient deficiencies can decrease yields, they can serve as a good soil health indicator.

2.3.12 pH

The pH level of soil plays a critical role in many chemical reactions including cation exchange capacity and the availability of nutrients in the soil solution (Krzic et al., 2021). Maintaining soil pH within an optimal range for plant productivity is an important soil characteristic. For this reason, soil pH was included as a soil health indicator in CASH (Moebius-Clune et al., 2016).

2.4 Management Practices

Agriculture is an important type of land management. Over the years soil health has been degraded on the Canadian prairies due to the use of destructive agricultural management practices (Senate Committee on Agriculture, 1987). This degradation, however, can be amended through the use of beneficial agricultural management practices. Knowing that management can improve soil health is important when evaluating soil health indicators. This section will review the beneficial management practices of manure application, perennial crops, cover crops, and tillage.

2.4.1 Manure Application

Livestock manures, such as dairy manure, pig manure, and poultry manure, can be used as a source of fertilizer. There are different application and storage methods for manure. Manure can be stored as a liquid in large, specially designed reservoirs or as a solid in large mounds. Liquid manure is injected into the soil using a variety of field openers, while solid manure is broadcasted and then incorporated into a field (Manitoba Agriculture, 2015).

There is evidence in the literature that manure application can improve soil health. Research by Miller et al. (2021) from Lethbridge, Alberta, found that continuous application of feedlot manure on a clay loam soil increased soil organic carbon by 200-260%. In the study, feedlot manure was applied at multiple rates (0 Mg ha⁻¹, 30 Mg ha⁻¹, 60 Mg ha⁻¹, 90 Mg ha⁻¹) to a dryland cropping system. The study found an increase in TOC between the unfertilized control and the manure application treatments. However, there were no differences in TOC among the different manure application rates.

A meta-analysis from 2014 by Maillard & Angers (2014) found that soil that received manure contained more SOC than non-manured soils. The meta-analysis analyzed 42 studies, totaling 130 observations. The studies in the meta-analysis ranged in length from 3 to 82 years, with the average being 18 years. The depth of the soil in the analysis ranged from 15 cm to 100 cm. Overall, the meta-analysis concluded that soil receiving manure contained higher SOC stocks than soil that received mineral fertilizers. Soil that received manure contained an average of 9.4 Mg ha^{-1} more carbon than soil that received no fertilizer. Compared to soil that received synthetic fertilizers, manure treatments contained more carbon by an average of 5.6 Mg ha^{-1} . The meta-analysis indicated that more research is needed regarding the effect of the manure form (liquid vs solid) and the source of the manure (dairy vs pig vs poultry) (Maillard & Angers, 2014).

A 2021 study found that straw and manure increased soil carbon (Wang et al., 2021). The study examined the effects of dry cattle manure and straw incorporation on carbon and nitrogen sequestration for two depths. The study lasted 360 days and contained two experiment sites, one in the Heilongjiang province and one in the Jilin province of China. The ^{13}C and ^{15}N tracing techniques were used to determine the amount of carbon and nitrogen sequestration. The study contained four amendment treatments and two incorporation depth treatments, 15 cm and 35 cm. The amendment treatments were straw and manure, straw, manure, and an unfertilized control treatment. After 360 days, the researchers concluded that deeper incorporation depth (35 cm) increased carbon sequestration as 36% of carbon was sequestered at a depth of 15 cm compared to 40 % at a depth of 35 cm depth. Incorporation depth had no effect on nitrogen sequestration. In addition, the researchers found that the application of straw and manure enhanced the sequestration of both carbon and nitrogen. Averaged across the two sites, the straw treatments

increased TOC by 2.2 g kg⁻¹ or 15.5%, the manure treatment increased TOC by 0.9 g kg⁻¹ or 5.8%, and the straw and manure treatment increased TOC by 3.4 g kg⁻¹ or 23.9%. For total nitrogen (TN), the straw treatment increased TN by 0.3 g kg⁻¹ or 26%, the manure treatment increased TN by 0.2 g kg⁻¹ or 14%, and the straw with manure treatment increased TN by 0.5 g kg⁻¹ or 40%. Overall, the researchers concluded that the addition of manure and straw led to the greatest increase of SOC and TN in soil (Wang et al., 2021).

2.4.2 Perennial Crops

From a soil health perspective, there is an advantage to growing a perennial crop compared to an annual crop. A perennial crop is a living mulch that provides constant organic cover and protects the soil year-round. The lack of tillage in a perennial crop system allows large and complex root systems to develop. These root systems build soil structure and provide carbon to the soil microorganisms (Baer et al., 2002). In addition to this, a perennial cropping system eliminates the shoulder season fallow period as there is live crop growth throughout the entire growing season.

The benefits of perennial crops to soil health are supported by the literature. A study from Lethbridge, Alberta, found that after 12 years, perennial crops improved soil carbon stocks in the top 30 cm. The study contained four rotations: a fallow-spring wheat rotation, a continuous spring wheat rotation, a crested wheatgrass stand, and a native grass stand. The crested wheatgrass stand increased soil organic carbon by 3 Mg ha⁻¹, while the native grass stand increased SOC by 8 Mg ha⁻¹ (Norris et al., 2023).

A study from Kansas found that converting annual cropland to perennial cropland increased SOC accumulation. The study investigated the effect of growing crested wheatgrass on a silt loam soil in Kansas. The conversion of annual cropland to perennial intermediate wheatgrass increased the SOC accumulation rate by $0.4 \text{ Mg C ha}^{-1} \text{ year}^{-1}$ compared to the annual cropland. The study also found that particulate organic matter carbon and nitrogen were both higher in the intermediate wheatgrass stand compared to soil under annual crop production (van der Pol et al., 2022).

A meta-analysis from the United States found that perennial crops improved multiple soil health indicators. The meta-analysis analyzed 302 studies and compared the effect of tillage treatments (perennial cropping system, chisel plow tillage, and no-till) to the moldboard plow for seven soil health indicators that included SOC, microbial biomass carbon, microbial biomass nitrogen, soil respiration, active carbon, beta-glucosidase, and ACE soil protein. The meta-analysis found that perennial crops improved all the soil health indicator values throughout the soil profile. The authors of the meta-analysis concluded that compared to reduced tillage and no-till, perennial cropping is the best practice for improving soil health (Márcio R. Nunes et al., 2020).

2.4.3 Cover Crops

Cover crops are crops that are grown in between cash crops. The goal of cover cropping is to provide soil cover, add soil carbon, reduce weed pressure, and limit erosion. Cover crops have been found to benefit soil health and prove to be a useful management tool to help improve soil health (Clark, 2007). However, in Manitoba, climate limitations due to the shorter growing

season limit the growth of fall cover crops, which may constrain the potential for soil health gains (Janzen, 2023).

There is evidence in the literature that cover crops can improve physical soil health in the Northern Great Plains. A 2018 study from Brookings, South Dakota, found that cover crops increased the infiltration rate, lowered bulk density, and lowered ground penetration resistance (Chalise et al. 2018). The infiltration rate of the cover crop treatment was 1.8 and 1.3 times higher than the non-cover crop treatment. Bulk density was significantly lower for the cover crop treatment at two depths. For the 0-5 cm depth, the bulk density of the cover crop treatment was 1.3 Mg m^{-3} compared to 1.39 Mg m^{-3} for the non-cover crop treatment. For the 5-15 cm depth, the cover crop bulk density was 1.33 Mg m^{-3} , and the non-cover crop treatment was 1.4 Mg m^{-3} . These bulk density results were supported by the ground penetration-resistance results. This study found that the ground penetration resistance was significantly lower in both the 0-5 cm and the 5-15 cm depths in the cover crop treatment when compared to the non-cover crop treatment. The ground penetration resistance in 0-5 cm depth was 2.2 MPa and 2.76 MPa for cover crop and non-cover crop treatments, respectively. In the 5-15 cm depth, the ground penetration resistance was 2.76 MPa and 2.92 MPa, respectively (Chalise et al. 2018).

A 2020 meta-analysis found that cover crops had a positive effect on the soil biological indicators, such as the soil microbiome (Kim et al., 2020). The meta-analysis examined 60 relevant studies on cover crops from the Web of Science, Scopus, and Google Scholar databases. For soil that had cover crops, the analysis determined that there was a 27%, 22%, and 2.5% increase in soil microbial abundance, activity, and diversity, respectively. The analysis also determined that factors such as climate, termination method, and tillage practices had a significant impact on the effect of the cover crops. A continental climate, chemical termination,

and conservation tillage were found to nullify the benefits of cover crops on the microbial biome. The authors of the study stated that more research is needed surrounding the effects of cover crops and the interactive effects of other soil conservation practices in concert with cover crops on the soil microbiome (Kim et al., 2020).

A second meta-analysis from 2020 examined the effects of cover crops on soil health and soil productivity. The meta-analysis reviewed 269 studies relating to the effect of cover crops on 38 soil health indicators. The meta-analysis used t-tests with an alpha of 0.05 and concluded that out of the 38 indicators, cover crops had a significant effect on 28 of the indicators when compared to the non-cover crop treatment. In terms of the three soil health property categories, the physical, chemical, and biological, cover crops produced a significant difference for all the physical indicators. For the chemical soil health indicators, cover crops significantly affected pH, CEC, and soil organic carbon. For the biological soil health indicators, cover crops significantly affected carbon mineralization, nitrogen mineralization, microbial carbon, and microbial nitrogen. The analysis also noted that the cover crops caused a significant reduction in weed pressure (Jian et al., 2020).

2.4.4 Tillage

Tillage is the process in which soil is physically disturbed using mechanical implements to control weeds and prepare the seedbed for planting. Understanding the negative impacts of tillage on soil health led to the development of conservation tillage practices, such as strip-till and no-till (Opara-Nadi, 1993). A 2018 study on a silt loam soil in Swift Current, Saskatchewan, examined the long-term impacts of rotation and tillage on soil organic carbon and found that no-till can increase soil organic carbon levels (Maillard et al., 2018). The experiment started in 1982

and contained three rotations: a wheat and fallow rotation, a continuous wheat rotation, and a pulse wheat rotation. Three tillage treatments were also present in the study: a conventional tillage treatment, a minimum tillage treatment, and a no-till treatment. After 21 and 25 years in the continuous wheat rotation, the no-till treatment contained significantly higher soil organic carbon over the minimum and conventional tillage treatments. For 21 years and 25 years, the no-till treatment contained 15% and 12% higher soil organic carbon stocks, respectively, when compared to the other tillage treatments (Maillard et al., 2018).

A 2018 study from New York State found that no-till improved soil health for three soil types: a clay loam, a loamy sand, and a silt loam. The study examined the long-term impact of cover crops and two different tillage treatments, no-till and plow-till, on multiple biological, physical, and chemical soil health indicators in a corn mono crop. The soil under no-till management contained 17% more organic matter, 65% more soil protein, and 76% higher wet aggregate stability than the soil under plow-till management (Márcio Renato Nunes et al., 2018).

A 2021 study from the Jilin province of China examined the effects of tillage systems on soil water retention curves for a silt clay loam soil in a corn production system in both the field and in the lab setting. The study contained a conventional tillage (CT) treatment, a rotational tillage (RT) treatment, and a no-till (NT) treatment. The study found that the no-till treatment retained more water across all matric potentials for all three depths, 0-5 cm, 5-10 cm, and 15-20 cm. For example, the average water content by the 9th of October was $0.25 \text{ cm}^3\text{cm}^{-3}$ for RT, $0.32 \text{ cm}^3\text{cm}^{-3}$ for CT, and $0.38 \text{ cm}^3\text{cm}^{-3}$ for NT. An interesting finding of this study was that the laboratory water retention analysis was less sensitive and found fewer differences between the tillage treatments than the field analysis. The researchers of the study noted that the differences between the laboratory and field results could be attributed to the differences in the level of air

entrapment in the mesopores, which was affecting the ability of those pores to retain water (Huang et al., 2021).

A 2020 meta-analysis of tillage practices across Canada found that on the Canadian prairies, no-till systems were effective at increasing soil organic carbon (SOC). The analysis found that soil on the prairies accumulated carbon at a higher rate in the first years after the switch from a conventional tillage (CT) system to a no tillage (NT) system. The study also found that the rates of SOC accumulation declined as time in NT progressed. The analysis stated that in the 3 to 10 year period after the switch from CT to NT SOC accumulated at a rate of 740 kg C ha⁻¹ year⁻¹ or 1.3%. During the 11 to 20 year period after the switch to NT the SOC accumulation rate decreased to 260 kg C ha⁻¹ year⁻¹ or 0.87% and after 20 plus years of being in a NT system, the rate of carbon accumulation decreased to 95 kg C ha⁻¹ year⁻¹ or 0.23%. Despite the inverse relationship between the length of time under NT and the rate of SOC accumulation, the study noted that SOC still accumulated in a NT system when compared to a CT system every year (Liang et al., 2020).

2.5 Summary

Progress has been made in public policy and public awareness surrounding the importance of soil health. This increased awareness has led to the creation of multiple groups that focus on studying and advocating for soil health. As a result of the increased awareness, numerous studies investigating soil health have been conducted over the past three decades. This has led to the development of methods for assessing soil health, such as the CASH and the Haney tests. Current research on soil health indicators has shown that they can be responsive to management practices, such as conservation tillage, cover cropping, and manure application. However, due to

the variability of agricultural growing regions and soil types, the ability of soil health indicators to quantify the effects of management practices on soil health is not fully understood. There is a gap in the understanding of how combinations of management practices affect soil in Manitoba and on the Canadian prairies. This gap demonstrates the need for more research surrounding the combined effects of multiple conservation practices on contrasting soil types.

In conclusion, there is still a research gap in the understanding of which soil health indicators are responsive to management and could be used to assess soil health in Manitoba. Moving forward, more research is needed to calibrate CASH to Manitoban soils. Other provinces, like Saskatchewan (Wu & Congreves, 2022) and Ontario (Congreves et al., 2015), have created a calibration dataset by collecting samples from a large number of commercial farm fields. Manitoba should take a similar approach and create a calibration dataset using on-farm data. With a properly calibrated Manitoban soil health test, farmers and agronomists would be able to accurately measure the impact of management practices on soil health.

3.0 Materials and Methods

3.1 Site Description

The National Center for Livestock and the Environment long-term nutrient management study (The NCLE experiment, hereafter) is located in Glenlea, Manitoba ($49^{\circ}38'15.2''\text{N}$ $97^{\circ}09'27.3''\text{W}$). The field soil for this experiment was mapped as an imperfectly drained gleyed black chernozem composed of 50% Scantebury and 50% Red River series. These soils have clay texture and were formed from calcareous lacustrine parent material deposited by ancient glacial Lake Agassiz (Manitoba, 2024). The NCLE experimental site is nearly level with 0-0.5% slope, is not stony, and has an agricultural capability rating of Class 2 with an excess water limitation (Manitoba Agriculture, 2011). The experimental site has a humid continental climate, receiving an average of 542 mm of precipitation throughout the year with 210-220 mm falling during the growing season from May-August. The site receives an average of 105-115 frost-free days. (Manitoba Agriculture, 2022). An aerial image of the site can be seen in Figure 3.1.



Figure 3.1. Aerial image of the National Center for Livestock and the Environment long-term nutrient management study in Glenlea, MB.

3.2 Experimental Design

The experiment was a randomized complete block design with four replications and a split-split plot treatment structure. Each replicate consisted of two main plot treatments arranged as a split block design, known as the crop history treatments (Figure 3.3). The two main plot treatments were annual crop history and perennial crop history. Each main plot strip was 20 m wide and 345 m long. There was a 30 m wide crop alley between the main plot strips. The alleys were managed by growing either alfalfa or an annual crop that was different from the experimental crop. The main plots were perpendicularly split into subplots known as the soil fertility treatments (Figure 3.3). Each subplot was 20 m wide and 345m long with a 5 m buffer strip between subplots. The buffer strips between sub plots contained the same crop as the subplots but did not receive a fertility application. These buffer strips were used to prevent edge effects

between the fertility treatments. Out of the 12 original crop fertility treatment levels present in the NCLE experiment (Figure A.1.1), only four treatments were examined in this current study. These treatments were: the unfertilized control, synthetic fertilizer, liquid pig manure applied to crop nitrogen demand, and solid dairy manure applied to meet crop nitrogen demand.

The soil management treatment was included in the experimental design by adding a second split parallel to the main plot crop history treatment to create sub-subplots (Figure A.1.1). The sub-subplots were 10 m wide and 345 m long and consisted of two soil management treatment levels: soil building (no-till plus cover crops during the shoulder seasons) and conventional (chisel plow cultivation plus fallow during shoulder season). An image of the sub-subplot split can be seen in Figure 3.2. The sub-subplot treatment was not randomized; rather the northern sub-subplot was always the conventional soil management treatment, and the southern sub-subplot was always the soil building management treatment.



Figure 3.2. Image from May 2022 of the second plot split. Left is the soil-building treatment, and right is the conventional treatment.

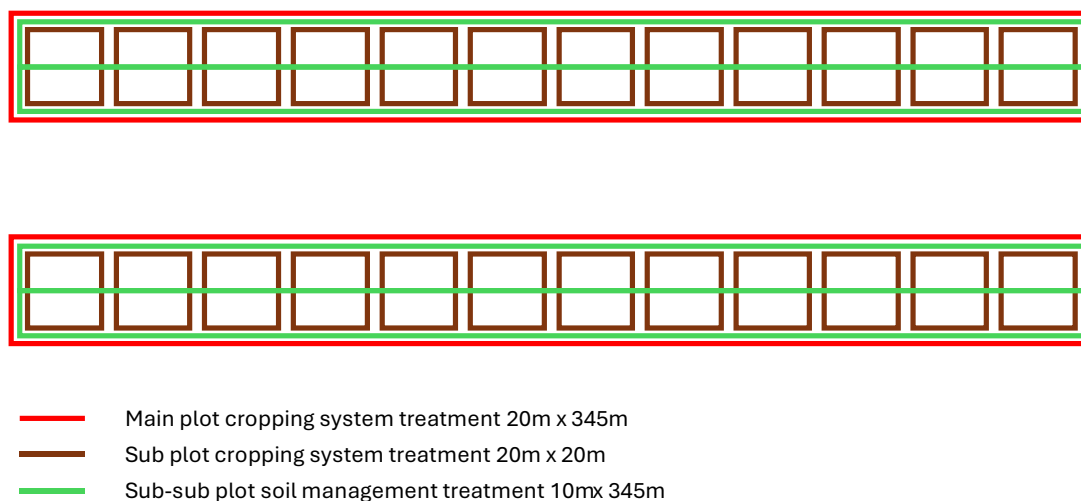


Figure 3.3. One replicate of the National Center for Livestock and the Environment long-term nutrient management study illustrating the split-split plot experimental design and size of experimental units. The red line outlines the cropping system history main plot treatment with annual and perennial cropping system treatment levels. The brown line outlines the soil fertility sub plot treatment with synthetic fertilizer, liquid pig manure, solid dairy manure, and unfertilized control treatment levels. The green line outlines the soil management sub-sub plot treatment with conventional and soil building treatment levels.

3.3 Experiment History

It is important to understand the detailed history of each of the treatments to help interpret the results of this study. Throughout the years, there have been various changes to the treatments at the NCLE experiment. The most notable changes were the termination of the perennial plots in 2019 and the cessation of manure application to the fertility treatments in 2015. A timeline of the NCLE treatment history can be seen in Figure 3.4. Overall, the NCLE experiment is a good place to study soil health because it has contrasting treatments that influence soil health and 13 years of well-documented experimental records.

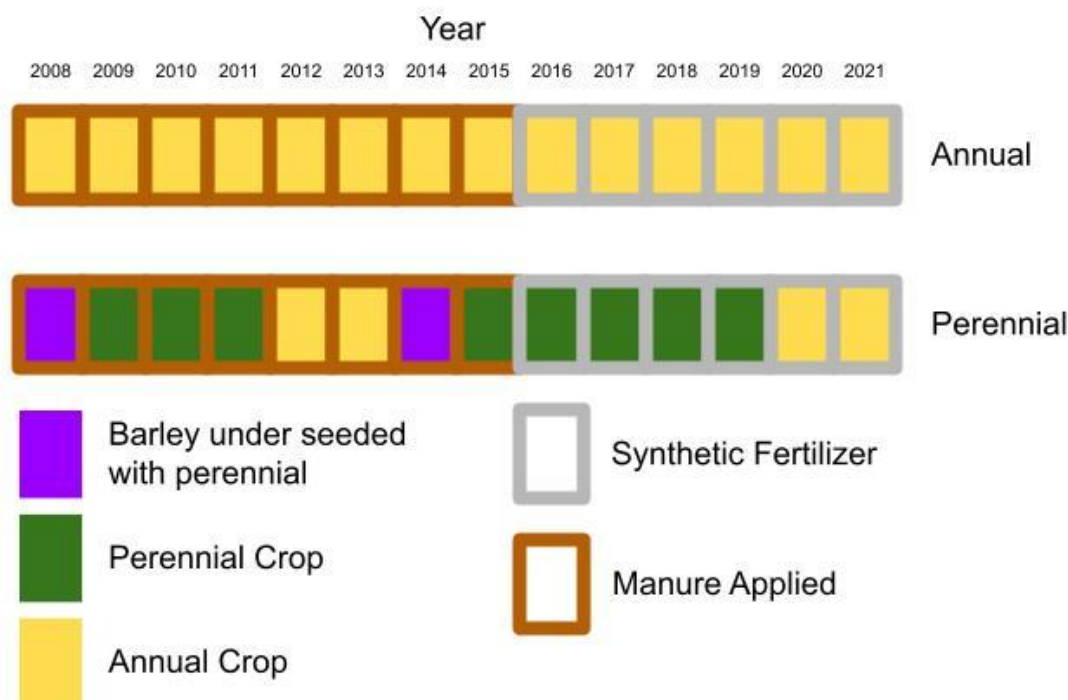


Figure 3.4. Crop history treatment timeline (2008-2021) for the annual and perennial crop main plot treatments in the National Center for Livestock and the Environment long-term nutrient management study. Annual crops were grown in the annual crop rotation treatment. In the perennial crop treatment, alfalfa was under seeded to barley in 2008 and 2014 for establishment. Perennial crops were grown for 3 year (2009-2011) and 5 years (2015-2019) periods. In the perennial crop treatment, annual crops were grown from 2012-13. Annual and perennial crops were grown with a range of fertility treatments including manure, synthetic fertilizer, and an unfertilized check from 2008 to 2015. In 2016, manure applications ended for the remainder of the study and synthetic fertilizer was applied annually to all treatments with the exception of the unfertilized check.

3.4 Soil Sampling

One 7.62 cm wide by 15.24 cm long by 15.24 cm slice of soil was taken as a sample from each plot on November 4th, 2020, using a square, straight cutting-edge spade. This sampling technique is known as the bread slice technique (Moebius-Clune et al., 2016). The samples were placed into large plastic bags and stored in a refrigerator at 4°C as soon as field sampling was completed.

3.5 Soil Processing

The samples were processed the week after they were collected. There were multiple steps to soil processing. In the first step, soil was passed through a 6.35 mm square mesh screen to homogenize the sample and help with drying. A portion of the 6.35 mm sieved sample was kept at field moisture content, while the remainder was moved to the next step. In the second step, the soil was air-dried for a week at 25°C, and samples were weighed to ensure the soil reached a constant weight. Some of the air-dried soil sample was pulverized using a 10000-rpm rotary grinder (Christie & Norris 8" Laboratory and Soil Mill, Ipswich United-Kingdom). A second portion of the air-dried sample was sieved to 2 mm. A flow chart of the soil processing is provided in Figure 3.5.

3.6 Soil Storage

Due to the individual requirements of the methods for the soil health indicator tests, the soil samples were stored at multiple temperatures and moisture contents. The 6.35 mm sieved soil was stored air-dried at room temperature and used for soil respiration analysis. Air-dried pulverized soil was stored at room temperature and used for TOC, soil protein, Olsen phosphorus, potassium, and micronutrient analysis. Some of the 6.35 mm air-dried soil was sieved to 2 mm and used for WAS, active carbon, and AWHC analysis. A portion of the 6.35 mm sieved soil was kept fresh frozen at -20°C; this was used for PMN analysis. The remaining 6.35 mm screened air-dried soil was stored in a refrigerator at 4°C as a reserve. A flow chart in Figure 3.5 depicts the soil health indicator test and its corresponding soil storage requirement.

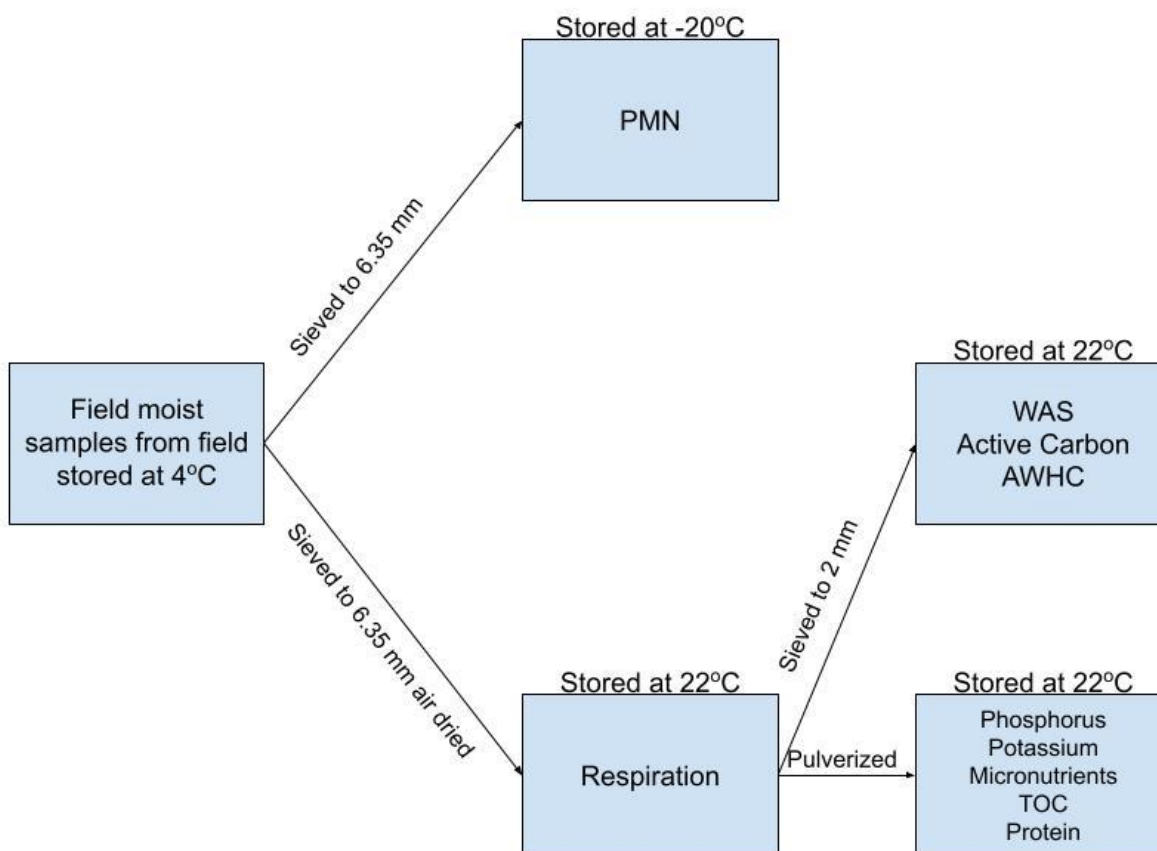


Figure 3.5. Flow chart of soil processing and storage for potentially mineralizable nitrogen (PMN), wet aggregate stability (WAS), available water holding capacity (AWHC), total organic carbon (TOC), active carbon, phosphorus, potassium, micronutrients, and ACE soil protein (Protein).

3.7 Soil Health Indicators

Thirteen different soil health indicators from the comprehensive assessment of soil health (CASH) were tested in this study. The two physical indicators that were measured are available water-holding capacity (AWHC) and wet aggregate stability (WAS). The five biological indicators tested for were active carbon (POXC), total organic carbon (TOC), ACE soil protein,

potentially mineralizable nitrogen (PMN), and soil respiration. The six chemical soil health indicators tested for were potassium, phosphorus, zinc, magnesium, manganese, and iron.

3.7.1 Available Water Holding Capacity

Available water holding capacity was measured using the pressure plate method described by Carter & Gregorich (2007). To test AWHC, 2 mm sieved air-dried soil was placed in a rubber retaining ring and lightly packed. The rings were prepared on either a high-pressure or low-pressure ceramic plate. The ceramic plates (Pressure Plate Extractor Soilmoisture Equipment Corp, Goleta USA) were hydrated with deionized water for eight hours before the rings were prepared. After the rings were prepared, the plates with the rings were hydrated for eight hours using deionized water. Figure 3.6 shows the hydrated discs and rings loaded in the pressure chamber. Two sets of pressure plates were used in this study. A low-pressure 0.1 bar plate representing field capacity, and the high pressure 15 bar plate representing the permanent wilting point. To create the pressure needed in the 0.1 bar chamber, pressurized air, an air regulator, and a mercury column were used. To achieve the target pressure in the 15 bar-chamber, a high pressure tank of nitrogen gas and a series of pressure regulators (Press plate extractor, Soilmoisture Equipment Corp, Goleta USA) were used. The threshold for a chamber to have reached equilibrium was two consecutive days without water being expelled from the chamber. Once the soil rings had reached equilibrium, they were weighed, and those weights were used to calculate AWHC using equation 3.1, where GWC is the gravitational water content in percent.

$$\text{AWHC\%} = (\text{GWC\% at 0.1 bar} - \text{GWC\% at 15 bar}) \quad [\text{Equation 3.1}]$$

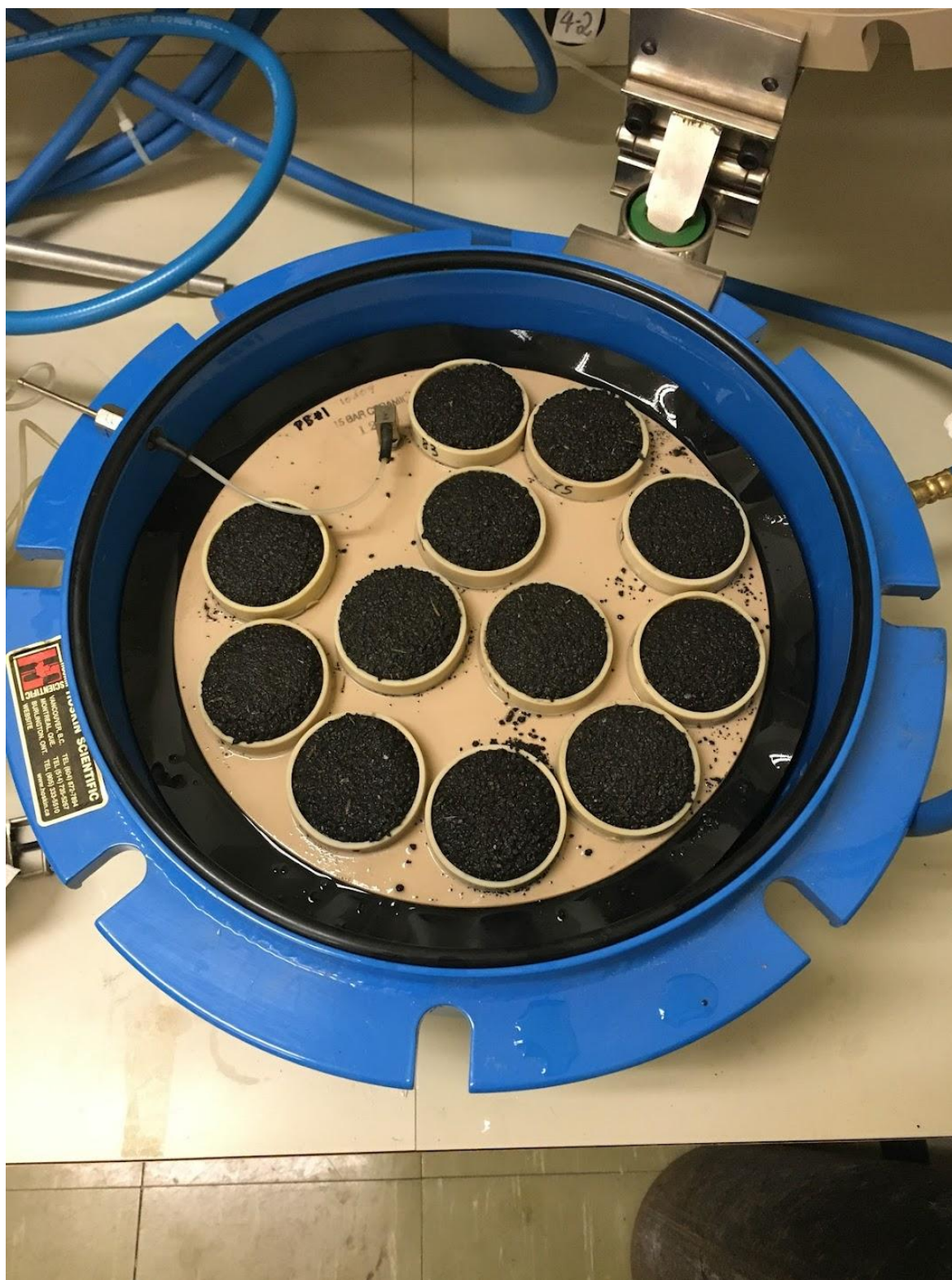


Figure 3.6. An open pressure chamber with hydrated ceramic disks and soil rings.

3.7.2 Wet Aggregate Stability

Moebius-Clune et al. (2016) described the wet aggregate stability water droplet method used in this study. For the method, 30 g of 2 mm sieved soil was placed on a 0.25 mm sieve. A filter paper was placed under the 0.25 mm sieve to catch aggregates that fell through the 2 mm sieve. The Cornell Infiltrator (Cornell Sprinkle Infiltrator, Cornell University, Ithica USA) was suspended above the sample from a height of 0.5 m (Figure 3.7) and ran at a target flow rate of 2.2 mm min⁻¹ for 5 minutes. While the infiltrator was running, it was given a very gentle push every minute to change the area of droplet impact. After 5 minutes, the soil in the filter below the sieve was collected and labeled as water-unstable aggregates. The soil remaining in the 0.25 mm sieve was collected and labeled as water-stable aggregates. Both were oven-dried at 105°C for 48 hours and weighed. The weights were used to calculate the wet aggregate stability using Equation 3.2 (Cornell University, 2022).

$$\text{Wet Aggregate Stability}\% = \left(\frac{\text{Water stable aggregates (g)}}{\text{Total soil (g)}} \right) \times 100 \quad [\text{Equation 3.2}]$$



Figure 3.7. Wet aggregate stability testing equipment consisting of a Cornell infiltrrometer suspended above soil on 0.25 mm sieve that is sitting on a funnel and trivet.

3.7.3 Total Organic Carbon

Air-dried and pulverized soil was used to measure total organic carbon. The analysis was performed by AgVise Laboratories (Soil Testing Agvise Laboratories Norwood USA). Nelson & Sommers (1996) described the dry combustion method used to determine total organic carbon. Because the soil might contain inorganic C in the form of salts of carbonate, a soil carbonate

correction was performed (Sherrod et al., 2002). Total soil organic carbon was calculated using Equation 3.3.

$$\text{TOC} \left(\frac{\text{g}}{\text{kg}} \right) = \text{Total Carbon} - \text{Calcium Carbonate Equivalent} \quad [\text{Equation 3.3}]$$

3.7.4 Active Carbon

Weil et al. (2003) described the method for active carbon analysis. For the method, 2.5 g of 2 mm air-dried sieved soil was placed in a 50 mL centrifuge tube with 20 ml of 0.02 M KMnO_4 and 0.1 M CaCl_2 . The tube was shaken and left to settle, and then 0.5 mL of the supernatant was added to a new 50 mL centrifuge tube. The supernatant was diluted 10:1 using deionized water (Figure 3.8), and a spectrometer cuvette was filled with the dilutant. The absorbance of the cuvettes was read at a 550 nm wavelength using a spectrophotometer (Ultrospec 2100 pro, Harvard Bioscience Inc, Holliston USA)

To produce the calibration curves needed to calculate the amount of active carbon in the samples, standard solutions were prepared along with the samples. Active carbon (POXC) was calculated using Equation 3.4, where a is the intercept of the calibration curve, and b is the slope of the calibration curve (Weil et al., 2003). For quality control purposes, the samples were run in triplicates, and the average value of the triplicate was used to calculate the mean. For the triplicates, if there was a technical replicate that deviated greater than 10% from the mean, the technical replicate was discarded.

$$\text{POXC mg Kg}^{-1} = (0.02 \text{ Mol L}^{-1} - (a + b \times \text{absorbance})) \times (9000 \text{ mg C Mol}^{-1}) \times \left(\frac{0.02 \text{ L solution}}{0.0025 \text{ Kg soil}} \right) \quad [\text{Equation 3.4}]$$



Figure 3.8. Image of diluted supernatant for active carbon analysis.

3.7.5 ACE Soil Protein

Moebius-Clune et al. (2016) described the method used to measure ACE soil protein. For the method, 3 g of air-dried and pulverized soil was placed into a 50 mL heat and pressure stable glass test tube. Next, 24 mL of 20 mM sodium citrate solution (pH 7) was added to the test tubes. The tubes were autoclaved for 30 minutes at 15 psi and 121°C. Afterward, the tubes were left to cool (Figure 3.9), and the contents were transferred to 50 mL centrifuge tubes. The samples were centrifuged to 10,000g rotational centrifugal force (RCF) to separate the soil particles from the autoclaved citrate extractant. A bicinchoninic acid (BCA) protein assay (Pierce BCA Protein Assay, Thermo Fisher Scientific, Waltham USA) was performed to measure the concentration of protein in the supernatant. For quality control purposes, an albumin bovine serum standard curve was created for each run of the samples. A (Ultrospec 2100 pro, Harvard Bioscience Inc, Holliston USA) was used to measure the absorbance of the assay dilutant at 562 nm. The ACE soil protein was calculated using equation 3.5, where Absorbance is the absorbance at 562 nm, m is the slope of the standard curve, and b is the intercept of the standard curve.

$$\text{ACE soil protein (mg g}^{-1}\text{)} = \frac{\left(\frac{\text{Absorbance}-b}{m}\right) \times \text{volume of supernatant (ml)}}{\frac{\text{weight of soil (g)}}{1000}} \quad [\text{Equation 3.5}]$$



Figure 3.9. Soil and citrate slurry in test tubes after autoclave treatment.

3.7.6 Potentially Mineralizable Nitrogen

Moebius-Clune et al. (2016) described the method used to measure potentially mineralizable nitrogen (PMN). Potentially mineralizable nitrogen was produced using frozen soil that was thawed for 24 hours at 25°C. For the method, 8 g of fresh frozen soil was placed in a 237 mL Boston round, and then 10 mL of distilled water was added. An anaerobic environment was created by filling the headspace gas of the Boston round with nitrogen gas, the nitrogen gas injection apparatus can be seen in Figure 3.10. The Boston round was then incubated for seven days at 30°C. After the incubation, ammonium and nitrate were extracted with 2 M KCl. Ammonium and nitrate were also extracted from the fresh frozen soil that had not been incubated.

For the ammonium and nitrate extractions, the soil slurries were shaken for one hour. The suspended soil was then separated from the extraction solution by centrifuging the solution to 3000 rpm for 2 minutes. The supernatant was frozen at -20°C for storage until ammonium and nitrate determination could be performed using the Technicon II segmented autoanalyzer (Technicon Auto Analyzer II, SEAL Analytical, Mequon United States). The nitrate concentrations from the fresh frozen and the incubated fresh frozen soil were used to calculate the amount of potentially mineralizable nitrogen using Equation 3.6.

$$\text{PMN (mg kg}^{-1}\text{days}^{-1}) = \frac{\frac{\text{Incubated soil nitrate (mg Kg}^{-1}) - \text{fresh frozen soil nitrate (mg Kg}^{-1})}{\text{Oven dry soil weight (g)}}}{\text{Incubation time (days)}} \quad [\text{Equation 3.6}]$$



Figure 3.10. Nitrogen gas injection apparatus for filling Boston rounds with nitrogen gas.

3.7.7 Soil Respiration

Moebius-Clune et al. (2016) described the method that was used to measure soil respiration. To measure soil respiration, a Whatman 40 filter paper was placed in a 250 mL canning jar. Then 20 g of air-dried 6.35 mm sieved soil was added to the jar, and 10 mL deionized water was poured gently onto the inside wall of the jar; this avoided any aggregate destruction. A stand with a beaker containing 0.5 M potassium hydroxide was placed in the jar. The jars were sealed and left to incubate in the dark for four days at 25°C. An image of the incubation jar with the beaker can be seen in Figure 3.11. After the four-day incubation period, the EC of the potassium hydroxide solution was measured using an EC meter (Accumet 500xl, Thermo Fisher Scientific Waltham USA). Soil respiration was calculated using Equation 3.7. Where EC Stock was the EC of the stock solution, EC Incubation was the EC of the solution after incubation, and EC saturated was the EC of a solution saturated with carbon dioxide. The EC of a potassium hydroxide solution fully saturated with carbon dioxide was assumed to be 42.6 ms cm⁻¹. (Schindelbeck & Kurtz, 2013).

$$\text{Respiration g Kg}^{-1} = \frac{\text{EC Stock} - \text{EC Incubation}}{\text{EC stock} - \text{EC saturated}} \times 82.519 \quad [\text{Equation 3.7}]$$



Figure 3.11. Jars containing soil and potassium hydroxide trapping solution used in soil respiration test.

3.7.8 pH

The pH level was measured using a pH meter (Accumet XL 500, accuTupH meter, Thermo Fisher Scientific Waltham USA). A 2:1 RO water to pulverized soil slurry ratio was used due to the nature of the clay soil (Figure 3.12). This methodology was based on that described by Carter & Gregorich (2007).



Figure 3.12 Image of pH meter testing soil slurry.

3.7.9 Phosphorus, Potassium and Micronutrients

Pulverized, air-dried soil samples were sent to AgVise Laboratories (Soil Testing Agvise Laboratories Norwood USA) to determine the plant available phosphorus, potassium, zinc, iron, manganese, and magnesium. The following methods were used for each nutrient. For soil phosphorus, the Olsen phosphorus method (Frank, Beegle & Joesph, 2015). For potassium, a 1 M ammonium acetate extraction with an atomic absorption spectrometer determination (Warncke & Brown, 2015). For iron and manganese, a diethylenetriamine pentaacetate (DPTA) extraction method (Whitney, 2015) with an inductively coupled plasma (ICP) determination. For zinc, a 0.1 M HCl extraction with an atomic absorption spectrometer determination (Whitney, 2015). For magnesium, a 1 M ammonium acetate extraction with an atomic absorption spectrometer determination (Warncke & Brown, 2015).

3.8 Statistical Analysis

The data was modeled as a Generalized Linear Mixed Model (GLMM) using Proc Glimmix in SAS 9.4. (SAS 9.4, SAS Institute Cary, USA). The fixed effects in the model were crop history, fertility history, and soil management. The random effects in the model were block, and the block by main effect interactions which included the following: block by crop history, block by crop fertility and block by soil management. Proc Glimmix was used to perform an analysis of variance (ANOVA) with a significance value of 0.05. The normality of the data was tested by performing a Shapiro-Wilk test using the Univariate procedure in SAS. The p-value from the Shapiro-Wilkes test was used to assess normality. The homogeneity of variance was assessed using lag plots of the residuals. If lag plots indicated that the variance of the residuals was heterogenous, then a transformation was performed. The two transformations were log-normal or

beta. Both the corrected and uncorrected models were run, and the Akaike Information Criterion (AIC) was used to determine if the transformation improved the model. The indicators modeled with a normal distribution were TOC, PMN, ACE soil protein, soil respiration, and pH. The indicators modeled with a log-normal distribution were active carbon, phosphorus, potassium, zinc, iron, manganese, and magnesium. The beta distribution was used for AWHC and WAS, a beta distribution was used for both these indicators as they're values were constrained between 0 and 1. Pairwise comparisons with a 0.05 significance level were then performed, and the Tukey-Kramer method was used to test for the least significant difference among treatment means. For the log-normal modeled estimates, the OMEGA back transformation method was performed (Edwards, 2017). Treatment main effect means were separated using a post hoc analysis with a significance level of 0.05.

4.0 Results and Discussion

This section will review the soil health indicator results from the NCLE experiment, discuss how they compare to current research, and determine if the hypotheses presented in the introduction of this thesis were rejected based on the results. The main objective of this study was to determine if the indicators found in CASH would be responsive to agronomic management on humic vertisol in the Red River Valley of Manitoba. Responsive soil health indicators in this study were the indicators that could detect differences among the treatments in the NCLE experiment using a Tukey LSD test with a significance level of $P < 0.05$. Unresponsive indicators were indicators that could not detect differences within treatments.

An ANOVA was performed on the 14 soil health indicators measured in the study with a significance level of $\alpha = 0.05$. Indicators were responsive to both the fertility and crop history treatments but not to the soil management treatment. This section will only focus on main effects as there were no significant treatment interactions. Of the 14 soil health indicators tested, nine were responsive to one or more of the study treatments. Four soil health indicators responded to the crop history treatment (WAS, soil respiration, PMN, potassium) and seven of the soil health indicators were responsive to the crop fertility treatment (TOC, active carbon, ACE soil protein, PMN, Potassium, Phosphorus, Zinc) (Table 4.1, 4.2). Two of the indicators (PMN and potassium) were responsive to both the crop history and the crop fertility treatments. None of the indicators were responsive to the soil management treatment.

In order to help explore the underlying mechanisms of the responses, the results will be organized according to the crop history and fertility treatments that the indicators were responsive to. Radar plots were used to explore treatment patterns for groups of soil health indicators.

Table 4.1. Analysis of variance (ANOVA) for biological and physical soil health indicators including wet aggregate stability (WAS), available water holding capacity (AWHC), total organic carbon (TOC), active carbon (POXC), ACE soil protein (soil protein), potentially mineralizable nitrogen (PMN), and soil respiration (Respiration) as affected by ...

Effect	WAS	AWHC	TOC	POXC	Soil Protein	PMN	Respiration
	-----Pr>F-----						
History [†]	0.0002	0.9481	0.8231	0.9534	0.6203	0.0385	0.0449
Fertility ^{††}	0.8189	0.3291	0.0138	0.0047	0.0465	0.0022	0.4876
Management ^{†††}	0.8981	0.1931	0.7152	0.8360	0.5408	0.8948	0.3688
History*Fertility	0.5851	0.9234	0.9130	0.9675	0.9174	0.6758	0.8333
History*Management	0.7044	0.5714	0.9388	0.4806	0.7345	0.4311	0.2703
Management*Fertility	0.0882	0.4273	0.6863	0.9372	0.8630	0.9215	0.8816
History*Management*Fertility	0.5318	0.2458	0.9217	0.9074	0.7858	0.5257	0.4317

[†] Cropping system history main treatment effect with annual and perennial cropping system treatment levels.

^{††} Soil fertility main treatment effect with synthetic fertilizer, liquid pig manure, solid dairy manure, and unfertilized control treatment levels.

^{†††} Soil management main treatment effect with conventional and soil building treatment levels.

Table 4.2. Analysis of variance (ANOVA) for chemical soil health indicators including potassium (K), phosphorus (P), zinc (Zn), iron (Fe), manganese (Mn), magnesium (Mg), and pH. As affected by ...

Effect	K	P	Zn	Fe	Mn	Mg	pH
	-----Pr>F-----						
History [†]	0.0366	0.9289	0.3877	0.6254	0.9035	0.6718	0.9481
Fertility ^{††}	<0.0001	0.0005	<0.0001	0.6071	0.1217	0.5746	0.3291
Management ^{†††}	0.7751	0.2355	0.3091	0.2889	0.1823	0.3291	0.1931
History*Fertility	0.6342	0.647	0.9211	0.9003	0.7805	0.9934	0.9234
History*Management	0.5447	0.5887	0.3506	0.7719	0.8904	0.2784	0.5714
Management*Fertility	0.9940	0.2600	0.7780	0.1743	0.9351	0.3594	0.4273
History*Management*Fertility	0.2880	0.4453	0.7544	0.8953	0.9275	0.5949	0.2458

[†] Cropping system history main treatment effect with annual and perennial cropping system treatment levels.

^{††} Soil fertility main treatment effect with synthetic fertilizer, liquid pig manure, solid dairy manure, and unfertilized control treatment levels.

^{†††} Soil management main treatment effect with conventional and soil building treatment levels.

Table 4.3. Main effects means and standard error of physical and biological soil health indicators including wet aggregate stability (WAS), soil respiration (respiration), potentially mineralizable nitrogen (PMN), and Potassium (K) as influenced by the treatments and treatment levels in the NCLE experiment.

Effect [‡]	Treatment level ^{‡‡}	WAS	AWHC	TOC	POXC	Soil protein	PMN	Respiration
		%	%	(g kg ⁻¹)	(mg kg ⁻¹)	(ug g ⁻¹)	(mg g ⁻¹ day ⁻¹)	(CO ₂ mg g ⁻¹ day ⁻¹)
History	Annual	92.98 a (0.56)^{††}	22.00 (0.94)	30.01 (2.4)	795.22 (14.15) [†]	3.56 (0.22)	2.36 b (0.16)	0.81 a (0.06)
	Perennial	95.99 b (0.38)	22.32 (0.95)	30.03 (2.4)	793.05 (14.11)	3.69 (0.23)	3.38 a (0.23)	1.02 b (0.06)
Management	Building	94.8 (0.39) [†]	21.57 (0.81)	30.03 (2.2)	791.80 (14.08)	3.67 (0.19)	2.83 (0.16)	0.90 (0.06)
	Conventional	94.56 (0.40)	22.75 (0.84)	30.00 (2.2)	796.48 (14.17)	3.58 (0.18)	2.82 (0.16)	0.93 (0.06)
Fertility	Control	94.51 (0.68)	20.93 (1.07)	20.68 b (2.4)	736.58 b^{††} (18.53)	3.00 b (0.25)	2.25 b (0.16)	0.93 (0.07)
	Liquid Pig	95.21 (0.61)	21.11 (1.08)	20.96 b (2.4)	807.29 ab (20.31)	3.91 ab (0.33)	3.07 a (0.22)	0.85 (0.07)
	Manure	94.57 (0.67)	22.96 (1.14)	30.51 a (2.4)	884.46 a (22.25)	4.24 a (0.36)	3.48 a (0.25)	0.94 (0.07)
	Solid Dairy	94.41 (0.69)	23.73 (1.16)	20.9 b (2.4)	756.70 b (19.04)	3.50 ab (0.30)	2.66 b (0.19)	0.94 (0.07)
	Manure							
	Synthetic Fertilizer							

[†] The value in the parenthesis represents the standard error of the soil health indicator value.

^{††} Values in a column with the same letter do not have a significant difference

[‡] The main treatment effects in the NCLE experiment are crop history (History), soil management (Management), and soil fertility (Fertility).

^{‡‡} Treatment levels in the NCLE experiment are annual crop history (Annual) and perennial crop history (Perennial) for the crop history treatment effect. Soil building (Building) and conventional for the soil management treatment effect. Unfertilized control (Control), liquid pig manure, solid dairy manure, and synthetic fertilizer for the soil fertility treatment effect.

Table 4.4. Means and standard error of chemical soil health indicators including potassium (K), phosphorus (P), zinc (Zn), iron (Fe), Manganese (Mn), Magnesium (Mg), pH.

Effect [‡]	Treatment level ^{‡‡}	K	P	Zn	Iron	Mn	Mg	pH
		(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	
History	Annual	603 (30.47)	28 (4.21)	3.37 (0.53)	34.88 (4.57)	6.38 (1.18)	1457.33 (46.08)	7.36 (0.15)
	Perennial	543 (27.47)	29 (4.29)	2.86 (0.45)	37.69 (4.94)	6.50 (1.19)	1433.32 (45.32)	7.34 (0.15)
Management	Building	575 (27.24)	27 (3.25)	2.97 (0.41)	36.99 (3.96)	6.58 (1.11)	1454.28 (39.16)	7.32 (0.11)
	Conventional	570 (27.01)	30 (3.59)	3.23 (0.45)	35.34 (3.78)	6.26 (1.05)	1435.93 (38.66)	7.38 (0.11)
Fertility	Control	530 bc (31.98)	25 bc (5.04)	2.16 b (0.39)	32.31 (5.57)	5.33 (1.03)	1489.38 (52.16)	7.56 (0.17)
	Liquid Pig Manure	592 b (35.72)	36 ab (7.26)	5.65 a (1.04)	43.22 (7.45)	7.35 (1.41)	1447.43 (50.69)	7.16 (0.17)
	Solid Dairy Manure	749 a (45.17)	57 a (11.37)	4.28 a (0.78)	34.76 (5.99)	5.97 (1.15)	1413.58 (49.50)	7.35 (0.17)
	Synthetic Fertilizer	458 c (27.6)	14 c (2.84)	1.81 b (0.33)	36.48 (6.28)	7.39 (1.42)	1432.44 (50.17)	7.33 (0.17)

† The Value in the parenthesis represents the standard error of the soil health indicator.

†† Values in a column with the same letter are not significantly different with a significance level of $p=0.05$.

‡ The main treatment effects in the NCLE experiment are crop history (History), soil management (Management), and soil fertility (Fertility).

‡‡ Treatment levels in the NCLE experiment are annual crop history (Annual) and perennial crop history (Perennial) for the crop history treatment effect. Soil building (Building) and conventional for the soil management treatment effect. Unfertilized control (Control), liquid pig manure, solid dairy manure, and synthetic fertilizer for the soil fertility treatment effect.

4.1 Soil Indicators Responsive to the Fertility Treatment

Seven soil health indicators were influenced by the fertility treatment levels in the NCLE experiment (Table 4.1, 4.2). The fertility treatment had four treatment levels that lasted seven years, from 2008 to 2015. The fertility treatment levels were solid dairy manure applied at crop nitrogen demand, liquid pig manure applied at the crop nitrogen demand, synthetic fertilizer applied at the crop nitrogen and phosphorus demand, and an unfertilized control. The seven indicators responsive to the soil fertility treatment were total organic carbon (p 0.0138), active carbon (p 0.0047), ACE soil protein (p 0.0465), potentially mineralizable nitrogen (p 0.0022), Olsen phosphorus (p 0.0005), soil test potassium (p <0.0001), and soil test zinc (p <0.0001). The response of these indicators to the fertility treatment demonstrates a legacy effect, as the fertility treatments ceased five years prior to sampling.

4.1.1 Total Organic Carbon

Total organic carbon was responsive to the fertility treatment (p 0.0138, Table 4.1). Total organic carbon in the solid dairy manure treatment was 31% greater than in the unfertilized control, 20% higher than the synthetic fertilizer, and 19% higher than the liquid pig manure treatment (Table 4.3). The other three fertility treatments (unfertilized control, synthetic fertilizer, and liquid pig manure) did not result in significant differences in TOC. This increase in TOC can be attributed to the high carbon content of the solid dairy manure. When a solid manure is applied at the agronomic nitrogen rate, a large amount of carbon is also applied. It is this carbon that contributes to the increased TOC in the solid dairy manure treatment. For comparison, in 2008, the dry matter content of the liquid pig manure applied to the NCLE experiment was measured as 0.8%, while the solid dairy manure treatment was measured at 21.3% (Flaten & Fraser, 2016).

The timeline for identifying these differences is notable, as the effect of the fertility treatment was detected five years after the last manure application. These results demonstrate the potential for manure application to create long-term increases in total organic carbon for humic vertisols of the Red River Valley. The longevity of the TOC increase from the solid dairy manure treatment may be attributed to the high clay content of the soil at the NCLE experiment. The soil in this experiment was a humic vertisol, which is characterized by a high capacity to accumulate soil organic carbon due to its clay mineralogy (Matus, 2021). No published studies in the scientific literature were found that quantified the long-term impact of solid dairy manure treatments on TOC for humic vertisols of the Red River Valley in Manitoba, making this the first report of these results. .

The TOC results from the NCLE experiment are supported by research from other growing regions with contrasting soil types. After 19 years of solid beef manure applied at double the corn nitrogen rate to a silty loam soil in South Dakota, soil organic matter (SOM) increased by 21.7 mg g^{-1} when compared to the unfertilized control (Gautam et al., 2022). In the South Dakota study, SOM levels were 65.2 mg g^{-1} in the unfertilized control, 65.6 mg g^{-1} in the synthetic fertilizer treatment, and 86.9 mg g^{-1} in the manure treatment. The proportional increase in soil carbon over the unfertilized control measured in the South Dakota (33%) was similar to that of the NCLE experiment (31%). This similarity is interesting considering that there were differences in the manure treatment length (8 vs. 19 years) and manure application rate (crop nitrogen requirement rate vs double crop nitrogen rate).

A study from southern Alberta found that 12 years of solid feedlot manure increased soil organic carbon in the top 10 cm of soil. The study took place on a clay loam soil in Lethbridge and applied $77 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ of feedlot manure to a field under continuous barley silage

production. The study found that there was a 350% increase in soil organic carbon for the feedlot manure treatment compared to the unfertilized control (Miller et al., 2014). This increase is consistent with the direction of the solid dairy manure result in the NCLE experiment, but the magnitude of the increase is 10 times greater than that in the NCLE experiment. A longer application period (12 vs. 9 years) and higher application rate ($77 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ vs. $56 \text{ Mg ha}^{-1} \text{ yr}^{-1}$) are two potential explanations for the greater increase in the Alberta study when compared to the NCLE experiment.

4.1.2 Active Carbon

Active carbon was responsive to the fertility treatment ($p = 0.0047$, Table 4.1). Solid dairy manure was the most effective at increasing active carbon. Active carbon in the solid dairy manure treatment was 20% greater than the unfertilized control, and 16% higher than the synthetic fertilizer treatment (Table 4.3). Similar to TOC, this result can be attributed to the high carbon content of the solid dairy manure.

This increase in active carbon from manure application is supported by research from southern Idaho. Dungan et al. (2022) found that seven years of a 17 Mg ha^{-1} dairy manure treatment increased active carbon in the top 15 cm of soil by 180 mg kg^{-1} and 120 mg kg^{-1} when compared to the unfertilized control and the synthetic fertilizer treatment, respectively. These increases in active carbon are similar to those found at the NCLE experiment, where the solid dairy manure treatment was 149 mg kg^{-1} higher than the unfertilized control and 128 mg kg^{-1} higher than the synthetic fertilizer treatment. Despite similar responses when amended, there were large differences in the baseline active carbon levels between the NCLE experiment and the Idaho study. The unfertilized control in the Idaho study was 385 mg kg^{-1} , which is 42% lower

than the unfertilized control of the NCLE experiment. These differences can be attributed to the higher clay and TOC content of the humic vertisol in the NCLE study, which can naturally retain more carbon than the silty loam soil in the Idaho study (1.3% TOC). Despite the difference in soil texture between the two studies, the absolute increases of active carbon in mg kg^{-1} were similar between the two studies, suggesting that manure had a similar effect on active carbon regardless of texture or initial TOC content.

One method used to make active carbon comparisons between soils with different properties is to compare the ratio of active carbon to TOC. This ratio can be used to quantify the size of the active carbon pool in soil. The results from this study found that the active carbon to TOC ratio was around 0.025 regardless of the crop or fertility treatments tested. In contrast, a 35-year long-term experiment by Wei et al. (2020) from China used a carbon mineralization test to estimate the active carbon pool. The study found that manure application increased the active carbon to TOC ratio of a manure treatment (0.0634) compared to the unfertilized control (0.0206) and a synthetic fertilizer treatment (0.0495). Differences between the Chinese study and the NCLE experiment could be attributed to the five-year gap between the last manure application and the sample collection at the NCLE experiment. This gap could have led to the soil carbon pools reaching an equilibrium at 0.025 active carbon to TOC in the NCLE experiment. Overall, the increase in active carbon from solid dairy manure application at the NCLE experiment is a unique finding there have been no studies that have investigated how active carbon responds to different fertility treatments in the Red River Valley.

4.1.3 ACE Soil Protein

Autoclaved citrate extractable soil protein was responsive to the fertility treatments ($p = 0.0465$, Table 4.1). For soil protein, the solid dairy manure treatment was 38% higher than the unfertilized control (Table 4.3). This result was hypothesized as manure contains a high amount of organic nitrogen, which is the form of nitrogen that ACE soil protein measures. These results are notable as there has been no previous published research investigating the impact of manure application on ACE soil protein for the humic vertisols of the Red River Valley of Manitoba. One unexpected result from this study was the absence of a statistical difference between the synthetic fertilizer or the liquid pig manure treatment when compared to the solid dairy manure or the unfertilized control treatments. This result is discussed further in section 4.4.5. As expected, there was no response in ACE soil protein to the crop history treatment. Only a 3% difference in ACE soil protein was observed between the annual and perennial crop treatments.

The increase in ACE soil protein found in the solid dairy manure treatment in the NCLE experiment agrees with findings reported in the current literature (Ros et al., 2009; Ozlu et al., 2019; Dungan et al., 2022). A study by Dungan et al. (2022) investigated the effect of a solid dairy manure treatment on ACE soil protein after seven years in an irrigated row crop system in Idaho. The study found that a solid dairy manure treatment at the agronomic nitrogen rate (17 Mg ha^{-1}) increased ACE soil protein by 46% compared to the unfertilized control. Another study by Ozlu et al. (2019) from South Dakota found that solid dairy manure applied at the crop nitrogen removal rate increased acid extractable nitrogen by 43% compared to the unfertilized control. It should be noted that acid extractable nitrogen is different from soil protein; however, both are measures of organic nitrogen in soil (Rovira and Vallejo, 2002). The agreement among the findings of Dungan et al. (2022), Ozlu et al. (2019), and the NCLE experiment demonstrates

that manure treatments can increase ACE soil protein across the great plains of North America to a similar magnitude when applied at the nitrogen requirement rate. Quantifying the connection between soil protein and nitrogen cycling will be a critical next step for the use of soil protein as a soil health indicator.

4.1.4 Potentially Mineralizable Nitrogen

Potentially mineralizable nitrogen (PMN) had a significant response to the fertility treatment ($p = 0.0022$, Table 4.1). The solid dairy manure treatment was 54% higher than the unfertilized control, and the liquid pig manure was 41% higher than the unfertilized control (Table 4.3). Both manure treatments left an organic nitrogen legacy in the soil, which were detected by PMN. This increase in PMN may be explained by the manure-derived soil organic nitrogen. This organic nitrogen would increase PMN as soil organic nitrogen is the substrate for the nitrogen mineralization process (Moebius-Clune et al., 2016).

The PMN results of the NCLE experiment are supported by the literature (Ndayegamiye and Cote, 1988; Sharifi et al., 2014; Dungan et al., 2022). A study from southern Alberta by Sharifi et al. (2014) found that seven years of beef cattle manure mixed with cereal straw applied at a rate of 77 Mg ha^{-1} on a clay loam increased 14-day mineralizable nitrogen compared to the unfertilized control by 168%. This increase in mineralizable nitrogen from this manure treatment found by Sharifi et al. (2014) was greater than the increase in PMN found in the NCLE experiment. The greater increase in the Alberta study could be attributed to methodological differences, including incubation length (7 vs 14 days), manure application rate (77 Mg ha^{-1} vs 50 Mg ha^{-1}), or manure type (solid dairy manure vs beef manure).

A study from Idaho by Dungan et al. (2022) found that seven years of solid dairy manure application at 17 Mg ha⁻¹ on a silty loam soil increased PMN levels by 120% compared to the unfertilized control and by 44% compared to the synthetic fertilizer treatment. The results from Dungan et al. (2022) are comparable to those of the NCLE experiment, as they both demonstrated that manure can increase PMN compared to synthetic fertilizer and an unfertilized control. There are differences in the magnitude of the increases between the Idaho study and the NCLE experiment. In the Idaho study, the PMN increase was three times greater than the increase in the NCLE experiment. Potential explanations for the difference between the study results were the five-year gap between the last manure application and sample collection in the NCLE experiment as well as the time of year when samples were collected (November for NCLE vs March for Idaho study).

A study on a silty loam soil in Quebec found that nine years of farmyard manure or liquid pig manure increased PMN compared to an unfertilized control (Ndayegamiye & Cote, 1988). Farmyard manure at a rate of 60 Mg ha⁻¹ increased mineralized nitrate by 126%, whereas liquid pig manure at a rate of 120 Mg ha⁻¹ increased mineralized nitrate by 114% when compared to an unfertilized control. The total increase in PMN seen in the Quebec study was approximately double that of the increase in the NCLE experiment. This greater increase could be attributed to the five-year gap between the last manure application and the time of sampling at the NCLE experiment or differences in methodology. The Quebec study used a 40-week incubation that measured nitrate production of silty loam soil, and the NCLE experiment used a 1-week incubation that measured ammonium production of a clay soil. Both the studies by Ndayegamiye & Cote (1988) and Dungan et al. (2022) found an increase in PMN that was 2-3 times greater than the increase in the NCLE experiment. These differences between studies may be

explained by a multiple factors. First, the five-year gap between the last manure application in the NCLE experiment and sampling may have reduced the PMN response to manure application. Second, the studies by Ndayegamiye & Cote (1988) and Dungan et al. (2022) had longer growing seasons and higher precipitation amounts, these environmental factors may be conducive to increasing PMN. Future research should investigate how manure application affects PMN over time and in different climates.

4.1.5 Potassium

The fertility treatment significantly affected soil test potassium ($p < .0001$, Table 4.2). Solid dairy manure was the most effective at increasing soil test potassium levels. Soil test potassium for the solid dairy manure treatment was 41% higher than the unfertilized control treatment, 63% higher than the synthetic fertilizer treatment, and 26% higher than the liquid pig manure treatment. The liquid pig manure treatment was significantly higher than the synthetic fertilizer treatment by 29% (Table 4.4). In addition to being responsive to the fertility treatment, soil test potassium was responsive to the crop history treatment. This will be discussed further in Section 4.2.4.

Differences among soil test potassium levels in the fertility treatment can be explained by potassium application rates for each fertility treatment. The solid dairy manure treatment applied more potassium than the liquid pig manure treatment when applied at the nitrogen rate. From 2007 to 2015 the cumulative potassium applied to the experiment for the solid dairy manure treatment was 2256 kg ha⁻¹ compared to 920 kg ha⁻¹ for the liquid pig manure treatment (Flaten and Fraser, 2016). The smectite clay mineralogy of soil in the NCLE experiment also contributed to the longevity of this soil potassium legacy as it can fix large amounts of potassium in between phyllosilicate layers (Franzen & Bu, 2018; Rich, 1968).

The results from this study are consistent with previous potassium nutrient balances calculated for the NCLE experiment. After the first eight years of the NCLE experiment, potassium in the control treatment dropped by 146 kg ha^{-1} in 2015. At the same time, there was a potassium surplus in the solid dairy manure treatment of 560 kg ha^{-1} (Flaten and Fraser, 2016). In addition to the previously reported results from the NCLE experiment, these results are supported by several studies reported in the literature. Chen et al. (2020) found that a composted chicken manure treatment increased soil test potassium by approximately 30% after six years, when compared to an unfertilized control. Yadav, Benbi & Toor (2019) found that a farmyard manure treatment increased soil test potassium by 34% after 14 years when compared to a synthetic fertilizer treatment. Qian et al. (2005) found that a solid cattle manure treatment increased soil test potassium by 64% after seven years when compared to an unfertilized control.

Although soil test potassium was responsive to the fertility treatments in the NCLE experiment, the utility of potassium as a soil health indicator for humic vertisols of the Red River Valley in Manitoba may be questioned. The effectiveness of a nutrient-based soil health indicator is often predicated on whether nutrient levels are sufficient or deficient to support crop growth (Karlen, Stott & Mikha, 2021). Although the synthetic fertilizer treatment contained the lowest soil test potassium level, it still contained sufficient soil-test potassium levels to support crop growth in Manitoba (Manitoba Agriculture, 2007). Because of this, the differences found in soil test potassium for this study would not likely influence soil health in the context of annual cropping systems.

4.1.6 Phosphorus

Soil Olsen-phosphorus levels were responsive to the fertility treatment ($p = 0.0005$, Table 4.2). The solid dairy manure treatment had Olsen-phosphorus levels that were 128% higher than the unfertilized control and 307% higher than the synthetic fertilizer treatment. Olsen-phosphorus in the liquid pig manure treatment was 157% higher than the Olsen-phosphorus in the synthetic fertilizer treatment (Table 4.4). For the crop history treatment, there was an observed Olsen phosphorus difference of 3% between the means. This small difference is likely the cause for there being no inference in the crop history treatment.

These increases in Olsen-phosphorus are a result of the historical manure application rates being determined by the nitrogen requirement of the crop instead of the phosphorus requirement. This management strategy resulted in phosphorus application rates that exceeded crop demand (Stumborg & Schoenau, 2008). For example, the cumulative phosphorus balance within the annual crop history treatment from 2008-2015 when the fertility treatments were applied was -64 kg ha^{-1} in the unfertilized control, -98 kg ha^{-1} in the synthetic fertilizer treatment, 198 kg ha^{-1} for the liquid pig manure treatment, and 576 kg ha^{-1} for the solid dairy manure treatment (Flaten & Fraser, 2016). The drawdown period from 2015 to 2020 was not long enough, so these historical differences in Olsen-phosphorus documented in 2015 were detected again five years later in 2020 (Table 4.4).

This increase in Olsen phosphorus from different manure sources has been reported in other studies on the Canadian prairies. A study by Stumborg & Schoenau (2008) in Saskatchewan on a black chernozem soil with a cereal oilseed crop rotation found that Olsen-phosphorus levels increased by 129% compared to a control when solid cattle manure was applied at a rate of 15.2 Mg ha^{-1} (double nitrogen rate). The study also found that liquid pig

manure applied at 148,000 L ha⁻¹ (four times nitrogen rate) increased Olsen phosphorus levels by 554% compared to an unfertilized control and by 362% compared to a synthetic fertilizer treatment. Thus, similar to the NCLE experiment, both the liquid pig manure and the solid cattle manure treatments in this Saskatchewan study increased soil phosphorus levels.

A unique consideration with manure application in the Red River Valley is the susceptibility of the Lake Winnipeg watershed to eutrophication. High levels of phosphorus in soil is an environmental issue, as there is the potential for phosphorus from the soil to runoff into waterways and cause eutrophication. (Schindler, Hecky & McCullough, 2012). This environmental relevancy of Olsen-phosphorus is very important to consider when interpreting soil phosphorus levels. The risk from high phosphorus levels means that an optimum phosphorus level is best for a soil to be healthy (Moebius-Clune et al., 2016).

4.1.7 Zinc

In addition to macronutrients, micronutrients are also used as soil health indicators. Out of the micronutrients tested, zinc was the only indicator that was responsive to the fertility treatment ($p < .0001$, Table 4.2). For soil test zinc levels, liquid pig manure was 160% higher than the unfertilized control and 212% higher than the synthetic fertilizer treatment. The solid dairy manure treatment was 98% higher than the unfertilized control and 137% higher than the synthetic fertilizer treatments (Table 4.4). For the crop history treatment, there was an observed difference of 18% between the means. This difference was not significant due to the variability within the treatments (0.45-0.53 ppm).

The historical zinc balance from 2007 to 2015 explains the differences in soil test zinc levels observed among the fertility treatments in 2020. In 2015, at the end of the eight-year manure application period, a 14.3 kg ha⁻¹ increase in available zinc in the solid dairy manure treatment and a 7.94 kg ha⁻¹ increase in the liquid pig manure treatment when compared to the start of the experiment were observed (Flaten & Fraser, 2016). These increases occurred because the amount of zinc applied to the soil from manure exceeded the crop removal rate when manure was applied at the nitrogen rate. The zinc crop removal from 2015 to 2020 was not high enough to draw down these increases in zinc levels, so the treatment differences persisted (Flaten & Fraser, 2016).

These results are supported by the findings of other researchers. A study from Saskatchewan by Lipoth & Schoenau (2007) found that five years of a manure treatment increased soil test zinc levels. Liquid pig manure applied at four times the nitrogen rate increased soil test zinc by 6% compared to an unfertilized control. Solid cattle manure applied at the nitrogen rate increased soil test zinc levels by 6% compared to an unfertilized control. The liquid pig manure treatment increased soil test zinc levels by 9%, and the solid cattle manure treatment increased soil test zinc levels by 10% compared to the synthetic fertilizer treatment. The changes in zinc levels for the NCLE experiment were greater than those of the Saskatchewan study. This may be due to the manure in the NCLE experiment having a higher zinc concentration or the ability of the clay soil at the NCLE experiment to retain more zinc than the loam soil in the Saskatchewan study.

4.1.8 Benefits of Manure Fertility Treatments on Soil Health Indicators

The dry matter in manure had a strong effect on all of the biological soil health indicators in this study. To visualize the connections between the fertility treatments and the indicators, these results were plotted in a radar plot (Figure 4.1). In the radar plot, the lines represent the treatments, and the axis represents the indicators responsive to the crop fertility treatment in the NCLE experiment. The solid dairy manure treatment line was the greatest for all of the responsive soil health indicators except for zinc where liquid pig manure was the greatest.

The organic carbon and nitrogen in the dry matter of the solid dairy manure is likely driving the responses in these indicators (Larney, Ellert & Olson, 2005). Four biological soil health indicators (TOC, active carbon, PMN, and ACE soil protein) were increased by the solid dairy manure treatment compared to the unfertilized control treatment (Table 4.3). The solid dairy manure used in the NCLE study had a dry matter content ranging from 16 - 23%, whereas the liquid pig manure dry matter content ranged from 0.6-6 % (Table 6.1) (Flaten & Fraser, 2016). The higher DM in the solid dairy manure increased carbon inputs, resulting in increases in the biological soil health indicators.

The liquid pig manure treatment contained some dry matter but less than the solid dairy manure. This is likely the reason for the absence of a difference between the liquid pig manure and the solid dairy manure for Active carbon and ACE soil protein. The lower dry matter levels in the liquid pig manure treatment prevented an increase in active carbon and ACE soil protein when compared to the synthetic fertilizer and the control treatments.

The importance of the dry matter in the manure can be seen in Figure 4.1. Solid dairy manure has a high rate of dry matter inputs, resulting in the solid dairy manure line being at the top for the biological soil health indicators. The liquid pig manure had a lower rate of dry matter

inputs, resulting in a lower impact on the biological soil health indicators. Both the synthetic fertilizer and the control do not have dry matter inputs, resulting in little activity on the biological soil health indicator.

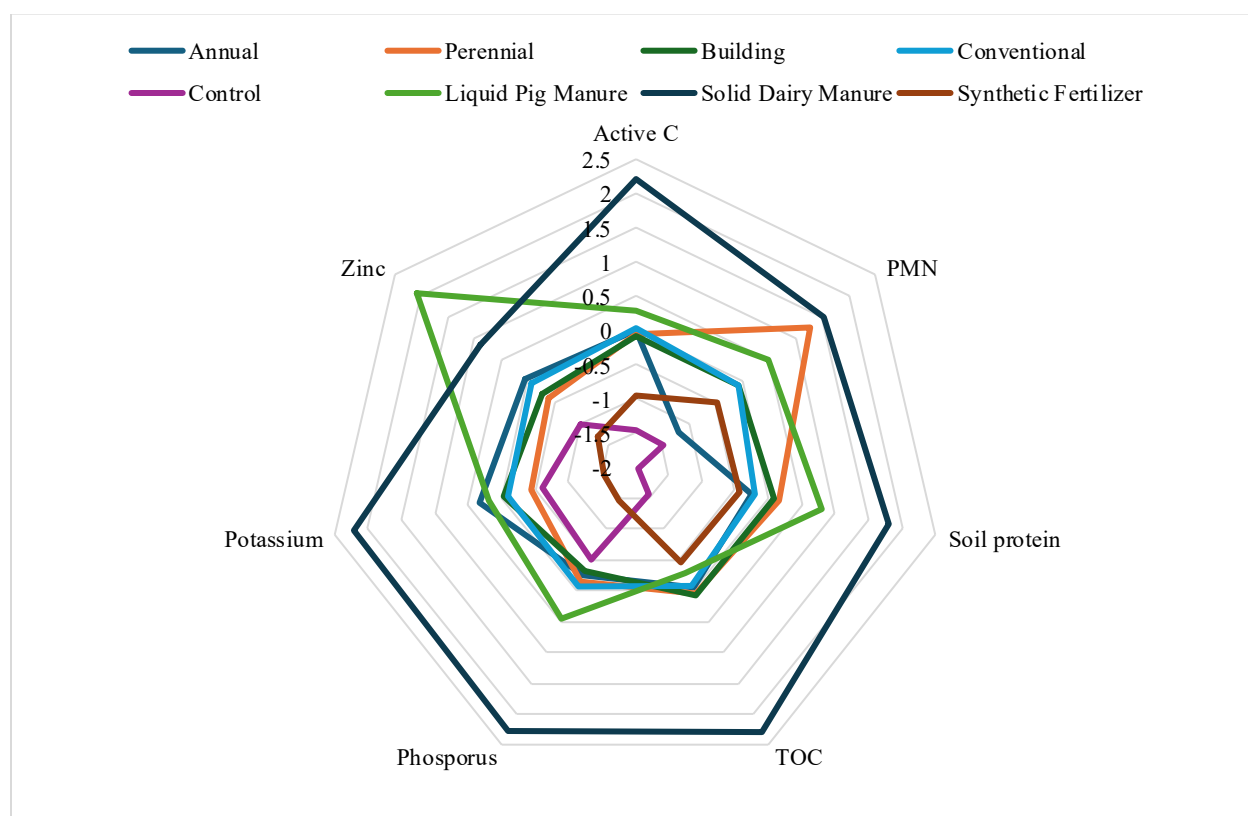


Figure 4.1. Radar plot of soil health indicators that were responsive to soil fertility treatments including active carbon (POXC), potentially mineralizable nitrogen (PMN), soil protein, total organic carbon (TOC), Olsen phosphorus (phosphorus), potassium, zinc. The radar plot is composed of the z-scores of the treatment means.

4.2 Soil Health Indicators Responsive to Crop History Treatment

The crop history treatments were annual and perennial crop history. These treatments were implemented from 2008 to 2019. Soil samples for this study were collected in the fall of 2020, one year after the perennial crop history treatment had been terminated and converted to annual crops. Thus, this study identifies which indicators can detect the short-term legacy effects of the crop history treatment. Of the soil health indicators examined, four were responsive to the crop history treatment: wet aggregate stability, soil respiration, potentially mineralizable nitrogen, and soil test potassium (Table 4.1, 4.2).

4.2.1 Aggregate Stability

Wet aggregate stability was affected by crop history treatment ($p = 0.0002$, Table 4.1). The perennial crop history treatment increased WAS by 3% compared to the annual crop history treatment (Table 4.3). Both the soil fertility and soil management treatments had no effect on WAS (Table 4.3). This result for the soil fertility treatments was unexpected and will be discussed further in section 4.4.4. The absence of a response to the soil management treatment was expected due to the short timeframe of treatment implementation (cover cropping and no-till) prior to sampling.

This increase in WAS from the perennial crop was expected as there are many benefits of perennial cropping systems to soil structure (Ernst and Siri-Prieto, 2009; Stainsby and Entz, 2022; Eze et al., 2023). It was hypothesized that these benefits from perennial crops would still be present after one growing season of annual crops in the crop history treatment. In addition to the results of this study, results from research in the neighboring Glenlea Long-Term Crop Rotation Study are consistent with those from the NCLE experiment (Stainsby, 2019). Stainsby (2019) found that after three years of an alfalfa crop, there was significantly higher aggregate

stability in the top 0-10 cm of soil when compared to the start of the rotation. The increase in aggregate stability found by Stainsby (2019) was greater than that found in the NCLE study. One potential explanation for this greater increase is the use of the wet sieving method, which tests all size classes of aggregates. Another is the fact that Stainsby (2019) sampled from an actively growing perennial crop, whereas in the NCLE experiment, the perennial crop had been terminated one year prior.

Not all research in Manitoba has found that perennial crops improve aggregate stability. Research on a sandy loam soil in Manitoba contrasts with the results of the NCLE experiment. A study by Adesanya et al. (2016) investigated the effect of a mixed perennial grass stand consisting of timothy (*Phleum pratense L.*) and orchard grass (*Dactylis glomerata L.*) on the aggregate stability of a sandy loam soil in Carman, Manitoba. The study by Adesanya, Akinremi & Zvomuya (2016) used the same aggregate stability method as Stainsby (2019) and found that five years of a perennial crop did not have a significant effect on aggregate stability compared to annual crops. These contrasting results are likely explained by soil texture. Clay soil has a high level of internal cohesion when compared to sandy loam soil. This cohesive nature of clay soil will lead to better soil aggregation than sandy loam soil. The study on the sandy loam soil in Carman had five years of perennial treatment history (Adesanya, Akinremi & Zvomuya, 2016) while results from the clay soil at the Glenlea Long-Term Rotation Study had three years of treatment effect (Stainsby, 2019). The clay soil in the NCLE experiment had eight years of treatment effect, split between a three-year phase and a five-year phase. Results from the NCLE experiment and the Stainsby (2019) experiment suggest that the humic vertisols soils found at Glenlea have a greater ability to build aggregate stability than the sandy loam soil from the experiment at Carman.

Other research within the Northern Great Plains supports the aggregate stability results of the NCLE experiment. A study by Eynard et al. (2004) investigated the impact of tillage and perennials on wet aggregate stability in South Dakota on soil types ranging from clay to sand. The study found that soil under a perennial grass system had 17% higher aggregate stability compared to soils under an annual cropping system. This 17% increase in aggregate stability is greater than the 3% increase found in the NCLE experiment. The possible reasons for the greater improvement in the South Dakota study are a longer treatment length (10 years vs 8 years at NCLE), soil type (clay vs various loams), and differences in the timing of sampling during the perennial phase in comparison to one year after termination of the perennial phase. In addition, the soil at the NCLE experiment started with a high WAS that was above 90%. This high starting point for aggregate stability limits the potential for improvements as aggregate stability has a maximum value of 100%.

The use of tillage to terminate perennials in the NCLE experiment may have limited the increases in aggregate stability measured. The treatment structure of the South Dakota study allowed for a comparison of the relative impact of tillage and crop history on aggregate stability. After 10 years, the no-till treatment increased aggregate stability by 32% compared to the tillage treatment, this increase is greater than the 17% increase from the perennial system treatment compared to the annual crop treatment. These results suggest that tillage is more detrimental to aggregate stability than perennial cropping is beneficial. Thus, the three years of tillage (2012, 2013, 2020) in the NCLE experiment perennial history treatment may have limited the increase in aggregate stability measured in this current study.

4.2.2 Soil Respiration

Soil respiration responded to the crop history treatment ($p = 0.0449$, Table 4.1). Soil respiration in the perennial crop history treatment was 26% higher than soil with an annual crop history (Table 4.3). For both the management and the fertility treatments, there were no significant differences among the treatment means. The lack of a response to the fertility treatment was unexpected and will be discussed further in section 4.4.3. The increase in soil respiration was expected as perennial crops benefit soil carbon and soil microbial populations, both of which have a positive effect on soil respiration (USDA NRCS, 2009; Alagele et al., 2020) and soil respiration has been found to be positively correlated with soil carbon (Wang et al., 2003; Lai et al., 2017). These benefits of perennial crops to soil respiration remained after the conversion to annual cropping at the NCLE experiment. This demonstrates the short-term legacy of perennial crops to soil respiration.

In contrast to soil respiration, the carbon-based soil health indicators of TOC and active carbon did not respond to the crop history treatment. Thus, it was more likely that differences in soil microbes were driving the increased soil respiration in the perennial crop history treatment. However, it is important to consider the potential impact of tillage and the conversion of perennial to annual cropping systems. Disturbance via tillage has been found to increase soil respiration in the short term on Red River Valley soils (Koiter, 2008). The conversion of a perennial crop system to an annual crop system has been found to result in increased CO_2 emissions for soils in the Red River Valley (Fraser & Amiro, 2013).

The results for soil respiration in the NCLE experiment are supported by the literature. Research from the Glenlea Long-Term Crop Rotation Study found that metabolic quotient, a metric similar to soil respiration, was 80% higher for soil under a rotation containing perennial

forages when compared to a rotation that contained only annual grain crops (Braman, 2012). This 80% increase in respiration is greater than the 25% increase found at the NCLE experiment. The soil from the study by Braman (2012) was sampled from an actively growing perennial system, while the soil from the NCLE experiment was sampled from a perennial stand that had been terminated the year prior. The difference in the size of the increases between the two studies suggests that soil respiration may have been reduced after one year of annual crop production at the NCLE experiment. Methodological differences may also be causing the differences between the two sets of results. For example, respiration for the NCLE experiment was measured using rewetted dried soil, while Braman (2012) used fresh soil.

A study by Wang et al. (2015) on the loess plateau in China investigated the effect of converting annual cropland to perennial grasslands on a silty soil. The study found that 15 years after an annual cropland had been converted to a grassland, there was a 12% increase in soil respiration. Despite having a shorter treatment period and the cropping system conversion being in the opposite direction, the increase in respiration from the perennial cropping treatment was greater in the NCLE experiment than in the Chinese study. This illustrates that soil texture may be an important factor when interpreting soil respiration as a soil health indicator. These findings also demonstrate the potential for changes in this indicator that can be expected as a result of management changes with perennial crops.

4.2.3 Potentially Mineralizable Nitrogen

In addition to being responsive to the fertility treatment, potentially mineralizable nitrogen (PMN) had a significant response to the crop history treatment ($p = 0.0385$, Table 4.1). Soil with perennial crop history had 43% higher PMN levels than soil with annual crop history (Table 4.3).

This difference in PMN was the largest increase of the four indicators responsive to the crop history treatment. Thus, PMN may be the best indicator to identify differences in the short-term legacy of cropping history. The same factors discussed in section 4.2.2 for soil respiration (soil carbon and soil microbes) are potential reasons for the increase in PMN. When respiration is higher, more soil organic matter is consumed, and this releases nitrogen that the PMN test is measuring. A study from 2001 found that nitrogen mineralization and respiration were correlated ($r^2=0.93$, Haney et al., 2001).

The positive effect of perennial crop history on soil nitrogen mineralization is supported in the literature. A study on a silty soil in Kansas by Means, Crews & Souza (2022) found that soil under actively growing intermediate wheatgrass mineralized 40% more nitrogen in the top 10 cm of soil compared to soil under an annual cropping system. The authors of the Kansas study concluded that the mineralization benefits seen in the intermediate wheatgrass treatment could be attributed to higher carbon inputs relative to the annual crop treatment. This result is similar to the 42% increase in the NCLE experiment and is interesting considering the Kansas study results were from an actively growing perennial crop.

A study in Wisconsin found that perennial-based forage cropping systems had higher PMN levels than annual cropping systems. The study surveyed 624 fields throughout Wisconsin and found that PMN levels were 10-90% higher in fields growing perennial forage compared to those growing annual crops (Augarten et al., 2023). The PMN increase found in the NCLE experiment falls within the range reported in the Wisconsin survey study.

4.2.4 Potassium

In addition to being responsive to the fertility treatment, soil test potassium was responsive to the crop history treatment ($p = 0.0366$, Table 4.1). The annual crop history treatment had 10% higher soil test potassium levels than the perennial crop history treatment (Table 4.3). These differences can be explained by the historical nutrient removal for these two treatments at harvest. In the perennial crop history treatment, whole plant biomass was harvested and removed on an annual basis from 2007 to 2015. In the annual crop history treatment, the grain was harvested and the residue was either removed (2008, 2010, 2012, 2014) or returned to the soil (2009, 2011, 2013, 2015). Considerably more potassium is removed when a perennial crop is harvested compared to when an annual grain is harvested (Manitoba Agriculture, 2007). According to the Manitoba soil fertility guide (Manitoba Agriculture, 2007), a 3-ton ac^{-1} grass hay harvest removes 117-143 lb ac^{-1} of K_2O , whereas a 35 bu ac^{-1} canola grain crop harvest removes 16-20 lb ac^{-1} of K_2O . In the NCLE experiment, the perennial crop treatment had a historically higher potassium removal compared to the annual crop treatment. This matches with the observed lower soil test potassium levels in the perennial crop history treatment.

The soil test potassium results of this current study are supported by the literature. A study by Havlin, Westfall & Golus (1984) on a clay loam soil in Colorado found that soil test potassium levels were lower after six years of alfalfa harvest relative to the start of the experiment. The study was designed to investigate if potassium fertilization could compensate for potassium removal when growing alfalfa. They concluded that despite the alfalfa harvest drawing down soil test potassium levels, there was no yield response to potassium fertilizer. This study also suggested that the drawdown of potassium from the unfertilized control and synthetic fertilizer treatments were reduced by the release of potassium from the interlayers of the clay

phyllosilicates. This, in effect, buffered the drawdown of soil test potassium for the unfertilized control and synthetic fertilizer treatments. This finding may be important when interpreting results from the NCLE experiment. As indicated in Section 4.1.5, the 2:1 clay mineralogy of the soil found at the NCLE experiment can fix and release potassium in the interlayer space. Despite the response of soil test potassium to the crop history treatment in this study, soil test potassium may not be a useful indicator of soil health in the Red River Valley.

4.2.5 The Benefits of Perennial Crop History to Soil Health.

Multiple soil health indicators were able to detect the legacy of the perennial crop one year after termination. Soil respiration, PMN, and WAS were all improved by the perennial crop history treatment when compared to the annual crop history treatment. These trends are visualized in a radar plot (Figure 4.2). In the radar plot, the lines represent the treatments and axis represent the indicators that were responsive to the crop history treatment. For three of the four indicators, the perennial treatment had the greatest z-scores, inferring a strong effect of the perennial treatment on the indicator. Potassium is the one indicator axis that the perennial treatment did not have the greatest z-score. Instead the annual treatment had the greatest z-score on the potassium axis.

The similar pattern among soil health indicators in the radar plot can be attributed to the types of benefits that perennials contribute to soil physical and biological properties. Perennials are beneficial to soil micro-organisms as they create a stable environment that provides carbon for microbes to consume (Alagele et al., 2020). These soil micro-organisms are responsible for the functional processes that soil health indicators are measuring (Henneron et al., 2015). For example, soil respiration and potentially mineralizable nitrogen rely on the ability of soil microorganisms to decompose organic matter and produce carbon dioxide and ammonium

(Vicena et al., 2022). While aggregate stability is promoted by the organic compounds that soil organisms produce and is enhanced by the reinforcing matrix of fungal hyphae and plant roots. Overall, the biological soil health benefits from the perennial cropping system promotes physical soil health and creates a positive feedback loop. For example, improved soil biology increases the abundance of macroaggregates and improves soil structure (Yudina & Kuzyakov, 2023) leading to better soil gas diffusion, water infiltration, and carbon storage (Perret et al., 1999; Mangalassery et al., 2013; Kravchenko et al., 2015; Wang et al., 2019). The improved soil physical properties then, in turn, benefit soil micro-organisms, which further improves the soil physical properties.

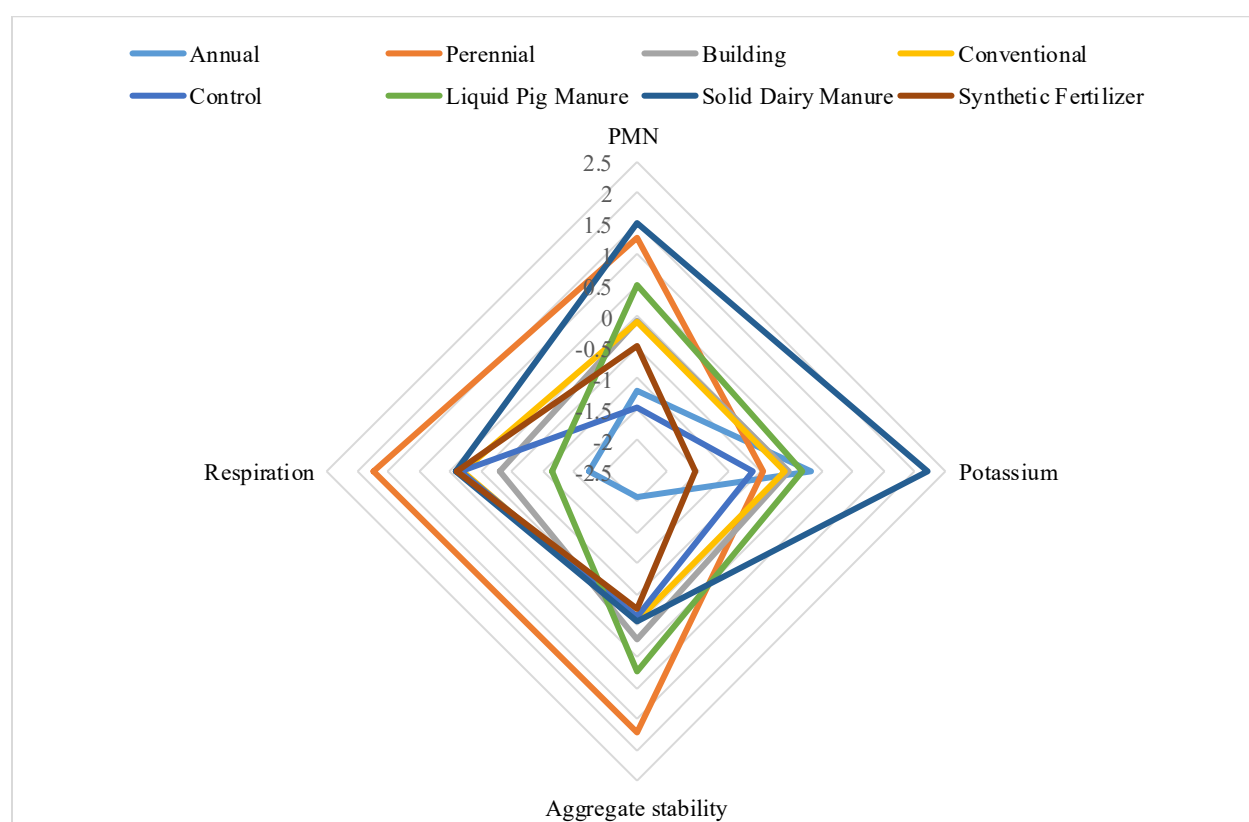


Figure 4.2. Radar plot of indicators responsive to the crop history treatment including potentially mineralizable nitrogen (PMN), potassium, aggregate stability, soil respiration (respiration). The radar plot is composed of the z-scores of the treatment means.

4.3 Unresponsive Soil Health Indicators

Multiple soil health indicators did not respond to any of the treatments in the NCLE experiment. An unresponsive indicator was an indicator that did not show a significant response to any of the experimental treatments. These unresponsive indicators included: available water holding capacity (AWHC), pH, manganese (Mn), iron (Fe), and magnesium (Mg). This section will explore the potential reasons for the unresponsive nature of these indicators to the treatments in this study.

4.3.1 Available Water Holding Capacity

Available water holding capacity did not respond to any of the management treatments in the NCLE experiment (Table 4.1). This result was unexpected as it was hypothesized that the fertility treatments involving manure would increase AWHC. The absence of a significant difference among the history and management treatment means is likely due to the small observed differences, which were 5% or less. There were greater observed differences among the fertility treatments, with mean differences as high as 13%. However, variability was also higher in the fertility treatment with the standard error ranging from 1.07-1.16%. This variability was higher than the variability of the management (0.81-0.84%) or crop history treatments (0.94-0.95%, Table 4.3).

The hypothesis that fertility treatments involving manure would increase AWHC was based on previous studies that have found a relationship between organic amendments like manure and improved soil water-holding capacity (Nyamangara, Gotosa & Mpofu, 2001; Larney & Angers, 2012; Hosseinzadeh et al., 2021). A five-year study on sandy loam soil at Carman, Manitoba, found that manure application could increase available water holding capacity. This

study is the same one referenced in 4.2.1 by Adesanya, Akinremi & Zvomuya (2016). In the study, it was found that for an annual cropping system, AWHC was higher in the solid pig manure treatment compared to the control by 30% for the 10-20 cm depth. This result suggests that manure applied to sandy soil has the capacity to improve AWHC compared to the clay soil found at the NCLE experiment. A study by Blanco-Canqui, Hergert & Nielsen (2015) found that long-term manure application compared to non-manured soil increased available water-holding capacity by 16% in the top 30 cm of a fine sand loam after 71 years. The authors of this study cited that the increase in AWHC from manure application was due to the positive correlation between the AWHC and TOC levels.

The correlation between water-holding capacity and TOC has been demonstrated repeatedly in literature (Hudson, 1994; Acín-Carrera et al., 2013; Oldfield, Bradford & Wood, 2019; Eze et al., 2023). As discussed in section 4.1.1, results from the NCLE experiment found that the solid dairy manure treatment increased TOC by 0.8% compared to the control. However, this increase in TOC did not result in an increase in AWHC. There are two likely explanations for the absence of a difference in AWHC in the NCLE experiment. First, the high water-holding capacity of the humic vertisol in the NCLE experiment may limit the contribution of increased soil organic matter to the overall AWHC of this soil. A meta-analysis by Minasny & McBratney (2018) found that the benefits of TOC to AWHC were greater for sandy soil than they were for clay soil. A 1% increase in TOC resulted in an increase of 1.94% for sandy soil and an increase of 1.41 % for clay soil. The 1% TOC increase is similar to the 0.8% increase in the solid dairy manure treatment however, an increase in TOC did not translate into an increase in AWHC for the soil at the NCLE experiment.

Methodology is a second possible reason for the absence of a significant difference in AWHC. As prescribed by the CASH, disturbed soil cores were used to measure AWHC. There are practical and logistical reasons to use disturbed soil cores when running a commercial soil health testing lab, however the disturbed core method may be less accurate. (Aina, 1985; Duan et al., 2010). This conflict when measuring AWHC was documented for soils in Manitoba by Shaykewich (1970), who concluded that disturbed soil cores do not accurately represent the available water holding capacity of soil in the field. Despite the inaccuracy of disturbed core AWHC, the creators of CASH included disturbed core AWHC because it gives an estimate of the ability of a soil to store water for crop production and was found to be a responsive indicator within the scope of the New York State soils used in the CASH calibration data set (Idowu et al., 2009). The unresponsiveness of AWHC within the NCLE experiment and the literature surrounding the limitations of the disturbed core AWHC method indicate that the extra effort required to take undisturbed cores may be warranted to test for AWHC on the humic vertisol of the Red River Valley.

4.3.2 pH

Soil pH was unresponsive to the treatments in the NCLE experiment. There were small observed differences in pH among the means that were 5% or less. This result was expected due to the minerology of the soil in NCLE experiment. The Scanterbury soil series has a high clay content and is formed from calcareous parent material (Manitoba Agriculture, 2011). This combination of soil characteristics in the NCLE experiment results in a soil with a high pH buffering capacity (Krzic et al., 2021), making it difficult to change pH through the addition of soil amendments or cultural practices. Although pH can be responsive to management in some soil types (Moebius-

Clune et al., 2016), the lack of a difference in pH in the NCLE experiment is likely due to the natural buffering capacity of Red River Valley humic vertisols.

4.3.3 Micronutrients

Iron, manganese, and magnesium were unresponsive to all treatments in the NCLE experiment (Table 4.2). For iron, the absence of a significant difference in the history and management treatments was due to negligible observed differences that were less than 2%. For the fertility treatment, however, the observed difference between the control and the liquid pig manure treatment was 34%. The absence of a significant difference was due to the variability of the data. The standard error of the fertility treatment ranged from 5.57-7.45 ppm, which is higher than the variability of the soil management (3.78-3.96 ppm) or crop history treatments (4.57-4.94 ppm). For magnesium, the observed differences among the means were no greater than 5%. These negligible observed differences were the reason for the lack of a significant difference for any of the treatments. For manganese, there were negligible differences among the history and the management treatment means that were equal to or less than 5%. For the fertility treatment, there was a 39% observed difference between the synthetic fertilizer treatment and the unfertilized control treatment. The absence of a significant difference was due to the variability in the fertility treatment (1.03-1.42 ppm, Table 4.4).

The unresponsive nature of the micronutrient indicators was unexpected. It was hypothesized that magnesium, manganese, and iron would be responsive to the manure treatment because manure contains these three micronutrients (Manitoba Agriculture, 2015) and previous studies have found that manure can increase soil micronutrient levels (Rees et al., 2014; Wang et al., 2016; Indraratne, Spengler & Hao, 2021). A study by Nikoli & Matsi (2011) found that nine

years of a liquid dairy manure treatment increased manganese levels by 79% and iron levels by 56% when compared to an unfertilized control. One possible explanation for the absence of a significant difference is the unique soil type of the NCLE experiment. The humic vertisols of the Red River Valley have a high clay content, and the parent material contains dolomitic limestone. This, combined with high levels of organic matter, makes it naturally fertile with magnesium, iron, and manganese (Dhaliwal et al., 2019). This natural fertility may be buffering any potential micronutrient increase from the manure treatments. Ultimately, the lack of a response to the NCLE treatments makes magnesium, iron, and manganese ineffective soil health indicators for this soil type (Andrews, Karlen, and Cambardella, 2004).

4.4 Unexpected Findings

There were multiple indicators that did not respond as hypothesized to the treatments within the NCLE experiment. Indicators with unexpected results included TOC, active carbon, soil respiration, wet aggregate stability, ACE soil protein, potassium, and phosphorus. The unresponsiveness of the soil management treatment will also be discussed. In this section, potential reasons for the unexpected results will be explored. This will help identify the ability or limitations of the soil health indicators to measure differences between management practices for the humic vertisols of the Red River Valley.

4.4.1 Total Organic Carbon

It was hypothesized that the perennial crop history treatment would increase TOC compared to the annual crop history treatment. However, the ANOVA indicated there was no difference between crop history treatments (Table 4.1). This hypothesis was based on the known

relationship between higher carbon inputs from perennial crops and increased TOC. This relationship was observed by Fraser & Amiro, (2013) who found that perennial crops grown on a clay loam soil in Manitoba, sequestered 96 g C m^{-2} during the growing season while annual crops emitted 149 g C m^{-2} . Several additional studies have found this relationship (McGowan et al., 2019; Sprunger et al., 2019; Ledo et al., 2020). A Study evaluating the impact of management on soil carbon from research sites across Canada documented that perennial crops could increase soil organic carbon by 9 Mg C ha^{-1} compared to an annual crop treatment after 17 years (VandenBygaart et al., 2010).

The absence of a significant difference in TOC was likely due to the difference between treatments being less than 1%. This absence of a difference may be attributed to the 2020, 2013, and 2012 growing seasons where tillage and annual cropping was practiced in the perennial crop treatment. These two practices can decrease TOC levels (Nunes et al., 2020). This disturbance to the perennial cropping treatment likely created a soil environment that favored net respiration and resulted in the loss of soil carbon that had been potentially sequestered from 2007-2011.

One unexpected result of this study was that the perennial crop treatment increased soil respiration without showing any measurable increase in either TOC or active carbon soil health indicators. Soil carbon and soil respiration have been shown to be related (Wang et al., 2003; Lai et al., 2017). It was thus hypothesized that an increase in soil respiration would be accompanied by an increase in soil carbon in the NCLE experiment. The perennial cropping system may have had a positive effect on soil carbon fractions that were not quantified in this study.

4.4.2 Active Carbon

Similar to TOC, the absence of an increase in active carbon in the perennial crop history treatment was unexpected. The observed difference in active carbon between the annual and perennial crop history treatments was less than 1%. It was hypothesized that active carbon would be higher in the perennial crop history treatment for the same reasons as the hypothesized increase in TOC. The absence of an active carbon response and the presence of a soil respiration response to the perennial crop history treatment puts into question whether active carbon is a good estimate of microbially available carbon for the clay soils of the Red River Valley.

A second unexpected result was the absence of an increase in active carbon for the liquid pig manure treatment compared to the synthetic fertilizer or the control treatments. It was hypothesized that active carbon would be increased by the liquid pig manure treatment when compared to the control and synthetic fertilizer treatments. However, the liquid pig manure treatment had an active carbon value that was not different from the solid dairy manure, synthetic fertilizer, or unfertilized control treatments. There was an observed difference of 9% between liquid pig manure and the control treatments and a 7% difference between the liquid pig manure and the synthetic fertilizer. The variability within the fertility treatments (18.53-22.25 mg kg⁻¹) is likely the reason for an absence of a significant difference among these treatments.

4.4.3 Soil Respiration

It was hypothesized that soil respiration would be increased by the two manure fertility treatments. This hypothesis was rejected as there was no soil respiration response to any of the fertility treatments. There was an observed difference of 9% between the unfertilized control and the liquid pig manure treatments. This difference was not significant due to the variability of the

data ($0.07 \text{ CO}_2 \text{ mg g}^{-1} \text{ day}^{-1}$) in addition to this there may be high spatial variability within the NCLE experiment as the experiment spans 16 ha. The observed difference between the control and the solid dairy manure was small at 1%. This small difference between the means is likely the reason for the absence of a significant difference.

The hypothesized increase in respiration from manure application is supported by the literature and was based on the positive correlation between TOC and soil respiration (W. J. Wang et al., 2003). Other studies from North Dakota (Niraula, Rahman & Chatterjee, 2018) and Ontario (Rochette & Gregorich, 1998) found manure to increase soil respiration. The absence of a soil respiration response to the manure treatments in the NCLE experiment may be caused by the five-year time gap between the last manure application and sample collection. Similar to the North Dakota and Ontario studies, it is possible that soil respiration was higher in the manure treatments compared to the unfertilized control and synthetic fertilizer treatments during the years of manure application from 2007 to 2015. However, in the five-year period after the manure applications stopped, the microbial community may have consumed all the readily available carbon from the manure and reached an equilibrium, thus reducing differences in respiration among the fertility treatments. This absence of a soil respiration response conflicts with the active carbon results. There were differences in the active carbon levels between the fertility treatments, and active carbon is an estimate of microbially available carbon.

4.4.4 Aggregate Stability

It was hypothesized that fertility treatments involving manure would increase wet aggregate stability. However, this was not the case as there were no significant differences among the fertility treatments. The hypothesized effect of manure increasing aggregate stability was based

on previous studies that found increases in soil carbon from manure application resulted in increased soil aggregation (Riaz and Marschner, 2020). This relationship was also found by Ozlu & Kumar (2018) in South Dakota. When compared to soil that received inorganic fertilizer, seven years of a solid cattle manure treatment increased wet aggregate stability by 11.2% in the top 10 cm of a silty loam soil.

The methodology for measuring aggregate stability may be one reason for the absence of a response to the manure treatments. Simulated raindrops contain less kinetic energy than natural raindrops. Thus, the disruptive forces from the simulated rainfall used in this study may have been too weak to thoroughly test the strength of the aggregates for this clay soil. Wet aggregate stability was above 85% for all of the samples tested. Testing the aggregates with more destructive force might have allowed for WAS differences to be observed between the fertility treatments.

Aggregate size class may also have been a factor. In this study, aggregate stability was tested for one size fraction class (2 to 0.25 mm). There may be differences in the stability of aggregates larger than 2 mm or smaller than 0.25 mm that were not measured. Previous research on a similar soil found that differences in aggregate stability can exist for size fractions larger than 2 mm (Stainsby and Entz, 2022). It would be beneficial to compare methodological differences between the rain simulator method and alternative wet sieving methods for clay soils for a range of aggregate size classes (Kemper & Rosenau, 1986).

4.4.5 ACE Soil Protein

Although differences in ACE soil protein were identified for some fertility treatments, the absence of significant differences in ACE soil protein between the synthetic fertilizer, liquid pig manure, and the unfertilized control was unexpected. There was an observed difference of 17% between the control and the synthetic fertilizer and a 30% observed difference between the control and the liquid pig manure treatments. The absence of significant differences between these fertility treatments was likely due to the variability within the data (0.25-0.36 $\mu\text{g g}^{-1}$). It was hypothesized that the treatments nitrogen inputs, liquid pig manure and synthetic fertilizer, would have higher levels of soil protein than the unfertilized control. This hypothesis was based on the synthetic fertilizer and liquid pig manure treatments being fertilized to meet crop nitrogen demand. The fertilization would limit the drawdown of organic nitrogen in the soil (Eldor Alvin Paul, 2014; Geisseler et al., 2019). Whereas it was expected that the unfertilized control would draw down the soil organic nitrogen pool as there was no nitrogen fertilization to replenish the nitrogen removed during grain harvest for 13 years.

The absence of a significant difference among the liquid pig manure, synthetic fertilizer, and unfertilized control can be attributed to varying crop nitrogen removal rates for the range of fertility treatments tested in the experiment. Throughout the manure application phase of the experiment (2008-2015) both the synthetic fertilizer and the liquid pig manure treatments had yields over 200% greater than the unfertilized control (Flaten & Fraser, 2016). These high yields mean that nitrogen removal rates with grain harvest are high, and these high nitrogen removal rates were not conducive to building or maintaining soil organic nitrogen levels. The solid dairy manure treatment yielded 163% of the unfertilized control (Flaten & Fraser, 2016), likely indicating that a portion of the nitrogen present in solid dairy manure was not readily available

(Azeez & Van Averbek, 2010). Differences in nitrogen availability and removal rates are reflected in the historical nutrient balances. From 2008 to 2015, the synthetic fertilizer and liquid pig manure treatments accumulated 165 and 418 kg ha⁻¹ of nitrogen, respectively, whereas the solid dairy manure treatment accumulated 2290 kg ha⁻¹ of nitrogen (Flaten & Fraser, 2016). Ultimately, a smaller percentage of the nitrogen in the synthetic fertilizer and liquid pig manure treatments was likely incorporated as soil protein when compared to the solid dairy manure.

4.4.6 Potassium

The absence of a difference between the soil test potassium of the unfertilized control and that of the liquid pig manure treatment was unexpected. This result was unexpected because the unfertilized control received no potassium inputs throughout the duration of the experiment, which should have led to a drawdown of soil test potassium. There was an observed difference of 12% between the liquid pig manure and the unfertilized control treatment. However, the variability of the data prevented inference. The standard error of the fertility treatments (27.6-45.17 ppm) was greater than the history (27.47-30.47 ppm) and management treatments (27.01-27.24 ppm).

This absence of a difference is likely due to two factors. First, the removal rate in the unfertilized control is lower because the unfertilized control yielded less throughout the NCLE experiment. Second, there are high amounts of extractable and fixed potassium naturally found in this soil, which gives the soil a high potassium buffering capacity, potentially masking any differences between the control and the liquid pig manure. For example, the potassium levels of the unfertilized control were 530 ppm, which is well in excess of the crop requirements for all the commercially grown crops in the Red River Valley.

4.4.7 Phosphorus

The absence of a significant difference in Olsen phosphorus between the liquid pig manure and the control was an unexpected result. This was likely due to the high standard error of the treatment (11.3-7.26 ppm), which prevented inference between the treatments. It was hypothesized that the liquid pig manure treatment would be higher than the unfertilized control as research has shown that liquid pig manure can increase soil phosphorus levels (Stumborg & Schoenau, 2008). The absence of a difference between the liquid pig manure treatment and the control is supported by historical data from the NCLE experiment. In 2015, during the last year of manure application for the perennial rotation, there was no statistical difference in Olsen-phosphorus between the liquid pig manure and the unfertilized control treatment (Flaten & Fraser, 2016).

4.4.8 Soil Management Treatment

None of the soil health indicators were responsive to the soil management treatment. It was hypothesized that the soil-building treatment implemented in 2020 would maintain soil health indicators following the conversion to annual crops in all treatments. This hypothesis was based on previous literature documenting the ability of conservation tillage and cover cropping to improve soil health indicators, such as TOC, active carbon, AWHC, and WAS (Liu, Ma and Bomke, 2005; Sheehy, Regina & Six, 2012; Aziz, Mahmood & Islam, 2013; Maiga et al., 2019; Hashimi et al., 2023; Qin et al., 2023). For the majority of the indicators tested in this study, there were small observed differences among the means. Observed differences were less than 1% for TOC, active carbon, PMN, potassium, WAS, and pH. Observed differences were 5% or less

for ACE soil protein, AWHC, iron, manganese, and magnesium. The small observed differences among the means of these indicators was likely the reason for the absence of a difference. Two indicators had higher observed differences, including phosphorus and zinc with differences of 11% and 9%, respectively. For these indicators, variability likely prevented inference among the treatment means.

The short timeline between treatment initiation and sample collection for this study is the most plausible explanation for the absence of a soil health indicator response to the soil management treatment. There was only one growing season for the treatment to take effect, compared to seven years for the soil fertility and nine years for the crop history treatments. More time is likely required before soil health indicators will be responsive to the soil building treatment in the NCLE experiment. There are multiple examples in the literature of longer treatment lengths leading to an increase in soil health from either conservation tillage or cover cropping treatments. Iheshiulo et al. (2024) found that four years of no-till increased aggregate stability in Alberta. Dai et al. (2024) found that three years of cover crops increased aggregate stability in Mississippi. Sekaran, Sagar & Kumar (2021) found that six years in no-till increased SOC by 33% in South Dakota. Chahalid & Van Eerd (2020) found that six years of cover crops increased TOC, WAS, and soil respiration in Ontario.

5.0 General Discussion

The goal of this general discussion is to explore the implications of this study to soil health in the Red River Valley. This general discussion is divided into four main sections. The first is a summary of the results. The second is the suitability of the CASH soil health indicators to evaluate the humic vertisols found in the Red River Valley of Manitoba. The third is recommendations to improve soil health using management practices. The fourth is the adoption of soil health practices and management in the Red River Valley.

5.1 Summary of Results

Indicators from all of the CASH soil health indicator categories were responsive to one or more of the management treatments at the NCLE experiment (Table 5.1). The biological indicator group had the highest percentage of responsive indicators with all five of the indicators (TOC, active carbon, PMN, ACE soil protein, soil respiration) responding to either the fertility or the crop history treatment. The chemical indicator group had the lowest percentage of responsive indicators with only three of the seven indicators (potassium, phosphorus, zinc) responding to the imposed treatment. The physical indicator group was in the middle with one of the two indicators (WAS) being responsive to treatments. The seven indicators that were responsive to the fertility treatments were: active carbon, TOC, ACE soil protein, PMN, potassium, phosphorus, and zinc. The four indicators that were responsive to the crop history treatment were: PMN, soil respiration, WAS, and potassium. Two indicators (potassium and PMN) were responsive to both the fertility treatment and the crop history treatment. The unresponsive indicators were: AWHC, pH, Fe, Mn, and Mg.

Table 5.1. Summary of physical, biological, and chemical indicators responsive to management treatments.

Effect	Physical	Biological	Chemical
Number of responsive indicators			
History†	1	2	1
Fertility††	0	5	3
Management†††	0	0	0

† Cropping system history main treatment effect with annual and perennial cropping system treatment levels.

†† Soil fertility main treatment effect with synthetic fertilizer, liquid pig manure, solid dairy manure, and unfertilized control treatment levels.

††† Soil management main treatment effect with conventional and soil building treatment levels.

5.2 Suitability of Soil Health Indicators for Use on Clay Soils in the Red River Valley

This section addresses the suitability of the soil health indicators included in CASH to evaluate humic vertisols from the Red River Valley of Manitoba. To be considered suitable in this study, an indicator must be responsive to one of the treatments within the NCLE experiment. This section will also discuss potential barriers to the adoption of the indicators for on-farm use.

5.2.1 Suitable Soil Health Indicators

Eight soil health indicators in this study were suitable for identifying management differences in the Red River Valley. These indicators were: WAS, active carbon, TOC, ACE soil protein, PMN, soil respiration, Olsen-phosphorus, and zinc. Despite being responsive, some of the indicators may be better suited for on-farm use than others due to the potential ease of adoption. Total organic carbon, Olsen-phosphorus, and zinc could be adopted faster than the other indicators

because they are already standard in the soil nutrient tests available from commercial soil testing labs. For these three soil health indicators, the creation of interpretive soil health scoring functions is the only factor currently limiting adoption.

Some of the responsive indicators identified in this study have barriers to adoption. Three of the indicators need method standardization: wet aggregate stability, soil respiration, and potentially mineralizable nitrogen. The benefit of having a standardized method is that it enables different labs to replicate the same test, allowing for data from multiple labs to be compared. The ability to compare soil health indicator results is crucial to creating the soil health indicator scoring functions. . Wet aggregate stability currently has three different methods: the raindrop simulation method, the wet sieving method, and the slake test. Potentially mineralizable nitrogen and soil respiration are both incubation-based tests that need standardization for their run time (eg. 24 hr vs 4 day), temperature (eg. 25°C vs 40°C), and redox status (anaerobic vs aerobic). Criteria such as cost and throughput time will likely determine which method becomes adopted as a standard method.

A second barrier to adoption for five of the indicators is redundancy. Redundancy is when pairs of indicators are related and thus provide similar insights into soil health. Based on the results of this study, there are three pairs of potentially redundant indicators. These pairs are TOC with active carbon, PMN with soil respiration, and PMN with ACE soil protein. This redundancy is inefficient as it adds cost to the analysis without adding new information. Cost can be a barrier to adopting soil health testing, as cost already prohibits soil nutrient testing, which has been demonstrated to provide a defined return on investment. Deciding which redundant soil health indicator to use will likely be based on analysis costs and lab throughput time.

Along with responsiveness to management, another criterion for a suitable soil health indicator is relevance to crop production. For the nutrient soil health indicators, their relevance to crop production depends on if they are sufficient or deficient for crop growth. Potassium meets the first criterion as it was responsive to both the fertility and the crop history treatments. However, for the humic vertisols of the Red River Valley, potassium may not be a suitable soil health indicator as the mineralogy of the Red River Valley soil has naturally high levels of potassium that are sufficient to meet crop demand. Another indicator that may not be relevant to crop production in the Red River Valley is magnesium; there is a sufficient amount in the soil to meet crop growth, and there is an excellent supply from the subsoil.

5.2.2 Soil Health Indicators that are not Suitable

One of the key criteria for evaluating soil health indicators in this study was responsiveness to management practices. Five soil health indicators were unresponsive to any of the treatments at the NCLE experiment. These indicators were AWHC, pH, Mg, Mn, and Fe. The unresponsiveness of each indicator can likely be attributed to the following characteristics of the clay soil specific to this study: high water holding capacity for AWHC, high pH buffering capacity, and high SOM for the micronutrients.

It is difficult to conclude that these unresponsive soil health indicators should be removed from soil health testing for humic vertisols in the Red River Valley or for soils in southern Manitoba as they may be responsive under different soil textures or a longer treatment time frame. There are contrasting soil textures that are found in or on the border of the Red River Valley (but not in this experiment) that may be responsive to management based on these indicators. For example, sandy soil types can be found just outside of the Red River Valley

around the towns of St. Claude MB to the west and Grunthal MB to the east. These sandy soil types will have a different response to management than the clay soils found in the Red River Valley. The time frame of the treatments is also important to consider, as unresponsive soil health indicators from the NCLE study may become responsive to management in the future. For example, soil pH may become a responsive soil health indicator if soil acidification from high rates of nitrogen fertilization becomes an issue in the Red River Valley. Or micronutrients may become deficient after many decades without micronutrient fertilization. Overall, these five unresponsive indicators may still provide valuable insight into the productive capability of a given soil type at a regional level or may be responsive in the future.

5.3 Recommendations to Improve Soil Health Using Management Practices

Improving soil health is important for the future success and sustainability of agriculture because healthy soil can help mitigate the challenges posed by climate change while also reducing the environmental impact of agriculture. One such challenge is the increased unpredictability of precipitation. This unpredictability was observed over the course of 12 months from June 2021 to June 2022, where in 2021, there were severe drought conditions at the NCLE experiment as shown by the deep slickensides (Figure 5.1). The following spring in 2022, the Red River Valley experienced heavy flooding that left parts of the Glenlea research station underwater (Figure 5.2). The rest of the 2022 spring was wet, and there were often saturated soil conditions throughout the first half of the growing season at the NCLE experiment (Figure 5.3). This 12-month period of drought to flood may be foreshadowing what normal weather is in the future.

Climate scientists predict that the total amount of precipitation and the intensity of precipitation events on the prairies will increase with climate change; however, the frequency of

the precipitation events is predicted to decrease. Thus, for agriculture to prosper, soil must be able to withstand challenging environmental conditions. Increasing soil health will help accomplish this goal. As shown by the findings of this study, growing perennial crops and applying manure are practices that farms in the Red River Valley can implement to improve soil health and manage extreme weather events like drought and excessive moisture.



Figure 5.1. Image of drought conditions on July 29, 2021, where deep slickensides can be seen in the field.



Figure 5.2. Aerial image from NCLE experiment on May 25, 2022, looking east towards the spring flooding of the Red River.



Figure 5.3. Aerial image of the NCLE site on June 15, 2022, after a rainfall demonstrating saturated soil conditions.

5.3.1 Perennial Crops

To improve soil health, farmers in the Red River Valley should grow more perennial crops. The results from this study have demonstrated that a mixture of perennial grasses can improve soil health by improving aggregate stability, potentially mineralizable nitrogen, and soil respiration. The ability of perennial crops to improve soil health can be attributed to the lack of soil disturbance, constant ground cover, and diversity of crop species. These characteristics of perennials help increase soil carbon levels and are beneficial to soil organisms whose importance to soil function cannot be overstated. It is important to remember that perennial forage crops are only an option for select farming operations that have grazing animals or are in an agricultural region that contains strong local demand for forages. Other agriculture production systems that contain perennial crops are biofuel crops and perennial grain crops. These crops are not very prevalent in the Red River Valley but do present a potential opportunity to improve soil health.

5.3.2 Manure Application

Second, farms should apply manure to improve their soil health. The results from this study have shown that both solid dairy manure and liquid pig manure can improve soil health, with solid dairy having the greater benefit to soil health. The benefits of manure can be attributed to the carbon and nutrients that are in manure, which creates a legacy of soil health benefits. This legacy is demonstrated by the fact that the results of this study were from soil that had not received manure in five years. Because manure application can increase the biological function of soil while also fertilizing the soil, applying manure has the economic advantage of reducing fertilizer costs while also improving soil health. It is important to remember that manure application is only a viable option for the areas of the Red River Valley where there is a high density of livestock producers

that provide a local supply of manure. Despite the logistic benefits, farmers should not always apply manure to the closest field, there are agronomic benefits of spreading out your manure to every acre possible.

Despite all the positive soil health benefits, there are issues associated with the over-application of manure that need to be kept in mind when selecting application rates and application frequency. Phosphorus loading in soil is an environmental issue if phosphorus runs off the field and ends up in waterways. This leads to eutrophication, which is an environmental issue for Lake Winnipeg. Greenhouse gas emissions of nitrous oxide and methane pose an environmental threat and must be managed to maximize the benefits of manure. Designing manure application strategies that are balanced and maximize the benefits of manure application while minimizing the potential risks will be important moving forward.

5.3.3 Cover Crops and No-Till

The soil-building treatment that includes no-till and cover crops had no effect on any of the soil health indicators in this study. This was likely due to the short time frame of one year for the soil-building treatment to take effect. More time will be needed to evaluate the soil building treatment as both practices in the soil building treatment, cover cropping, and no-till, can and have been shown to improve soil health. The impact of the soil building treatment should be investigated in 3 to 5 years once there has been more time for the treatment to take effect at the NCLE experiment.

Overall, the manure treatment after eight years and the perennial treatment after 10 years have both been proven to increase soil health. The soil-building treatment after one year did not

increase soil health in this study, but may do so in the future. There are options for farmers to improve their soil health however, these practices require some level of animal integration into a farming system. Farmers should implement small, incremental changes first to ensure that the changes are effective. What is important is a farmer's awareness of soil health and how their agronomic practices affect soil health. This awareness will enable them to improve the health of their soils and is paramount to the future of soil health in the Red River Valley.

5.4 Adoption of Soil Health Practices and Management

The future of soil health in the Red River Valley is predicated on the agricultural industry managing soil health and implementing soil health-building practices. The results of this study demonstrate that soil health indicators can be responsive to long-term management practices such as manure application and perennial cropping. To further increase the adoption of soil health practices, three things are needed: research surrounding soil health indicators and scoring functions, incentives to promote the adoption of soil health programs, and extension to increase the understanding of soil health.

5.4.1 Future Research for Soil Health Testing

Future research needs to focus on whether the soil health indicators in this current study are responsive and best suited for use on the humic vertisol throughout the Red River Valley. The Red River Valley is a large area, and the results of this study cannot be applied to the entire Red River Valley region of Manitoba as this study represents only one research site. More research sites and site years from other experiments will be required to definitively determine which soil health indicators are responsive to management on the clay soil of the Red River Valley. The

need for more research sites and site years is particularly important for the unresponsive indicators in this study, AWHC, pH, manganese, magnesium, and iron. The results from this study cannot be used to definitively answer whether these unresponsive soil health indicators are not suitable for use in the Red River Valley.

A second area that research should focus on is creating an interpretative framework for local soil health indicator values. This research would allow farmers and agronomists to make informed soil health management decisions in the Red River Valley. Without interpretation, the values are not useful for making on-farm management decisions. When interpreting soil health indicator values in the CASH, the indicators fall into one of three interpretation frameworks: higher indicator values improve soil health, lower indicator values improve soil health, or an optimum value is best for soil health. The knowledge gap for the Red River Valley is the threshold level at which the indicator values are good or bad. Without this interpretation framework, soil health indicators are not very informative outside of a research setting that has various contrasting treatments. To create the interpretation framework, a large local calibration dataset is needed for each indicator. This local data set can be used to create scoring functions that allow for the interpretation of the indicator values. To create the local dataset, samples should be taken from farm fields. The use of farm fields to create the calibration dataset has multiple benefits. First, it would allow for the immediate collection of data. Second, it would create farm-ready soil health scores as the test would be calibrated to soil from local farms. Third, it is a method that has already been used by the authors of the CASH to refine the CASH.

5.4.2 Incentives

Efficient well structured incentive programs will be integral to increasing the adoption of soil health practices in the Red River Valley. Farmers can be hesitant to adopt new practices, so incentive programs are commonly used in Canada and the US to get farmers to adopt new practices. There have been incentive programs available to Manitoban farmers for adopting soil health practices in the Red River Valley. In the past, these incentives were through the Ag Action Manitoba Program. The practices incentivized in the program include resource management planning, cover crops, intercropping, perennials in rotation, perennial cover on sensitive land, and improved pasture management.

Considering the findings of this current study, future incentive dollars should focus on the following: manure management, perennial crop establishment, and crop livestock integration. Incentivizing farmers with manure to spread out their manure across all their acres would be of great benefit as some fields may not get manure applications due to their distance from the barn. The multi-year soil health legacy of manure application identified in this study indicates that manure application in every year is not required to result in soil health benefits.

Creating incentives for farms to establish more perennials would improve soil health, especially for fields that have spent a lot of time under high-disturbance annual crop production. Incentives should be created for growing perennial forages or perennial biofuel crops in rotation, as a phase of a perennial crop in rotation can leave a legacy of soil health benefits. Crop-livestock integration will be essential to soil health in the future. Encouraging livestock farms to work collaboratively with grain farms would allow for the implementation of soil health practices. The practices of manure application and growing perennial crops are more common on livestock farms than on grain farms. If these two farming systems were incentivized to

collaborate, there would be increased opportunity for soil health practices. Overall, the incentive programs should be created with consultation from all the stakeholders involved in order to effectively increase the adoption of soil health practices.

5.4.3 Extension

People are an essential component of agriculture, they make the on-farm management decisions and these on-farm management decisions directly impact soil health. Improving their understanding of soil health will lead to improved soil health management. Currently, there is a need for a robust set of local agronomic best management practices that improve soil health. If there was a set of local agronomic best management practices, then both farmers and agronomists would be more comfortable making soil health-conscious management decisions. In the future, Manitoba Agriculture and Agriculture and Agri-Food Canada should focus on developing extension resources that would educate farmers and agronomists about soil health. These extension resources should address how improving soil health impacts common agricultural challenges that are at the forefront of farmers and agronomists minds. Examples of these challenges include drought stress, flooding stress, weed management, insect management, and disease management.

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7.0 Appendices

A1 NCLE Experiment Plot Plan

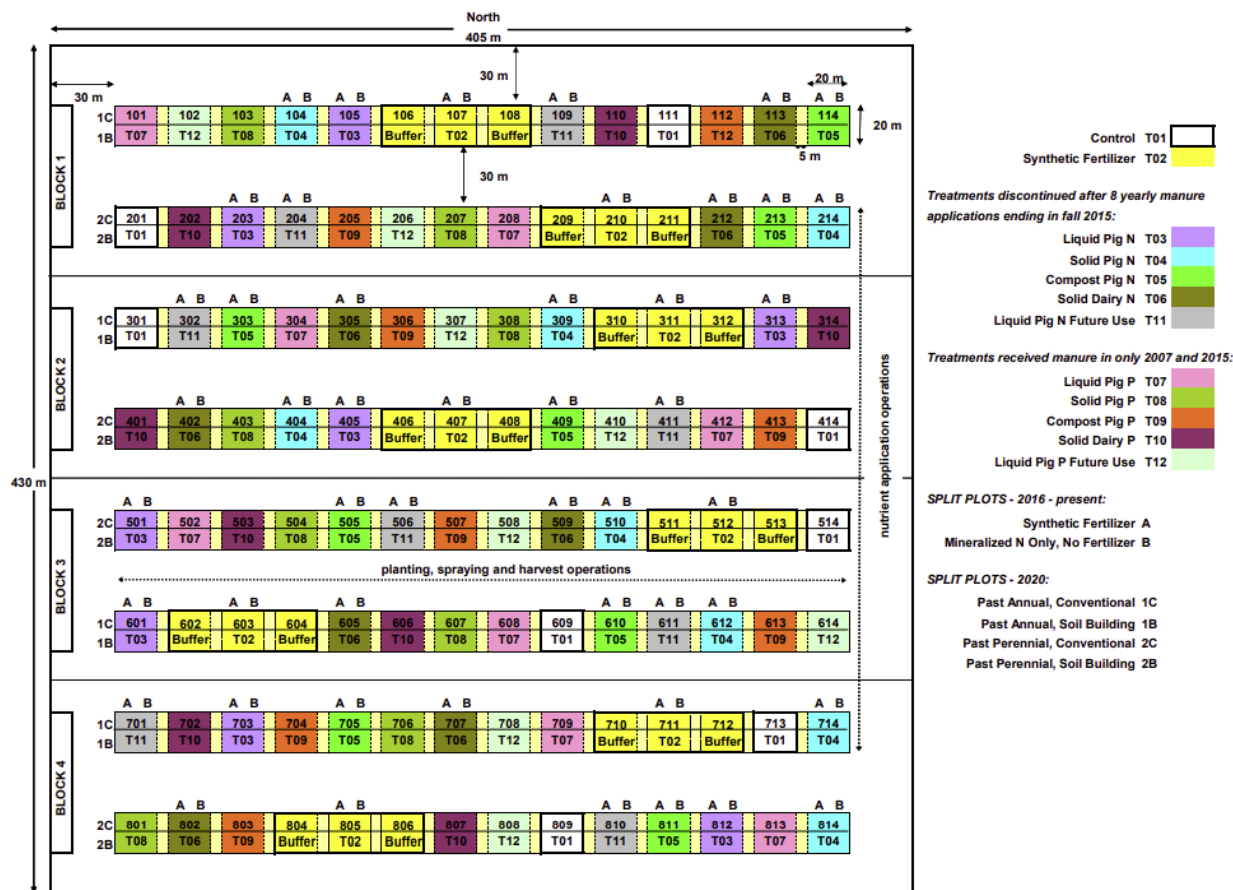


Figure A1.1. Detailed plot map of the National Center for Livestock and the Environment long-term nutrient management study. The treatments sampled for this study included T01 (no fertilizer control), T02 (synthetic fertilizer), T03 (liquid pig manure with the application rate based on crop nitrogen needs) and T10 (solid dairy manure with the application rate based on crop nitrogen needs).

A2 Properties of Applied Manure

Table A2.1. Dry matter content of manure applied to NCLE site from 2008 to 2016. There were no manure applications from 2017-2020 (adapted from Flaten and Fraser, 2016).

Year	Liquid Pig	Solid Dairy
	-----% DM-----	
2008	0.8	21.3
2009	0.6	23.2
2010	missing	missing
2011	0.7	20.1
2012	5.5	23.4
2013	0.9	22.8
2014	0.9	17.2
2015	0.6	18.5
2016	6.1	16.3
Average	2.0	20.3

Table A2.2. Manure application rate, amount of total nitrogen applied, and total organic nitrogen applied to the annual and perennial cropping system treatments for both liquid pig and solid dairy manure fertility treatments in the NCLE long-term study from 2008 to 2015.

Cropping System Treatment		Annual			Perennial		
	Year	Manure Application Rate	Total Nitrogen Applied	Total Organic Nitrogen Applied	Manure Application Rate	Total Nitrogen Applied	Total Organic Nitrogen Applied
		tonnes ha ⁻¹	kg ha ⁻¹	kg ha ⁻¹	tonnes ha ⁻¹	kg ha ⁻¹	kg ha ⁻¹
Liquid Pig Manure	2008	79	119	4	78	117	4
	2009	73	80	15	34	37	7
	2010	75	56	11	75	56	11
	2011	212	276	104	227	296	112
	2012	102	205	97	131	262	124
	2013	50	67	12	52	71	12
	2014	90	148	28	89	146	28
	2015	108	165	12	62	95	7
	Total	-	1116	283	-	1080	305
Solid Dairy Manure	2008	56	418	368	56	418	368
	2009	40	291	206	20	145	103
	2010	45	290	247	45	290	247
	2011	49	257	184	88	460	329
	2012	71	422	312	113	669	496
	2013	58	396	335	54	369	312
	2014	56	328	275	51	294	246
	2015	46	238	187	22	114	89
	Total	-	2640	2114	-	2759	2190