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SOIL HEALTH INDICATORS AS TOOLS TO UNDERSTAND THE EFFECTS OF DISTURBANCE IN AGROECOSYSTEMS

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Abstract

Soil health refers to a soil's ability to sustain biological life into the future while maintaining water and air quality. No-till agriculture has become a primary strategy to improve soil health in row-crop production in the United States. Growers who have adopted no-till practices are typically highly reluctant to use any type of tillage out of concern for its effects on the health of their soil. The two primary objectives of this thesis research were 1) to examine the effects on soil health of one-time inversion tillage in a six-year rotation including canola, corn, soy, cover crops and perennials and 2) to compare soil health effects of contrasting fertilization methods, involving either surface applications or incorporation using reduced disturbance, in a corn-soy rotation. These objectives were approached using four soil health indicators: total organic carbon, bulk density, labile carbon, and aggregate stability, to determine the impacts of different management strategies implemented at the Dairy Cropping Systems Experiment (DCSE) at the Penn State Agronomy Research Farm at Rock Springs, PA. This experiment had been initiated in 2010 as a full crop entry experiment, with all phases of the crop rotations planted every year in a randomized complete block design, replicated four times. The channery silt loam soil at this site was sampled in spring 2010 prior to the start of the experiment and in 2013, 2016, 2019, and 2021 at two depths: 0-5 and 5-15 cm for labile and total carbon and to 15 cm for aggregate stability.

The research conducted under Objective One is described in Chapter Two of this thesis. The management system under study was a six-year crop rotation that included two approaches to terminating the perennial forage crop—termination by standard herbicides or by inversion tillage. The aim was to assess soil health effects of once-in-six-year tillage as a strategy for herbicide reduction (T1/6) when compared to no-tillage using standard herbicide treatment (NT).

Although tillage initially reduced total soil organic and labile carbon, plots that were tilled showed similar soil health levels as the continuous no-till plots in all four indicators after two full years in perennial forage. Results from this analysis indicated that soil health can return to no-till levels despite a tillage event if rotated to perennial forage for sufficient years.

The research for Objective Two is described in Chapter Three and involved a more traditional corn-soy rotation, which had been included in the Dairy Cropping Systems Experiment because it is common among grain crop growers. Soil health indicators were compared in soils subjected to four fertilizer application strategies: no-till with broadcast manure (NT-BM), chisel disk with broadcast manure (CD-BM), no-till with broadcast synthetic fertilizer (NT-SF), and no-till with injected manure (NT-IM). Despite the classification of chisel-disk as a type of conservation tillage, the CD-BM strategy had the highest expected potential to reduce soil health because of its increased level of disturbance. Investigating the impact of CD on soil health was the primary focus of this chapter. There was also some expectation that injected manure would reduce soil health due to disturbance associated with injection, which also motivated comparison of the three different no-till strategies. Soil total organic carbon, labile carbon, and aggregate stability were all reduced in the CD-BM strategy, though no effects due to tillage were observed at the 5-15 cm depth. Additionally, there were no differences between the effects of the three no-till strategies on soil health. Results from this analysis suggest that soil health is negatively impacted by chisel disking compared to no-till, but that manure injection does not appear to affect soil health.

The concluding chapter of this thesis summarizes the results of both studies and provides recommendations for farmers and future research. Reducing tillage intensity is critical to improving soil health, though strategic timing of one-time tillage events may alleviate some of the herbicide requirement typically associated with no-till, particularly when these events are coupled with perennials in rotation. Chisel-disking may be a positive alternative to more intensive tillage practices, but it showed short-term negative impacts compared to no-till. Overall, this thesis supports the idea that reducing disturbance and increasing perenniality of systems is the key to long-term improvements to soil health.

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Chapter 1

Introduction: Soil Health Indicators and Management Practices in the Dairy Cropping Systems Experiment

Soil health, “the continued capacity of soil to function as a vital living ecosystem that sustains plants, animals and humans, and connects agricultural and soil science to policy, stakeholder needs and sustainable supply chain management” (Lehmann et al., 2020), is currently a popular subject among the public and the agricultural community. However, soil health is not a new concept; one of the earliest references to the term soil health was in an unpublished dissertation by Henry Wallace in 1910. Early uses of the term focused mainly on a soil’s physical and chemical properties—it was not until the 1990s that the modern understanding of soil health was popularized in science (Brevick, 2018). John Doran’s definition, first published in 1996, expanded on previous understanding of soil health as a means to sustain agricultural productivity by including greater responsibility for overall ecosystem health as a part of the definition: “the continued capacity of soil to function as a vital living system, within ecosystem and land-use boundaries, to sustain biological productivity, maintain the quality of air and water environments, and promote plant, animal, and human health” (Doran et al., 1996). Today, concerns about climate change have resulted in expanding the definition of soil health to incorporate climate resilience (Ramborun et al., 2019). Soil, particularly in the agricultural context, is and will continue to be affected by climate change—growing seasons may be longer or shorter, some areas may experience more rain than normal, whereas others may need to adapt to longer periods of drought (IFPRI, 2009; Ramborun et al., 2019). It is therefore necessary to evaluate soil management techniques that will enhance soil health and, as a result, the long-term productivity of the soil. This is particularly important in agricultural systems as agricultural soils produce our food supply and are the foundation for the livelihood of many farmers.

Before the term soil health gained wider usage within the USDA as well as the public, the term soil quality was more common, as it was employed in the NRCS soil quality test kit, a well-known resource for on-farm soil testing methods (USDA, 2001). The change in the uses of the terms, soil quality and soil health, was due mainly to recognition and growing concern for the biological aspects of soil health rather than just the physical and chemical aspects (Stott & Moebius-Clune, 2017). The now three-pronged approach promoted by the USDA's Division of Soil Health and applied in the Cornell Soil Health Testing Manual, uses soil physical, chemical, and biological indicators to evaluate a soil's health. Each category has a suite of individual indicators that can be measured to quantify the health of the soil (**Table 1.1**).

Research publications in the last decades have emphasized the benefits of three main management practices to improve soil health: decreasing tillage intensity; increasing cropping system diversity; and perenniality (Nunes et al., 2020; Sprunger et al., 2021). Although the benefits and drawbacks of different management practices can depend on soil type and regional climatic variables, several general trends can be drawn upon to determine the direction of studies aimed at improving soil health. These observed trends in the literature are consistent with the four principles of soil health (**Figure 1.1**) outlined by the USDA-NRCS: 1) maximize continuous living roots, 2) minimize disturbance, 3) maximize soil cover, and 4) maximize biodiversity (USDA-NRCS, 2018).

A study by Sprunger et al. (2021) of organic corn systems, which surveyed 195 organic farmers in the Midwest region, concluded that reducing tillage intensity and incorporating perennials into crop rotations were the two main drivers of improved soil health across soil textures. The study also noticed that soil texture was the factor that caused the most variation in soil health differences, with fine and medium textured soils typically having higher soil carbon and nitrogen than coarse textured soils, emphasizing the importance of taking these differences into account when comparing soil health between systems with different soil textures. Despite crop diversity usually being considered as a positive contributor to soil health, the organic systems in this study showed a negative correlation between crop diversity and soil health. The explanation for this finding was that organic systems apply higher intensity of tillage as more diverse crops are used in organic rotations. Additionally, the study found increased mineralizable carbon, which is considered a positive effect on soil health, was associated with perennial crops, particularly when left in rotation for longer periods of time. This study deemed perenniality a driving factor of soil health in organic systems, as perennials were associated with reduced tillage in the farms surveyed as well as the increased belowground biomass inputs associated with perennial root systems. A nationwide meta-analysis by Nunes et al. (2020), also concluded that increased perenniality and reduced tillage were two main driving factors in improved soil health over a wider variety of cropping systems and disturbance types. Over the 302 studies assessed, the researchers observed improved biological health in annual systems that had reduced tillage intensity, planted cover crops, and minimized residue removal. Additionally, perennial systems had consistently better soil health than annual systems. Management practices aligned with these trends were implemented in Penn State's Dairy Cropping Systems Experiment (DCSE) (Malcolm et al., 2015). They include no-tillage, reduced tillage, diverse crop rotations, cover cropping, and inclusion of perennials.

This thesis is based on evaluating soil health indicators associated with selected management practices in the DCSE. This chapter will explain aspects of the experimental design and the specific soil health indicators analyzed. The goal of this chapter is to provide an overview of the DCSE and the rationale for choosing the indicators and management practices investigated in this thesis.

Introduction to the Dairy Cropping Systems Experiment

The Sustainable Dairy Cropping Systems Experiment (DCSE) was established in 2009 on Penn State's Agronomy Research Farm in Rock Springs, Pennsylvania (40.721384, -77.919952). In this experiment, three rotational cropping systems suitable for the Northeastern U.S. region were established to assess their capabilities to integrate the goals of producing sufficient forage, feed, and fuel for a 65-milking cow herd while minimizing off-farm inputs (Malcolm et al., 2015). Additionally, the cropping systems in this project were intended to reduce nutrient and soil loss, build soil health, and increase biodiversity while remaining energy efficient, productive, and both ecologically and economically sustainable (Busch et al., 2020).

The DCSE was designed based on sustainable management practices and the advice of an advisory panel consisting of both local farmers and invested researchers to ensure the system provided useful information for both parties. The systems aimed to replicate conditions of a 97-ha farm at 1/20th the scale to better understand the outcomes of improved management practices for dairy farmers. The goals of the dairy cropping systems were achieved by comparing two six-year rotations and one two-year rotation in a randomized complete block design with each year of each rotation represented every year in four replicate blocks. The two diverse, six-year rotations included legumes, cover crops, and perennials. One system, designated the Manure Rotation, compared broadcast and injected manure application methods. The other six-year rotation, designated Weed Rotation, compared standard and reduced herbicide application with full tillage once in six years. The simpler, two-year system was designated Corn-Soy and represents a common rotation in this region. The latter rotation included four management sub-treatments: yearly chisel disking with broadcast manure (CD-BM), no-till with broadcast manure (NT-BM), no-till with synthetic fertilizer (NT-SF), and no-till with injected manure (NT-IM). For this thesis, soil health indicators were evaluated only in the Weed and Corn-Soy Rotations, because they offered the opportunity to assess the effects of varied levels of disturbance (by tillage and fertilizer application approach) on soil health.

Published research on the DCSE includes a study by Malcom et al. (2015), which provided an energy analysis of the management practices used in the system. Another study by Busch et al. (2020), compared the high and low diversity crop rotations and their respective methods of insect management to assess the ability of high diversity crop rotations to suppress insect pests while maintaining yield advantages. Additionally, Snyder et al. (2016) and Summers et al. (2021), published studies examining the costs and benefits of reducing herbicides in the weed rotation. The Sustainable Dairy Cropping Systems serves as an opportunity for interdisciplinary studies of important questions in agriculture. Much of the available literature concerning soil health has reported on short term studies (five years or less) following implementation of specific management practices, but these do not offer insights into how soil health may change over longer time periods under conservation management (Reynolds et al., 2007; Franzluebbers et al., 2008). The dairy cropping systems in this study provide an opportunity to examine changes in soil properties over a longer period, namely nine years. Having the means to evaluate changes in soil health following implementation of specific agricultural management in the DCSE is an important opportunity to evaluate soil health over the longer term. As climate change continues to cause weather patterns to shift and fluctuate, having a clear foundational understanding of soil health and its management will be increasingly important for farmers and researchers alike (Ramborun et al., 2019).

Management practices at the DCSE

No-tillage

In recent years, no-till agriculture has gained much more attention as a management practice for reducing erosion and conserving soil. However, several studies referenced in the USDA-NRCS Soil Health Literature Summary (2015) indicate that no-till as a practice alone does not guarantee improved soil quality and that a combination of practices may be needed. The specific practices that will improve a soil's quality are dependent upon the land use history, crops being grown, and regional factors such as soil type and climate (Evett et al., 1999; Jiang et al., 2007; Hubbard et al., 2013; Olson et al., 2013). In addition, long-term studies are necessary to evaluate the effectiveness of a management technique as some reports have found that practices such as no-till take upwards of five years for soil quality improvements to be evident (Reynolds et al., 2007; Franzluebbers et al., 2008).

No-tillage, or no-till, agriculture has become a common way to improve soil health because it helps maintain a more porous soil structure (Stubbs et al., 2004). As early as 1994, improvements to aggregate stability as a result of no-till were documented when compared to conventionally tilled systems (Batey, 2009). In the nationwide meta-analysis by Nunes et al. (2020), the researchers found that switching from a moldboard plow tillage system to a no-till system improved all seven soil health indicators evaluated in the study. However, the improvements in soil properties following no-till depended on the length of time a system had been under no-till and the cropping system used. Soil organic carbon typically improved after only three years of no-till management and additional conservation practices such as addition of cover crops and increased retention of crop residues enhanced the positive effects of no-till on soil health.

Because farmers use multiple management practices at once, and we know that the benefits of no-till are enhanced by other positive soil health management practices (Basche and DeLonge, 2019), it is important to study no-till effects in the context of different cropping and fertilization practices. For this thesis project on soil health indicators, tillage effects on soil properties were studied in the six-year Weed Rotation and the Corn-Soy Rotation. In the Weed Rotation, soil health indicators in continuous no-till (NT) were compared to indicators in otherwise no-till soils that were subjected to once-in-six-year inversion tillage (T1/6). With continuous NT, standard application rates of herbicide were used to terminate the perennial crop, while in T1/6, plowing was used to terminate the perennial crop to permit reduction in herbicide use. In the Corn-Soy Rotation, soil health indicators under four fertilizer application strategies were compared: manure broadcasted on continuous no-till (NT-BM); synthetic fertilizer applied to continuous no-till soils (NT-SF); manure injected into continuous no-till soils (NT-IM); and broadcasted manure incorporated into soil by chisel-disking (CD-BM). These fertilization application approaches involved contrasting levels of physical disturbance, which provided the opportunity to evaluate effects of disturbance on soil health indicators. The Corn-Soy Rotation permitted a comparison of combined management practices that are more similar to on-farm conditions. By looking at systems more holistically, we are able to achieve results that are relevant to both researchers and farmers. The specific practices are reviewed below.

One-time tillage

One-time tillage, also referred to as occasional or strategic tillage (among other names), is the practice of tilling otherwise no-till soil at strategic intervals in a cropping rotation, usually to mitigate problems with plant or insect pests, incorporate stratified nutrients, or as in the case of the present study, to terminate a perennial or cover crop. While no-till is often touted as the ideal tillage system for improving soil health, occasional tillage could alleviate the pest and weed problems associated with no-till which would otherwise require heavy herbicide or pesticide applications. Occasional tillage has also been effective at incorporating nutrients, such as phosphorus, that are stratified at the soil surface (Scheiner et al., 1998; Grove et al., 2007; Quincke et al., 2007).

The Weed Rotation in this experiment used inversion tillage, once in the six-year rotation, specifically after the third year of perennial growth, as the means to terminate the perennial and reduce herbicide use. Because perennial roots are deeper, denser, and more resistant to disturbances than annual roots, full inversion tillage with a moldboard plow is needed to effectively terminate the crop. While regular use of moldboard plow tillage has clear detrimental effects on soil health (Nunes et al., 2020), there is not a clear consensus on the use of moldboard plow as a one-time intervention strategy, though there is evidence to suggest that its impact on soil health is minimal (Kettler et al., 2000; Wortmann et al., 2010). The objective of this study system was to assess the impact of one-time tillage events on otherwise no-till soil, when coupled with cover crops and perennials, to determine if one-time tillage is an effective way to reduce herbicide use in no-till systems.

Conservation tillage

Conservation tillage is a broad term defined by the USDA (2000) as "Any tillage and planting system that covers 30 percent or more of the soil surface with crop residue, after planting, to reduce soil erosion by water." Chisel-disking falls under this definition and as it is the only conservation tillage approached examined in the dairy cropping system, it will be the focus of this section. A 1992 study by Carter compared conventional tillage practices to shallow and chisel plowing—both of which are considered conservation tillage—and found that soil structural stability improved significantly in three to five years in the conservation tillage plots (Carter, 1992). The meta-analysis by Nunes et al. (2020) found that systems converting from moldboard plow tillage to chisel-disk tillage improved in three out of the seven soil health indicators tested—not as successful soil recovery as the conversion to no-till, but still a significant increase. When Duiker and Beegle (2006) compared no-till, chisel-disk, and moldboard plow tillage, they found that moldboard plow tillage had a significant negative impact on soil health, but no significant differences between the no-till and chisel-disk treatments.

When systems are converted from a more intensive tillage practice to chisel-disking, the evidence suggests that chisel disking improves soil health. However, the differences between chisel disking and no-till are less clear—while Duiker and Beegle (2006) found no differences, the meta-analysis of 302 studies by Nunes et al. (2020), indicated that soils converted from moldboard plow to no-till improved considerably more than soils converted from moldboard plow to chisel disk tillage. Based on the soil health principle of minimizing disturbance (**Figure 1.1**), we would expect that chisel-disk would have a negative impact on soil health compared to no-till as it is introducing more disturbance to the system, despite being considered conservation tillage. To elucidate the differences between no-till management and chisel disk management, the present study compares these two tillage practices, coupled with fertilizer management, to contribute to the body of knowledge on the effects of chisel disking. We expect the soils receiving the chisel-disk tillage to have reduced soil health compared to the no-till soils.

Manure application

Manure application is both a management practice and a necessity for most dairy farmers, due to the large amounts of manure produced on-farm. Manure contains large stores of nitrogen which can be beneficial to crops, but depending on the time and method of application, the nitrogen can be lost to leaching in the form of nitrate or volatilization in the form of ammonia or nitrous oxide (Morken and Sakshaug, 1998; Van Es et al., 2006). This is a concern not only economically for the farmers, but also ecologically, as excess nitrogen pollutes nearby water ways and nitrous oxide, a potent greenhouse gas, is released to the atmosphere (Gupta et al., 2004). Although broadcasting manure over fields is the easiest and most common method of manure application, an alternative method, manure injection, was developed in 1998 by Morken and Sakshaug. This method uses specialized equipment that creates slits to inject liquid manure several inches into the soil where it will be most accessible to plant roots. This creates small pockets of manure distributed along the crop rows rather than being evenly distributed across the field as it is when broadcast. Injected manure has been shown to reduce ammonia volatilization and phosphorus run-off, but in some cases can increase nitrous oxide emissions (Dell et al., 2011). The effect of injected manure on soil health is only partially understood—some studies claim the effect on soil health is negligible if it exists, while others claim the machinery required for injection is a cause of soil compaction that can be detrimental (Batey, 2009; Huijsmans et al., 2016; Duncan et al., 2017).

In the Corn-Soy Rotation, the effects of four fertilizer application strategies on soil health were evaluated, including broadcasted manure (with no-till or chisel disk), injected manure (with no-till), and synthetic fertilizer (with no-till). We expected to see reduced soil health as a result of the chisel-disk tillage, but due to the potential for disturbance by the manure injection machinery, it would be possible for soil health indicators to decline in the plots receiving injected manure as well. The other potential effect of the injected manure would be increased soil carbon at deeper depths as the manure is injected into the soil rather than being broadcast only on the surface. We were also interested in the potential differences in soil carbon between applying manure and applying synthetic fertilizer. Although Abiven et al. (2009) noted no differences in soil aggregate stability based on organic inputs, this study measured soil carbon and the addition of manure may add more carbon than synthetic fertilizer. Ideally, the comparison of these four strategies will elucidate any potential differences on soil health of not only tillage strategy, but fertilizer choice as well.

Cover crops and perennials

Many benefits from cover crops exist but are dependent on the type of crop selected and the time of year they are planted and terminated (Snapp et al., 2005). Generally, all cover crops serve to protect soil from erosion, but specific crops are usually chosen based on the needs of the soil or farmer. For example, some crops are planted for weed suppression (Stivers-Young, 1998), while others are planted for nitrogen management—either to add nitrogen or to take up excess nitrogen (Ketterings, 2015). Other benefits include improved soil tilth, soil moisture, and increased pest suppression (Snapp et al., 2005; Krueger et al., 2011). The effectiveness of cover cropping is not only dependent on crop selection, but also the regional climate. A potential risk to cover crops is slower soil warming in early spring as a result of crop residue left on the soil surface acting as insulation from the sun (Snapp et al., 2005). This can decrease the effectiveness of cover crops in cooler climates, especially where growing seasons are short (Stivers-Young and

Tucker, 1999). Cover cropping has proven an effective method in most soils in the Northeastern United States (Ketterings et al., 2015).

Similar to cover crops, perennial crops can provide multiple benefits to the soil and tend to improve all aspects of soil health due to their longer and denser root systems as well as their longer residency in the soil without disturbance (Cates et al., 2016; Nunes et al., 2020; Sprunger et al., 2020). While Basche and DeLonge (2019) found no effect due to no-till alone, no-till combined with perennial crops or cover crops led to significantly higher soil infiltration rates. Unlike cover crops, perennials reside in the soil throughout the year and therefore limit the space farmers can use to grow cash crops, whereas cover crops can be planted to overwinter or between cash crops. Additionally, perennial forage crops have limited economic value as they cannot be easily stored or transported and are not subsidized in the United States. These limits explain why perennials are not more commonly included in rotations; the aforementioned benefits to soil health would otherwise be desirable.

The Weed Rotation used both cover crops and perennials to increase the diversity of the rotation—in line with the four soil health principles. The six-year rotation included three years of mixed annual cash and cover crops followed by three years of perennial crops. The two treatments included two different termination methods for the perennials—herbicide termination or termination via tillage. The inclusion of perennials allows us to see not only how the moldboard plow event impacts the soil health compared to the no-till system, but also to determine if the perennial crops can mitigate any negative soil health consequences derived from the moldboard plow tillage and if three years in perennial crops is sufficient to mitigate these negative effects. Based on the body of research showing the positive effects of perennials on soil health, we expect that three years in perennial cover in addition to the three years with annual cash and cover crops, will be sufficient to return soil health to pre-tillage levels.

Soil Health Indicators Selected for Measurement in the DCSE

Four soil health indicators were examined: total organic carbon, bulk density, water stable aggregates, and labile carbon (**Figure 1.2**). Because management changes typically require several transition years to produce differences in soil properties, soils were sampled every three years. Indicators were evaluated using data from samples taken in the following project years: 2010, 2013, 2016, 2019, and 2021.

Total organic carbon

Total organic carbon, which is considered a biological soil health indicator by the USDA-NRCS (**Table 1.1**), refers to the carbon stored in organic matter. Because organic matter is most abundant at the soil surface, carbon can be readily lost to erosion or following disturbance. Because soil organic matter is the primary food source for microorganisms (Sikora & Stott, 1996), it is important to take measures to protect it using management practices that maintain good soil cover. Plant cover also serves to protect carbon because root growth improves soil structure and increases soil aggregate formation as organic matter stored within soil aggregates is better protected from degradation. Additionally, crop residues left on the soil surface will eventually breakdown and contribute to the organic carbon pool. Practices that leave roots in or residues on the soil will enhance soil organic carbon over time, as with the use of perennial crops in the Weed Rotation. Total organic carbon changes occur at a very slow rate, making even small increases important (USDA, 2009).

A common thread with biological indicators of soil health is that they can be difficult to measure and measurements are often indirect approximations. The only biological indicator, measured as part of the dairy cropping systems experiment, was total organic carbon, which was evaluated both as a percentage and by volume after correcting the percentage for bulk density. In this study, soil organic carbon percentages based on volume could only be reported for years in which bulk density tests were performed. Additionally, because organic carbon is an important food source for microorganisms, it is an important indicator to monitor in a long-term system. While labile carbon tends to show more dramatic increases and decreases as a result of management, even incremental changes in total carbon can be important (Culman et al., 2012).

Bulk Density

Soil compaction is quantified by a soils’ bulk density which is a measure of a soils’ dry weight per unit volume. The sampling method for bulk density can be time and labor intense and is often not measured for this reason, however, it is an important soil health indicator. Like other soil health indicators, it is most helpful to consider a soils’ change in bulk density over time rather because changes to bulk density happen slowly.

No-till is often put forth as a solution to many soil health problems, yet in the NRCS Soil Health Literature Summary (2015), of 14 studies of no-till soils reviewed in which bulk density was a metric, nine studies reported increased bulk density (higher compaction) or no change. Of these studies, those that did report lower bulk density over time integrated some sort of cover crop into their no-till system (Dao, 1993; Francis & Knight, 1993; Rhoton et al., 1993; Franzluebbers, 2002; Stipešević & Kladivko, 2002; Villamil et al., 2006; Reynolds et al., 2007; Franzluebbers, 2008; So et al., 2009; Schwen et al., 2011; Raczkowski et al., 2012; Kahlon et al., 2013). Although tillage can lower bulk density in the short term, its long-term impacts raise bulk density (Raczkowski et al., 2012, Kahlon et al., 2013). Because the evidence suggests that no-till alone does not guarantee improved bulk density, it is important to consider additional management strategies to lower bulk density over time. The Dairy Cropping Systems experiment considers this by including both cover crops and perennial crops in the Weed System, though the Corn-Soy System includes no additional cropping diversity.

Another reason bulk density serves as an important soil health indicator is that it can help to measure the total organic carbon by volume of a soil which is how carbon sequestration is tracked (FAO, 2019). If only the total organic carbon percentage is measured in an experimental setting, this could bias results if treatments have varying bulk densities. To get an accurate picture of how much carbon is being stored in the soil, bulk density must be included in the methodology.

Labile carbon (POXC)

Labile carbon, also referred to as reactive (**Table 1.1**), or active carbon, is the fraction of soil carbon most readily available to react with the surrounding soil matrix, including both microbial life and charged mineral particles. Although labile carbon is officially listed as a chemical indicator by the NRCS, a 2012 study found that reactive carbon reflects the microbial biomass carbon, making it a possible proxy to measuring soil microbial activity (USDA, 2014; Culman et al. 2012). This proxy can be useful, especially in resource-limited situations, because measures of microbial activity are often more complex and/or expensive. Additionally, the permanganate oxidizable carbon (POXC) method allows the labile carbon fraction to be estimated relatively easily compared to older methods of measuring labile carbon (Weil et al., 2003). Due to its reactivity, this carbon fraction changes more rapidly than the total organic carbon fraction and may show changes due to management over shorter time periods (USDA, 2014b).

Labile carbon was measured by the POXC method. The POXC method allows for relatively cheap and easy estimation of soil labile carbon and gives an idea of the soil microbial activity as well. Therefore, this is a good indicator for studying the overall health of a soil and for understanding more rapid changes in carbon dynamics.

Water stable aggregates

Soil aggregates are an important structural component of a soil. A high stability of aggregates in soil is generally a desirable trait, but the processes which build and maintain stable soil aggregates are complex and often nuanced. As with many soil health indicators, what is considered a high aggregate stability can vary widely depending on soil type and texture, function, and land-use history. Aggregate stability can be measured through a variety of methods, which usually include measuring the amount of soil that slakes off upon wetting (Kemper & Rosenau, 1986; USDA, 2001; Cornell University, 2021).

The NRCS Soil Health Literature Summary (2015) documents six studies that included aggregate stability as a metric for soil health—five out of these six studies reported decreased aggregate stability in conventionally tilled systems compared to no-till systems (Stipešević and Kladivko, 2005; Franzluebbers and Stuedemann, 2008; So et al., 2009; Aziz et al., 2013; Kahlon et al., 2013). The study that did not report a significant difference in aggregate stability between conventional tillage and no-till evaluated differences after only five years of management and indicated that this was not a long enough time interval to detect changes due to management (Rhoton et al., 1993). Kahlon et al. (2013) compared three tillage methods: no-till, ridge-till, and conventional plow-till—aggregate stability was the highest in the no-till treatment, intermediate in the ridge-till treatment, and lowest in the conventional-till treatment. It is generally agreed upon that the disturbances caused by tillage negatively impact soil aggregate stability and that the severity of that impact corresponds to the intensity of the tillage, which is supported by the "minimize disturbance" principle (**Figure 1.1**).

While no-till has proven to be beneficial to soil aggregate stability when compared to tillage, no-till alone is not guaranteed to improve aggregate structure (Evett et al., 1999; Jiang et al., 2007; Hubbard et al., 2013; Olson et al., 2013). One strategy that is thought to improve aggregate stability over time is the use of perennials in a crop rotation sequence. Because perennial roots remain in the soil year-round, their root exudates combined with the physical binding power of the roots themselves are believed to increase the stability of the aggregates in the soil (Quincke et al., 2007; Nunes et al., 2020). Perennial grasses particularly are of interest when attempting to improve aggregate stability as grasses tend to have more densely packed root systems than legumes or other perennial crops.

As explored previously, the inclusion of perennial grasses in a six-year rotation sequence is one soil health management strategy employed in the Dairy Cropping Systems—the effects of perennial crops on aggregate stability improvement will be investigated further in Chapter 2, though we expect that the use of perennial crops in the rotation receiving moldboard plow tillage once in the six-year rotation will return soil aggregate stability to pre-tillage levels.

Aggregate stability is a helpful measurement because it is related to many of the other physical soil indicators—for example, higher aggregate stability corresponds to a greater water holding capacity, faster infiltration rate, lower degree of slaking, lower possibility of forming a soil crust, and increased macroporosity. In addition, macroaggregates in the soil protect organic matter and make it less susceptible to loss via erosion (Elliott, 1986). Gupta et al. (2015) highlighted the fact that soil aggregates are highly influenced by both chemical and biological factors, making it a particularly useful physical indicator as it can be correlated to many other aspects of the soil environment.

While aggregate stability covers a lot of ground in terms of being a good indicator of soil physical health, it cannot provide direct information about soil compaction. Soil bulk density is a measure of how compacted a soil is and is therefore able to round out the coverage of soil physical health indicators. Additionally, bulk density can be used in conjunction with the biological indicator, total organic carbon, to determine total organic carbon by volume. Knowing the total organic carbon by volume allows us to measure the carbon sequestration potential of the dairy cropping systems soils.

Rationale and Significance

As the threat of climate change continues to accelerate, management of agricultural ecosystems has become increasingly complex (Nelson et al., 2009; Ramborun et al., 2019). Understanding the factors contributing to soil health is essential for maintaining productivity in the agricultural sector. Improving management techniques will not only benefit the soil, but will also improve water-use efficiency, reduce dependence on applied fertilizers, as well as reduce overall energy use on farms (Malcom et al., 2015; USDA-NRCS, 2015; Busch et al., 2020). Conclusions from this research will help to assess how soil properties respond to management practices intended to promote overall soil health, a widely applicable subject for farmers in Pennsylvania and similar climate regimes throughout the northeast.

The specific project objective in Chapter 2 was to look at the effects of one-time tillage in a six-year rotation that includes cover crops and perennials. Because soil health is negatively correlated with increased disturbance, we hypothesized that 1) soil health indicators of soil subjected to inversion tillage would decline directly following tillage (Bhardwaj et al., 2011; Cates et al., 2016; Nunes et al., 2020). Additionally, because perennial roots have been shown to improve soil carbon and soil stability we hypothesized that 2) returning these soils to no-till with cover crops and perennials would allow them to recover to similar levels by the end of a six-year rotation compared to soils that were not tilled (Quincke et al., 2007; Nunes et al., 2020). In Chapter 3, the project objective was to evaluate whether and how drastically soil health indicators are affected by chisel disking as a fertilizer application strategy. Because tillage can decrease soil compaction in the short term, we hypothesized that 1) bulk density would decrease in the chisel disk strategy at the 0-5 cm depth (Raczkowski et al., 2012, Kahlon et al., 2013). Next because disturbance typically reduces soil carbon, we hypothesized that 2) carbon would be reduced at the 0-5 cm depth but increased in the 5-15 cm depth in the chisel disk strategy as a result of buried residue at the deeper depth (Kettler et al., 2000; Duiker and Beegle, 2006; Nunes et al., 2020). Because injected manure introduces carbon directly into the soil, we hypothesized that 3) there would be increased carbon in the 5-15 cm depth in the injected manure strategy compared to the other two no-till strategies, but otherwise no differences between no-till fertilizer application strategies (Batey, 2009; Huijsmans et al., 2016; Duncan et al., 2017). Finally, due to the negative correlation between soil health and increased soil disturbance, we hypothesized that 4) labile carbon and water stable aggregates would be reduced as a result of chisel disking (Duiker and Beegle, 2006; Nunes et al., 2020).

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Figures

Figure 1.1 The four soil health principles identified by the USDA-NRCS include maximizing continuous living roots, minimizing disturbance to the soil, maximizing soil cover, and maximizing crop rotation biodiversity.

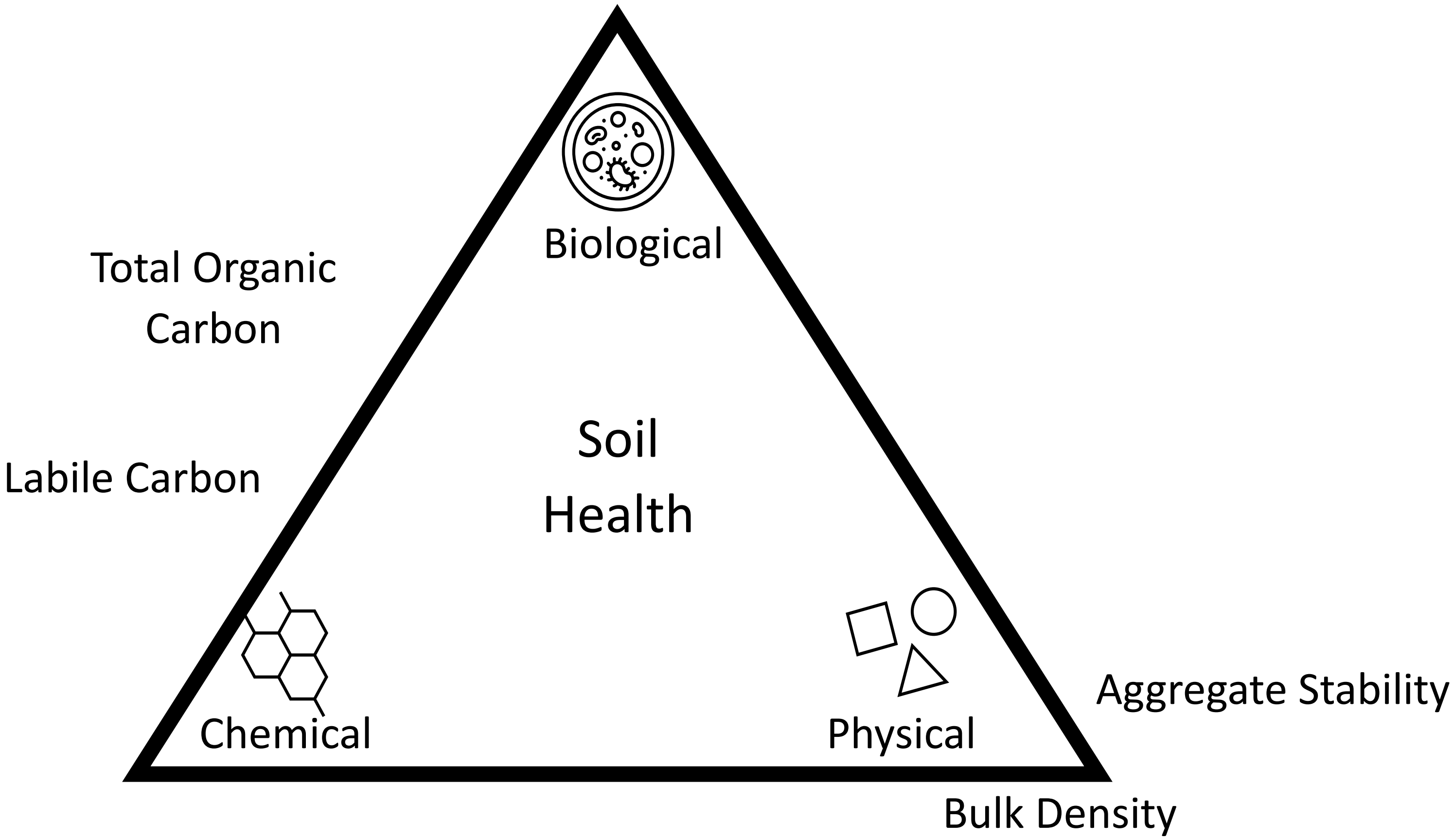

Figure 1.2 Soil health indicators addressed in this thesis. Because each indicator is interconnected by physical, chemical, and biological aspects, we propose that a triangle is a helpful visualization of this interconnectedness rather than putting each indicator into a fixed category.

Tables

Table 1.1 Soil health indicators recognized by the NRCS, separated into three categories: physical, chemical, and biological. *denotes that indicator is evaluated in the Penn State Dairy Cropping Systems experiment in proceeding chapters as well as described in this chapter.

Soil Health Indicators		
Physical Indicators	**Chemical Indicators**	**Biological Indicators**
Aggregate Stability*	Reactive (Labile) Carbon*	Earthworms
Available Water Capacity	Soil Electrical Conductivity	Particulate Organic Matter
Bulk Density*	Soil Nitrate	Potentially Mineralizable Nitrogen
Infiltration	Soil pH	Soil Enzymes
Slaking		Soil Respiration
Soil Crusts		Total Organic Carbon*
Soil Structure and Macropores		

Chapter 2

Soil Health Indicators Under Continuous No-Till vs. Integrated Weed Management with Strategic Tillage

Introduction

No-till agriculture introduces seeds into soil without plowing or disking, thus maintaining soil structure and leaving previous crop residues on the surface. Besides saving fuel and protecting soil from erosion, no-till farming conserves soil carbon by reducing the oxidation and loss of soil organic matter when soils are mixed by tillage (Stubbs et al., 2004). No-till farming is therefore considered an important management tool to improve soil health. When no-till management is adopted, however, the lack of soil disturbance increases weed pressure, reliance on herbicides, and potential for development of herbicide resistance (Ketter et al., 2000; Quincke et al., 2007). No-till also increases nutrient stratification in the soil because it allows fertilizers and residues from terminated crops to accumulate on the surface (Scheiner and Lavado, 2008). No-till farmers who also practice cover cropping typically apply herbicides to terminate cover crops and control weeds. Concerns that herbicide use adversely affects soil organisms and soil health, therefore, have presented a conundrum for no-till farmers. These issues have led to the consideration of using strategic disturbance events, such as occasional inversion tillage and cultivation, to distribute nutrients and reduce the frequency and rate of herbicide applications.

In a 2014 survey by the USDA, 66% of crop acreage in Pennsylvania was managed with no-till, and organizations such the Pennsylvania No-Till Alliance have played an important role in advocating its use. No-till farmers are often averse to using any type of tillage, due to the perceived potential to destroy the benefits gained from continuous no-till. An abundance of literature exists documenting the negative effects of tillage on soil health indicators, with soil health tending to worsen with increased tillage intensity (Bhardwaj et al., 2011; Cates et al., 2016; Nunes et al., 2020; Sprunger et al., 2021). A nationwide meta-analysis (Nunes et al., 2020) examined the effects of converting from moldboard plowing (MP) to either chisel plowing (CP), no-till (NT), or perennial systems (PER), on seven soil health indicators. The authors found that converting from MP to CP improved three soil health indicators in the first 0-15 cm of soil, but that converting from MP to NT improved all seven soil health indicators at 0-15 cm. Additionally, conversion to PER improved all soil health indicators at all depths sampled (0-40 cm). The analysis also found that combining cover crops with the transition to no-till enhanced soil health more than switching to no-till alone.

The addition of cover crops and perennials into cropping systems was studied under several tillage strategies at a site in Wisconsin, with soil health indicators tending to improve with increasing crop diversity and perenniality (Cates et al., 2016). Six treatments were tested ranging from continuous maize with yearly chisel plowing to more diverse rotation with less frequent chisel plowing and perennial crops. Findings suggested that increased diversity and perenniality of the systems enhanced soil health. The study hypothesized that soil health would be reduced in proportion to tillage intensity. That hypothesis was only partially supported, however, because in the systems that included high biomass inputs from cover crops and perennials, soil organic matter was maintained at similar levels to the completely perennial system, even though the former systems were tilled every one to three years.

Despite the clear benefits associated with reducing tillage, the issues of weed pressure, herbicide reliance and resistance, and nutrient stratification are still persistent. One-time tillage, also referred to as strategic tillage, occasional tillage, and single inversion tillage, etc., is a potential alternative to continuous no-till, however, disagreement exists as to the efficacy of one-time tillage on otherwise no-till land. Some studies report little to no effect of one-time tillage on soil health, whereas others report a persisting decrease in soil health following tillage (Wortmann et al., 2010; Stavi et al., 2011). A review by Blanco-Canqui and Wortmann (2020) looked at the impacts of occasional tillage on soil health. Though results varied from study to study, the general trend showed that occasional tillage does not seem to have a lasting effect on soil health—on average Blanco-Canqui and Wortmann reported that issues with no-till persisted less than two years. Additionally, occasional tillage was effective at reducing nutrient stratification and suppressing weed populations for several years following tillage. The review concluded that the benefits of one-time tillage depend on the type, timing, depth, and frequency of the tillage and that the ideal type of one-time tillage will vary. These findings explain the variability in the results of other studies but are encouraging for the use of one-time tillage when properly planned.

One-time tillage has been proven effective as a means of reducing herbicide dependence. A study in Nebraska (Kettler et al., 2000) looked at one-time tillage using a moldboard plow on otherwise no-till soil to control downy brome, an annual grassy weed. The study reported a 20% decrease in soil organic carbon at 0-7.5 cm, five years after the tillage event, though tilling the soil did have the desired effect of controlling weed populations. Additionally, at the 7.5-15 cm range, soil organic carbon increased by 15% compared to the continuous no-till treatment, which suggests that carbon may be redistributed, rather than lost, in the soil profile as a result of tillage.

Though many studies include one-time tillage or cover crops and perennials as a factor, a research gap exists concerning the interaction between one-time tillage and perennial crops (Osterholz et al., 2021). In the present report, we aimed to look at the effects of one-time tillage in a six-year rotation that includes cover crops and perennials. Based on the evidence described above, we expect that the inclusion of cover crops and perennials in the rotation will increase the resilience of soil following the tillage event. To that end, we hypothesized that 1) soil health indicators of soil subjected to inversion tillage would decline directly following tillage and 2) returning these soils to no-till with cover crops and perennials would allow them to recover to similar levels by the end of the same six-year rotation that was not tilled.

Materials and Methods

The experiments were carried out as part of the Dairy Cropping Systems (DCS) project established in 2010 at the Pennsylvania State University Russell E. Larson Agronomy Research Farm near Pennsylvania Furnace, PA (40.72˚N, -77.92˚W). This project simulated a 97-ha dairy farm at 1/20th the scale (approximately 5-ha) which could provide all forage and grain needed for a simulated 65-cow dairy herd with the goal of minimizing off-farm inputs. The project aimed to evaluate three systems for their ability to reduce nutrient and soil loss, build soil health, and increase biodiversity while remaining energy efficient, productive, and both ecologically and economically sustainable (Busch et al., 2020). The cropping system in this study consisted of the same six-year crop rotation of annual and perennial crops but contrasting weed management strategies, referred to as the Weed rotation (Summers et al., 2021). Crop entries in the six-year rotation were split plots (18 x 27 meter) to compare continuous no-till and standard herbicide management with strategic soil disturbance to reduce herbicide application.

The weed control management strategies were as follows: i. standard herbicide application rates to control weeds and terminate the perennial alfalfa and rye and oats cover crops (continuous no-till system) and ii. integrated weed management which included reduced herbicide applications in conjunction with termination of the perennial crop (alfalfa-orchardgrass) by inversion tillage using a moldboard plow once in six years (once-in-six-year tillage system) as well as shallow-disk cultivation in the corn and soybean between rows twice a year early in the growing season for weed control (Summers et al., 2021) in the corn and soybean entries. for the first 3 years and from 2013-2018 in one of two nested split-split (9 by 27 meter) plots described in Summers et al. (2021). The other nested split-split plot received a post-herbicide application instead of shallow-disk cultivation between the rows twice in the early growing season. Soil samples from these nested split-split plots were combined in 2019 for the once-in-six-year tillage system canola and perennial forage plots (AO2 and AO3).

The predominant soil series at the experimental site is Murrill (Fine-loamy, mixed, semiactive, mesic Typic Hapludults), with Hagerstown, Buchanan, and Opequon soil series also present (**Figure 2.1**).

Experimental Design

As a full-crop entry experiment, all six phases were planted every year in a randomized complete block design and replicated four times. Crop entries represented the main plots (37 by 27 m) with weed control/tillage management deployed in split-plots (18 by 27 m). The six-year sequence (**Figure 2.2**) consisted of: (1) canola (*Brassica napus* L.) or canola plus oats followed by a rye cover crop (2) soybean [*Glycine max* (L.) Merr.] followed by rye cover crop (3) corn grain or corn silage (*Zea mays* L.) followed by (4-6) perennial forage. The once-in-six-year tillage system (T1/6) included alfalfa (*Medicago sativa* L.) and orchard grass (*Dactylis glomerata* L.) as the perennial crop throughout the duration of the experiment (**Table 2.1**). The continuous no-till system (NT), receiving herbicide as the method of perennial termination, included alfalfa as the perennial crop until 2016 when orchard grass was added to standardize the systems. The NT and T1/6 systems refer to the suite of management practices incorporated throughout a six-year rotation, rather than the tillage treatment alone. Crop entry refers to the crop phase in the rotation at which soil samples were collected.

Crop termination via either moldboard plow or herbicide occurred in late August. The NT system was sprayed with 0. 9 kg ae ha−1 of glyphosate and (N-[phosphonomethyl]glycine) and 0.5 kg ae ha−1 2-4-D LV4, and 0.3 kg ae ha−1 dicamba to terminate the perennial alfalfa. Termination via tillage included passes with (1) a moldboard plow, (2) a disk, (3) a S-tine chisel, and (4) a cultimulcher. Crop termination via tillage or herbicide occurred in late summer or early fall. Additional disturbance to the T1/6 system by a high residue cultivator occurred in the soybean and corn crop entries (**Figure 2.2**). Pre- and postemergence herbicides were broadcast as part of the NT system weed management strategy. Preemergent herbicide was banded over corn and soybean in the T1/6 system and a high residue cultivator was used in place of postemergence herbicide; herbicide reduction varied depending on type but was reduced to as much as 45% the amount used in the NT system (Summers et al., 2021).

When soil was managed without tillage, manure was injected—in the NT system once following perennial termination prior to planting canola, and once before planting a rye cover crop and once in the T1/6 system before planting a rye cover crop (**Figure 2.2**). Manure was broadcast in the T1/6 system before tillage and incorporated by the tillage event prior to planting canola. Manure management practices were chosen to best reflect typical on-farm practices.

In 2010 at the start of this experiment, the rotation system was intended to compare weed management strategies of a standard herbicide application with continuous no-till and a reduced herbicide application with moldboard plow tillage once in the six-year rotation in addition to shallow high-residue cultivation between the rows of corn and soybean twice in each crop year to control weeds (**Figure 2.2**). Other weed control strategies that reduced herbicide use in and did not introduce soil disturbance are described in Summers et. al. (2021). The systems were managed consistently between 2010 and 2019 with the exception of the perennial mixture that is planted for three years in the rotation. Between 2010 and 2015 in the NT system, alfalfa was grown for the three perennial years. Then, from 2016 to 2019, alfalfa + orchardgrass was grown for the three years, standardizing the two systems (Summers et al., 2021). Because the alfalfa + orchardgrass was consistent throughout the T1/6 system and for the last three years of the NT system, all plots list alfalfa + orchardgrass (AO) as the perennial crop.

Soil sampling procedures

Plots were sampled in 2010, 2013, 2016, and 2019, however, only 2013 and 2019 data are presented here. Across all indicators and depths, no significant differences between systems or crop entries were observed in 2010, indicating that differences in following years were due to management. Data was not sufficient for analysis in 2016 across all indicators, so this year was excluded from analysis. Additionally, samples from only two crop entries were analyzed for TOC percentage, labile carbon, and WSA indicators—canola was sampled and represented the year directly following tillage in the T1/6 system, and third-year alfalfa orchardgrass (AO3) was sampled and represented five years after tillage in the T1/6 system and two years of perennials in both systems. Bulk density and TOC by volume also included the second-year alfalfa orchardgrass (AO2) crop entry, which represented four years since tillage in the T1/6 system and one year of perennials in both systems. Composite samples were collected from plots receiving broadcast manure by mixing ten, 7.5 cm diameter soil cores taken randomly throughout the plot with a soil probe—in 2016 and 2019 a tractor mounted Giddings soil probe was used to obtain soil cores. After 2010 (time = 0), soil sampling of plots receiving injected manure differed to account for manure distribution by injection. When sampling these plots, 18 cores were taken in three sets of six in lines perpendicular to the injection band (six inches apart) to obtain a more representative sample. Soil cores sampled for total organic carbon (TOC) and permanganate oxidizable carbon (POXC) were split into the 0-5 and 5-15 cm portions in the field and kept separately. Soil cores for aggregate analyses were sampled to 15 cm depth, with ten cores composited per plot and kept in cool, airtight containers prior to processing to minimize microbial activity.

Bulk density

Bulk density samples were collected from three crop entries: canola, and both the second and thirds years of alfalfa and orchardgrass (AO2 and AO3, respectively). Bulk density was measured a modified procedure of that described by Blake and Hartge (1986), which involves placing a 3-inch diameter metal cylinder on the sampling area and pounding it uniformly into the ground. To measure the bulk density from 0-15 cm, two cylinders were placed on top of each other and a large weight slightly larger than the size of the cylinders was dropped repeatedly onto the cylinders. Once the cylinder was fully in the ground, it was carefully removed, and any additional soil clinging to the top or bottom of the cylinder was sliced off using a knife. The two cylinders were then separated into 0-5 and 5-15 cm fractions again using a knife. The entire contents of the cylinders were then emptied into a labeled bag and sealed until further processing.

The weights of moist soil were recorded for each sample and then soils were wet sieved through a 2-mm sieve to exclude any large rocks. Rocks were then placed into a graduated cylinder with a known amount of water and the displacement of the water was measured to estimate the volume of the rocks. Moist soils were placed into a drying oven and reweighed after drying. The dry weight of the soil was used to calculate the bulk density using the formula: (oven dry weight of soil)/(volume of soil), where the volume of the soil is the known volume of the 3-inch diameter metal cylinder adjusted for the volume of any rocks or voids.

Total organic carbon

TOC was measured with a 2400 CHNS/O Series II Analyzer (Perkin Elmer, Waltham, MA, USA) in 2010, 2013, and 2016 and a Vario Max elemental analyzer (Elementar, Langenselbold, Hesse, Germany) in 2019 and 2021 and required 25 mg and 250-300 mg (respectively), ground soil samples. Carbon percentage was determined using high temperature combustion.

The bulk density value was used to determine the total organic carbon by volume. The carbon in grams per cubic centimeter was calculated by multiplying the percent carbon by the bulk density and dividing by 100. This number was then converted to metric tons per hectare (Mg/ha). The total organic carbon by volume was analyzed only for the year 2019. Due to the challenges presented by bulk density sampling in gravelly soils, 2019 is the only year that sufficient sampling for analysis was done.

Labile carbon (POXC)

Samples at 0-5 cm depth were tested for labile carbon, and at 0-5 and 5-15 cm depths for total carbon. Data from 2016 were excluded from analyses due to gaps in collection. Soils for carbon analysis (TOC and POXC) were air-dried and machine-ground. Soil POXC was measured using the Weil et al. (2003) method using a stock solution of 0.2 M potassium permanganate in 1 M calcium chloride (pH 7.2). A standard curve was created for each day samples were run. A handheld colorimeter (Hach, Loveland, CO) was used to measure absorbance at 550 nm. Absorbance values for each of the three standards were recorded and a standard curve was generated. The equation of the line was used to determine the constants for the equation for calculating POXC:

$$\text{POXC (mg kg–1)} = (0.02\ \text{Mol/L} - (a+b*\text{absorbance}))*(9000\ \text{mg C/Mol})*(0.02\ \text{L soln.}/0.0025\ \text{kg soil})$$

In this equation, the constant a is the y-intercept and b is the slope of the line generated by the standards. Absorbance is the value measured for each soil sample.

To measure the POXC, 2.5 g of soil was mixed with 2 mL $\mathrm{KMnO4}$ brought to 20 mL with water in a 50-mL conical tube and manually shaken at a rate of two shakes per second, timed for two minutes. The tube was uncapped then left undisturbed for ten minutes. Supernatant was diluted 1/10 in deionized water and mixed before measuring absorbance values used in the above equation.

Water stable aggregates

Samples were collected in June after crop planting at 0-15 cm for water stable aggregates. Data from 2016 were excluded from analyses due to gaps in collection. Field moist soils were sieved through 2-mm and 1-mm sieves, with material remaining on top of the 1-mm sieve retained and air-dried. To measure water stable aggregates (WSA), a modified version (Grover, 2008) of the Kemper-Rosenau (1986) method was used with a dispersing solution (2 g of sodium hexametaphosphate in 1 L of deionized water). Four grams of air-dried soils were added to sieve-bottom cups in an 8-cup wet-sieving apparatus (Eijkelkamp Soil & Water Giesbeek, Netherlands) (**Figure 2.3**). Tins containing deionized water were placed under the sieve-bottom cups, which were lowered to completely submerge the soils. After submersion without disturbance for five minutes, the cupholder was raised and lowered at a rate of 33 times per minute for another five minutes. Tins containing water were replaced with tins containing dispersing solution, and the process was repeated.

Once raised out of the solution, samples in the sieve-bottom cups were gently rubbed with a rubber-tipped rod for 20 seconds each. Samples were lowered again into dispersing solution and raised and lowered for a final five minutes. The two sets of tins, one containing water and unstable soil fraction and the other containing dispersing solution and the stable fraction, were removed and placed in a drying oven at 110℃ for two days. Any sand, rock, or particulate organic matter remaining in the sieve bottom cup was discarded.

WSA percentage was measured using the equation:

[Stable Aggregate/(Stable Aggregate + Unstable Aggregate)]*100

where stable aggregate refers to the fraction of the soil slaked off in dispersing solution and unstable aggregate refers to the fraction of the soil that slaked off in water.

Two replicates were performed for each plot and a percent difference between replicates was determined using the equation:

[(Rep 1 – Rep 2)/Average(Rep1,Rep2)]*100

If the percent difference between replicates was greater than 29%, a third replicate was performed. This was only necessary for one sample and a fourth replicate was never necessary. Results from this WSA procedure, which was carried out by different individuals in 2010, 2013, 2016, and 2019, can be subject to variation due to technique and handling practices. To account for this variation, standardized scores were generated for water stable aggregate data within each year. Standardized scores were calculated by subtracting the population mean (all blocks and both systems) from each WSA percentage of each sampled plot and dividing this value by the population standard deviation.

Statistical analysis

Statistical analyses were performed using the Standard Least Squares personality in JMP Pro 15 by SAS (SAS Institute, Inc. Cary, North Carolina). For labile C and TOC by percentage data, the fixed effects were experiment year, system, crop entry, and the two and three-way interactions between these terms with block, block x crop entry, and block x year as random effects.

Labile carbon and total organic carbon percentage data were analyzed for both 2013 and 2019 together, with year as a fixed effect. Additionally, total organic carbon by volume data were analyzed in just the year 2019 as this was the only year with sufficient bulk density data.

Due to a sensitivity in one step of the WSA analysis procedure, making statistical analysis between years unreliable, WSA were analyzed in 2013 and 2019 separately. Water stable aggregate percentages and the 2019 soil carbon by volume were both analyzed for each year separately using a simplified model including only crop entry and system and the interaction between them as fixed effects and block and the interaction between block and crop entry as random effects. Additionally, standardized scores were generated for water stable aggregate data to account for differences observed between years due to different people conducting the WSA analysis each year. Standardized scores were calculated by subtracting the population mean (all blocks and both systems) from each treatment plot’s WSA percentage and dividing this value by the population standard deviation. Standardized scores for each year were analyzed using the same model used for WSA percentage. Only two crop entries were analyzed for WSA, TOC by percent, and labile C: canola and third year alfalfa or alfalfa/orchardgrass. In the analysis of the total carbon by volume, second year alfalfa was also analyzed.

The SLICE test, which analyzes simple effects to separate LSmeans within an interaction, was used to test the pre-planned hypotheses. In the model used for labile C and TOC by percentage, the SLICE test was used on the three-way interaction term between crop entry, system, and year. The SLICE test was used on the two-way interaction between crop entry and system on the model used for WSA and TOC by volume. Means were considered significantly different at $p<0.05$.

Results and Discussion

Total organic carbon percentage

When TOC percentage was analyzed at the 0-5 cm depth, system was the only significant effect ($p=0.0014$), where the T1/6 system average was 18% lower than the NT system average. Canola TOC percentage in the T1/6 system decreased by 21% in 2013 ($p=0.0339$) and 29% in 2019 ($p=0.0039$) compared to the NT system (**Table 2.2**). Because no significant differences were observed between systems in the AO3 crop entry, we can assume that TOC percentage recovered to the same level as the NT system in the T1/6 system (**Figure 2.4**).

When TOC percentage was measured at the 5-15 cm depth, none of the fixed effects showed significant differences. The three-way interaction term showed no significant effects by either systems or crop entries (**Table 2.2**), meaning that no effects of management on TOC percentage were detected by our experiment at the 5-15 cm depth (**Figure 2.5**). Soil carbon percentage in the 0-5 cm range showed a similar trend to the labile C in that soil carbon in the

T1/6 system was significantly lower than in the NT system only in 2019 directly following tillage in the canola entry.

No significant difference existed between the two systems in the AO3 entry in either year, showing that TOC percentage recovered to pre-tillage levels even after a tillage event, after two years of perennial forage. As no significant changes occurred in TOC in the 5-15 cm range, tillage did not appear to have an effect at this depth. However, it is possible that TOC was redistributed to a deeper depth than what was sampled as part of our study as Ketter et al., (2000) noted a redistribution of carbon to deeper depths following tillage. Because the plow depth is closer to 30 cm, if buried residue increases TOC, this would likely be more apparent at a depth of 30 cm. Due to gravely soil conditions, sampling past 15 cm was not feasible on a wide scale and this possibility was not investigated further. While this potential for redistribution of carbon rather than outright loss of carbon due to tillage supports the idea that occasional tillage does not undo the benefits of no-till, this was not observed in our results. The TOC percentage results support Hypothesis 1) occasional tillage would initially result in a decline in TOC and Hypothesis 2) a return to no-till with cover crops and perennial forage would increase the TOC in the T1/6 system to similar levels as the NT system.

Bulk density

In the bulk density analysis, crop entry was a significant effect at the 0-5 cm range (p=0.00418), where the AO3 entry had 9% lower average bulk density than the canola entry and AO2 was not significantly different from either other entry (**Table 2.3**). Within the T1/6 system, the AO3 crop entry had a bulk density 8% lower than the canola entry (p= 0.0089) and the AO2 entry falling between the two, though not significantly different from either. Additionally, within the NT system, the AO2 crop entry had a bulk density 5% lower than the canola entry (p= 0.0089), and the AO3 entry had a bulk density 10% lower than the canola entry (p= 0.0005) (**Figure 2.6**). Because a lower bulk density score equates to a positive change in soil health, these results show that bulk density was improved after two years of perennial forage, regardless of tillage. The bulk density differences were only significant between the AO3 and canola in the T1/6 system, indicating that this system took longer for bulk density improvements than the NT system which had improved bulk density by the AO2 entry. As seen in the TOC percentage results, there were no significant effects observed in the 5-15 cm depth for bulk density (**Figure 2.6**).

Decreased bulk density following perennial cover was expected as the perennial roots are likely to add organic matter to the soil, reducing compaction and making the soil more porous and therefore lighter as well as improving fungal hyphae and biological activity that promotes soil porosity (Blanco-Canqui & Ruis, 2020). It is interesting to note that the bulk density did not increase significantly following tillage; there was no difference between the two systems in the canola crop entry. Tillage has been shown to decrease bulk density in the short term but increase it in the long-term (Logsdon et al., 2004). Bulk density was increased significantly following tillage compared to the AO3 entry in the T1/6 system, but the same trend was observed in the NT system. Therefore, it is possible that the main factor affecting the increase in bulk density was the termination of the perennial crop by either termination method. These data did not support Hypothesis 1) that bulk density would decrease immediately following tillage, however, we did see improvements in bulk density following perennial forage in both systems, which does support Hypothesis 2) that soil health can recover under no-till with cover crops and perennials.

Total organic carbon by volume

The bulk density data from 2019 was used to calculate TOC by volume (Mg/ha) which showed similar trends to the TOC percentage in both sample depths. System was a significant effect at the 0-5 cm range (p=0.00012), with TOC decreased by 20% in the T1/6 system compared to the NT system. The canola crop entry had 29% less TOC (p= 0.0003) and the AO2 entry had 17% less TOC (p= 0.0078) in the T1/6 system compared to the NT system (**Table 2.3**). In the AO3 crop entry, there were no differences between the two systems. These results show that the tilled soils required multiple years of perennial forage to recover to the same levels as no-till soils. In addition to the differences between the systems, within the NT system, the TOC by volume was 19% lower in the AO3 crop entry compared to the canola entry and 17% lower in the AO3 crop entry compared to the AO2 entry. No significant differences were found between systems or crop entries at the 5-15 cm depth (**Figure 2.7**).

Although TOC by volume was analyzed only in 2019, it is important to acknowledge, as correcting for bulk density can show different trends than percent carbon alone if compaction is different between systems or areas. Additionally, correcting for bulk density is how carbon sequestration is measured which is important when considering the potential for agricultural systems to sequester carbon (FAO, 2019). Though these findings show reduced TOC following perennial forage, these differences are explained by the positive soil health benefits of reduced bulk density. Because the bulk density of the NT AO3 entry is significantly lower than the canola entry, there is less carbon stored in this soil. This highlights the importance of correcting TOC percentage for bulk density as less carbon is stored in the 0-5 cm range than is indicated by the TOC percentage alone—this could be misleading for stakeholders interested in the carbon sequestration of soil. However, the results did support Hypothesis 1) TOC by volume would decrease immediately following tillage and Hypothesis 2) following a return to no-till with cover crops and perennials, TOC by volume in tilled soils would recover to similar levels as no-till.

Labile Carbon

In the analysis of POXC, system was a significant effect (p=0.00079), where the T1/6 system average was 27% lower than the NT system average. Canola POXC in the T1/6 system decreased by 30% in 2013 (p=0.0414) and 40% in 2019 (p=0.0039) compared to the NT system (**Table 2.2**). Because no significant differences were observed between systems in the AO3 crop entry, we can assume that POXC recovered to the same level as the NT system in the T1/6 system (**Figure 2.8)**. The POXC results show sensitivity to management in that there was an observable difference in canola in both systems in both 2013 and 2019. Additionally, absence of significant differences between the systems in the AO3 crop entry show recovery of labile carbon in tilled soil to similar levels as continuous no-till soil.

Quincke et al. (2007) also noted a significant decrease in labile carbon following tillage in the top few centimeters of soil, but found increased labile carbon in deeper layers that essentially offset the carbon lost from the more superficial layer, which suggested that labile carbon had been redistributed by moldboard plow rather than lost. In our study, POXC was only analyzed at the 0-5 cm depth, and it may have been possible that a similar redistribution of carbon occurred at deeper soil depth. However, because labile C behaved similarly to TOC at the 0-5 cm depth, it is unlikely that we would see the increased labile carbon in the 5-15 cm depth that was observed by Quincke et al. (2007), but it is still possible that both labile and total C were redistributed to a deeper depth than what was sampled as part of our study as Ketter et al., (2000) also noted a redistribution of carbon to deeper depths following tillage. Our results support Hypothesis 1) occasional tillage would initially result in a decline in labile C and

Hypothesis 2) following the return to no-till with cover crops and perennial forage, the T1/6 system labile C would recover to similar levels as the NT system.

Water stable aggregates

When WSA was compared in 2013 and 2019 separately, none of the main effects were significant in either year (**Table 2.4**). However, in the T1/6 system in 2019, the WSA in the canola crop entry decreased by 17% compared to the AO3 entry (p=0.017), showing that the presence of perennials had a significant impact on the physical stability of the soil following a tillage event (**Figure 2.9**). Additionally, the effect of system on the canola crop entry in 2019 was close to the threshold of significance with a p-value of 0.0553 and implied a downward trend in aggregate stability in the T1/6 system following a tillage event **(Figure 2.9**). To enable direct comparison of WSA data generated in different years by different operators, standardized scores were obtained by averaging raw scores in each year and measuring dispersion from the averages. The standardized scores enabled the data to be visualized on the same scale. (**Figure 2.10**), though the statistical outcomes were the same as the raw percentages (**Table 2.4**).

The lack of significant difference following tillage in the 2013 canola entry is explained by the presence of orchardgrass in addition to alfalfa in the T1/6 strategy. The NT strategy had only alfalfa as the perennial in rotation in 2013 and therefore may not have received as many benefits to soil structure as the T1/6 treatment. Because perennial grasses have more fibrous root systems, it has been suggested that they may promote higher aggregate stability than other types of perennials (Miller and Jastrow, 1990; Rachman et al., 2003). By 2019, the NT strategy also had both alfalfa and orchardgrass and therefore had experienced the benefits of both no-till and perennial grass roots to assist the formation of stable aggregates. In 2019 WSA in the T1/6 system was showing a downward trend compared to the NT system in the canola crop entry, which represented the spring immediately following tillage. The lower WSA may be explained by a combination of effects of the two tillage events as well as the increased stability from adding the orchardgrass to the NT system. In both 2013 and 2019 we saw no significant differences between systems in the AO3 crop entry, which could indicate perennial root action and associated fungal hyphae and soil microorganisms stabilize soil aggregates regardless of tillage. Further evidence of increased soil stabilization by perennial roots was the 21% increase in WSA between the canola entry and the AO3 entry in the T1/6 system. These results suggested that the perennial forage crops assisted with aggregate stability following a tillage event and partially supported Hypothesis 1) aggregate stability would decline following tillage and Hypothesis 2) soil health would increase in the T1/6 system to similar levels as the NT system following a return to no-till with cover crops and perennials. A 2010 study by Wortmann et al. found no significant differences in water stable aggregates across several tillage treatments, including no-till and moldboard plow tillage, five years following tillage. It is possible that the T1/6 system might have returned to similar levels as the NT system after several years without the aid of perennial roots, though Dougherty et al., (2022) suggests that only slight differences in soil health indicators can be expected within short timeframes, which may explain the lack of significant differences in the Wortmann et al., study. Additionally, the Wortmann et al. study used the water stable aggregate sampling method by Cambardella and Elliott (1994) which involves wet sieving soil and then adding together the weight of three different size classes of soils and may be less sensitive to management changes.

Conclusions

There are known benefits to the inclusion of one-time tillage events in no-till systems and the results of this study indicate that when including perennial forage crops in rotation with annual crops, the negative effects of one-time tillage on soil health can be mitigated. Water stable aggregates and bulk density were not affected by the management system (T1/6 or NT), but both showed improved soil health between the canola and third-year alfalfa-orchardgrass crop entries. The other indicators—labile carbon, total organic carbon percentage, and total organic carbon by volume—all showed significant decreases in soil health in the crop directly following tillage. None of the five indicators showed a significant difference between systems in the third-year alfalfa orchardgrass. The results from three of the five indicators supported our first hypothesis that soil health indicators of soil subjected to inversion tillage would decline directly flowing tillage. Results from all five indicators supported our second hypothesis that returning these soils to no-till with cover crops and perennials would allow them to recover to similar levels as soils that were not tilled. Additionally, this study revealed no effects of tillage on soil carbon at the 5-15 cm range, implying that tillage did not impact soil health beyond the 0-5 cm range in this study, though an investigation of soil carbon at a lower depth (15-30 cm) would be worth pursuing.

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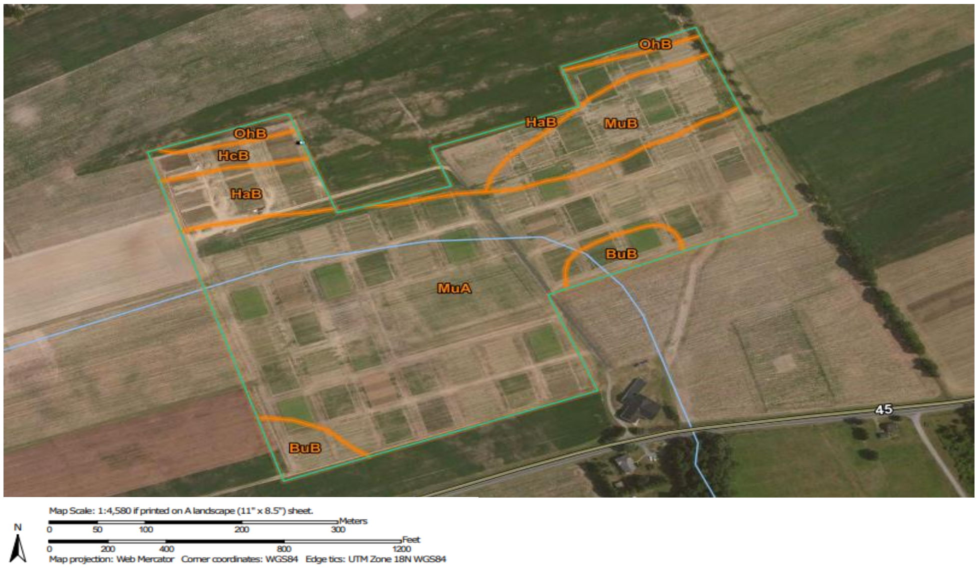
Figures



MuA: Murrill channery silt loam, 0 to 3 percent slopes

HaB: Hagerstown silt loam, 3 to 8 percent slopes

MuB: Murrill channery silt loam, 3 to 8 percent slopes

BuB: Buchanan channery loam, 3 to 8 percent slopes

HcB: Hagerstown silty clay loam, 3 to 8 percent slopes

OhB: Opequon-Hagerstown complex, 3 to 8 percent slopes

Figure 2.1. Soil map (NRCS SoilWeb) of the study site showing the soil types present. The primary soil type is the Murrill soil series.

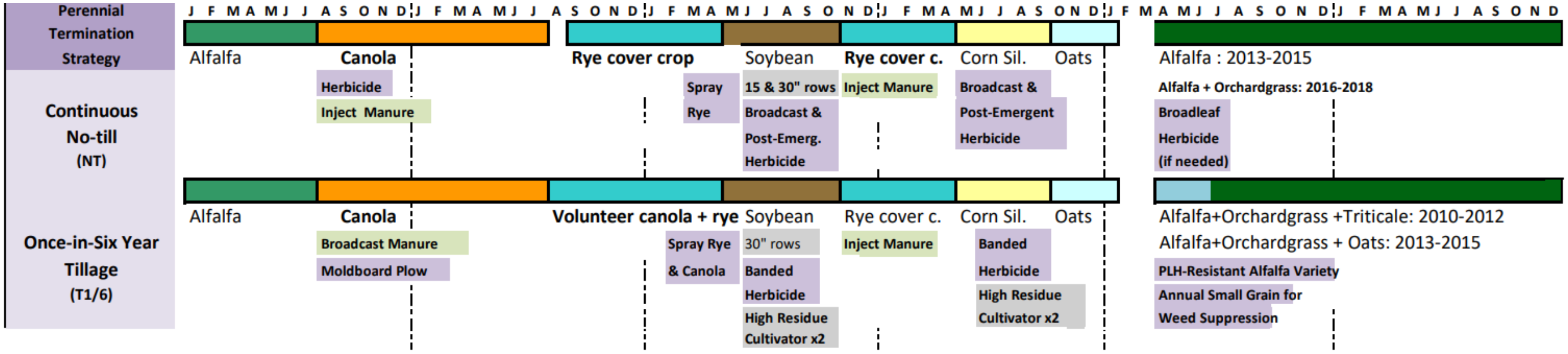


Figure 2.2. Six-year crop rotation sequence with continuous no-till (NT) and once in six-year tillage systems (T1/6).

Figure 2.3. Wet sieving apparatus (Eijkelkamp Soil & Water Giesbeek, Netherlands) used to measure water stable aggregates.

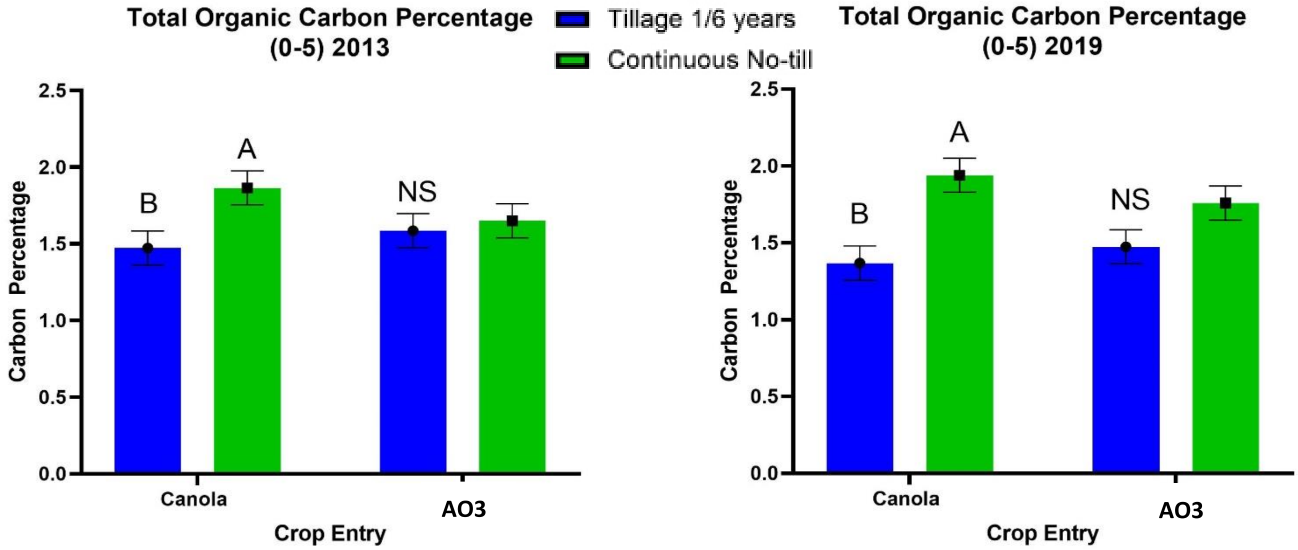


Figure 2.4 Total organic carbon measured as percentage in 2013 (right graph) and 2019 (left graph). TOC was significantly reduced by 21% in 2013 and by 29% in 2019 in the tillage system the spring after tillage. TOC did not differ in the AO3 before tillage or after tillage and two years of perennials in either 2013 or 2019. Uppercase letters (A, B) denote systems that differ at p < .05 within the same crop entry and year via the 'SLICE' procedure.

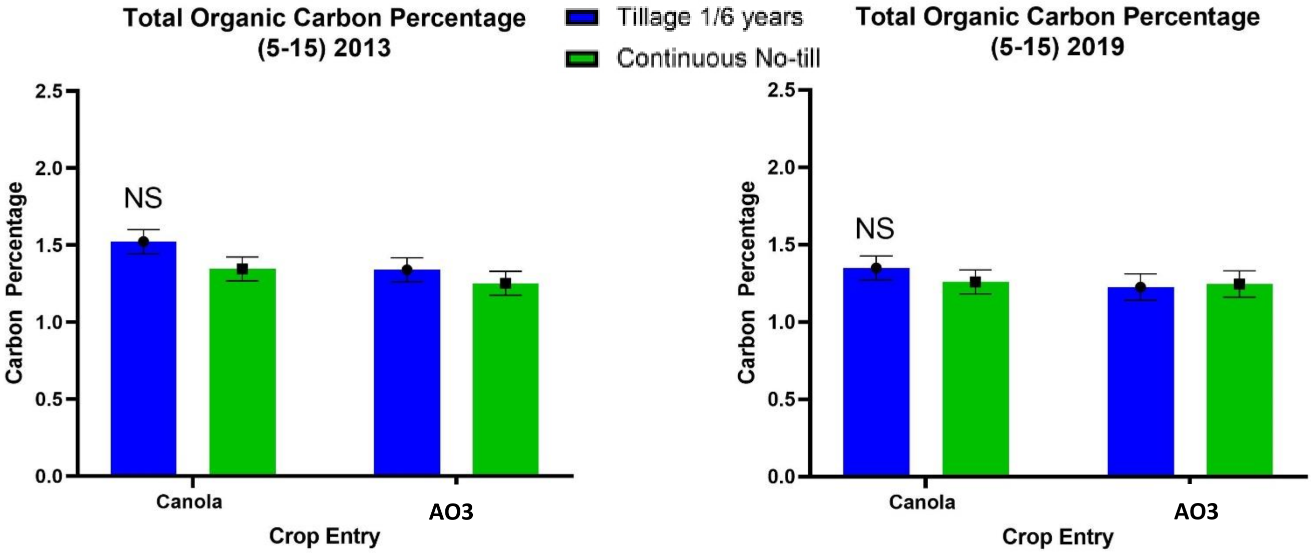


Figure 2.5 Total organic carbon at 5-15 cm measured as a percent in 2013 (right graph) and 2019 (left graph). TOC did not differ in either system in the canola entry, the spring after tillage in 2013 or 2019. TOC did not differ in the AO3 before tillage or after tillage and two years of perennials in either 2013 or 2019.

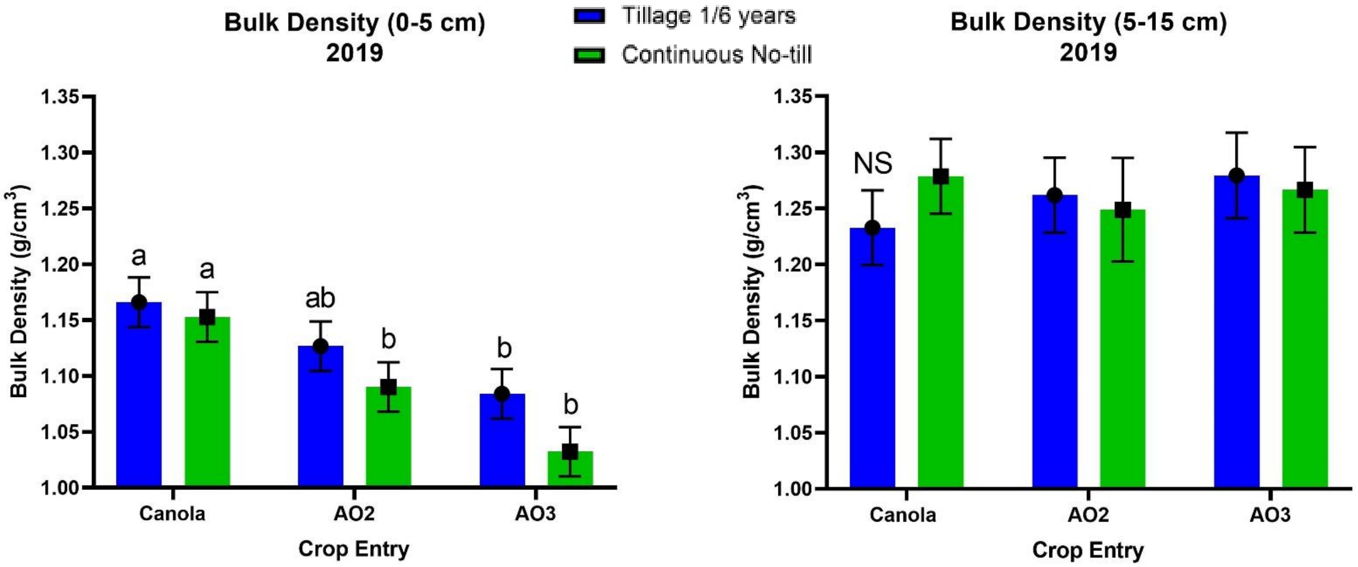


Figure 2.6 Bulk density (g/cm^3) in three crop entries at the 0-5 cm range (right graph) and the 5-15 cm range (left graph). There were no differences between systems in any of the crop entries, but in both systems, the canola year had significantly higher bulk density than in the AO3. There were no differences between systems or crop entries at the 5-15 cm depth. Lowercase letters (a, b) denote crop entries that differ at $p < .05$ within the same system via the 'SLICE' procedure.

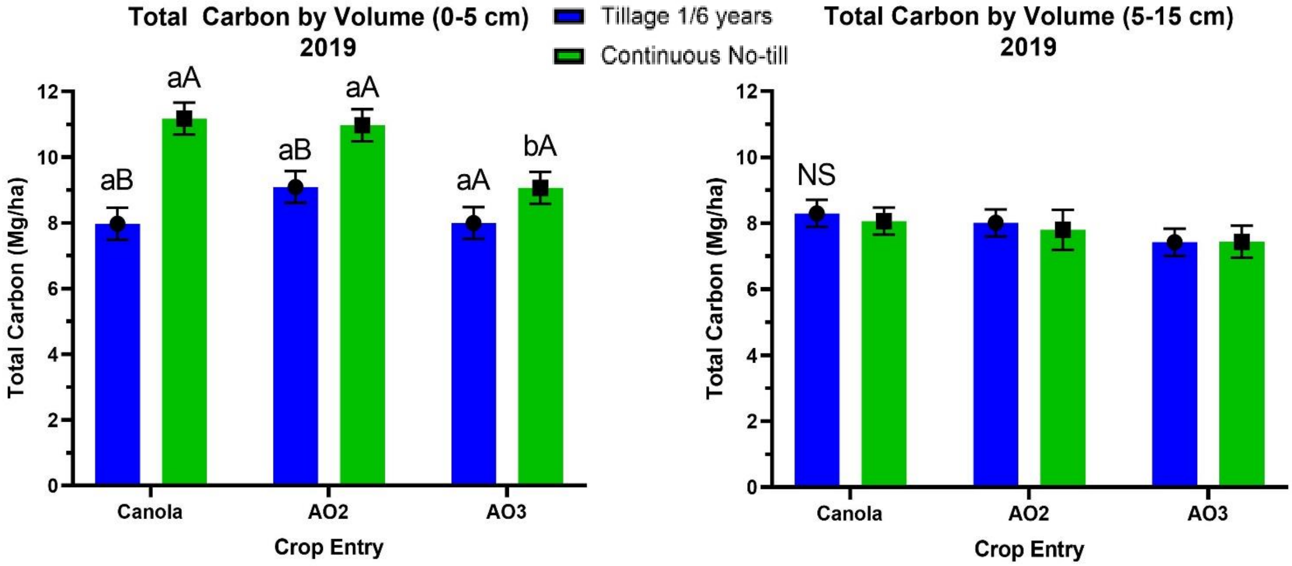


Figure 2.7 Total organic carbon by volume (Mg/ha) in three crop entries at the 0-5 cm range (right graph) and the 5-15 cm range (left graph). The two systems were significantly different in the canola entry, the spring following tillage, as well as in the AO2 entry, which is five springs after tillage. There was no significant difference in the AO3 crop entry, six springs following tillage or the spring directly before termination by tillage due to a decrease in carbon in the continuous no-till treatment. There were no differences between systems or crop entries at the 5-15 cm depth. Different lowercase letters (a, b) indicate that crop entries differ at $p < .05$. Different uppercase letters (A, B) indicate that systems differ at $p < .05$.

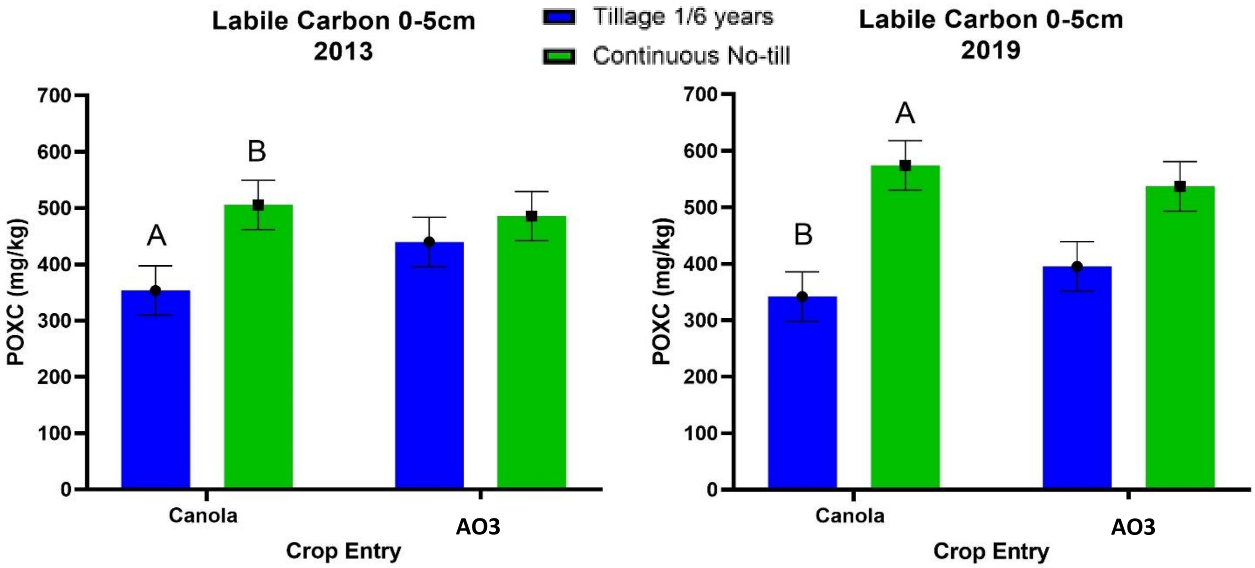


Figure 2.8 Labile carbon, measured as permanganate oxidizable carbon (mg/kg), in 2013 (left graph) and 2019 (right graph). Labile carbon in the T1/6 system was reduced by 30% in 2013 and by 40% in 2019 in the canola entry, the spring after tillage, compared to the NT system. Labile carbon did not differ in the AO3 entry, before tillage or after tillage and two years of perennials, in either 2013 or 2019. Uppercase letters (A, B) denote systems that differ at $p < .05$ within the same crop entry and year via the ‘SLICE’ procedure.

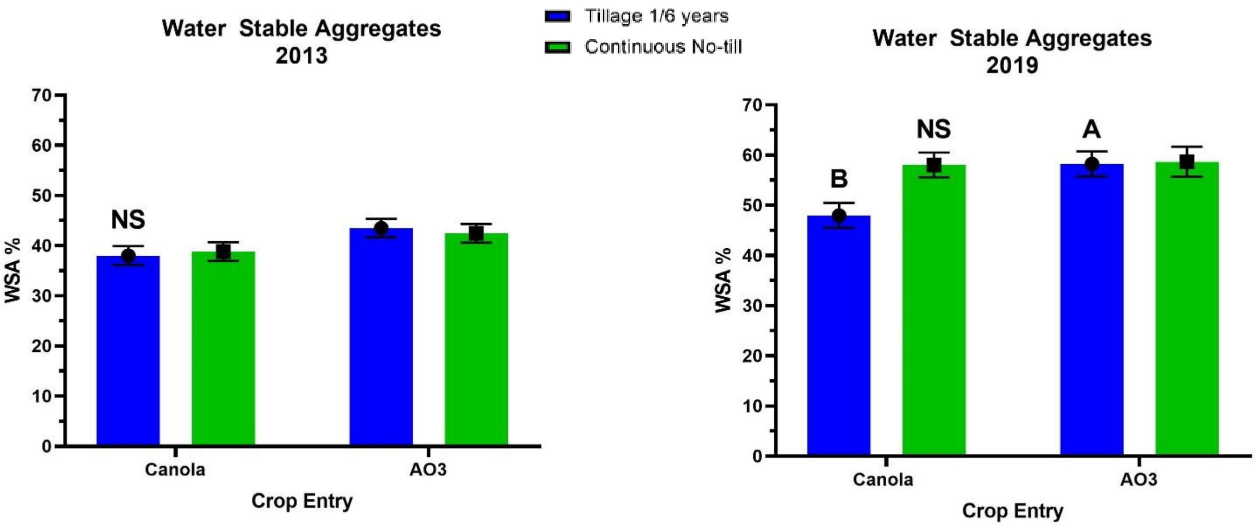


Figure 2.9 Water stable aggregate raw percentages in 2013 (left graph) and 2019 (right graph). In 2013, there were no differences between systems in either crop entry. In 2019, the systems were not significantly different in either crop entry, but there was a 21% increase in aggregate stability between the canola and AO3 crop entries in the T1/6 system. Different uppercase letters (A, B) indicate a significant difference between crop entries within a management system at the $p<0.05$ level.

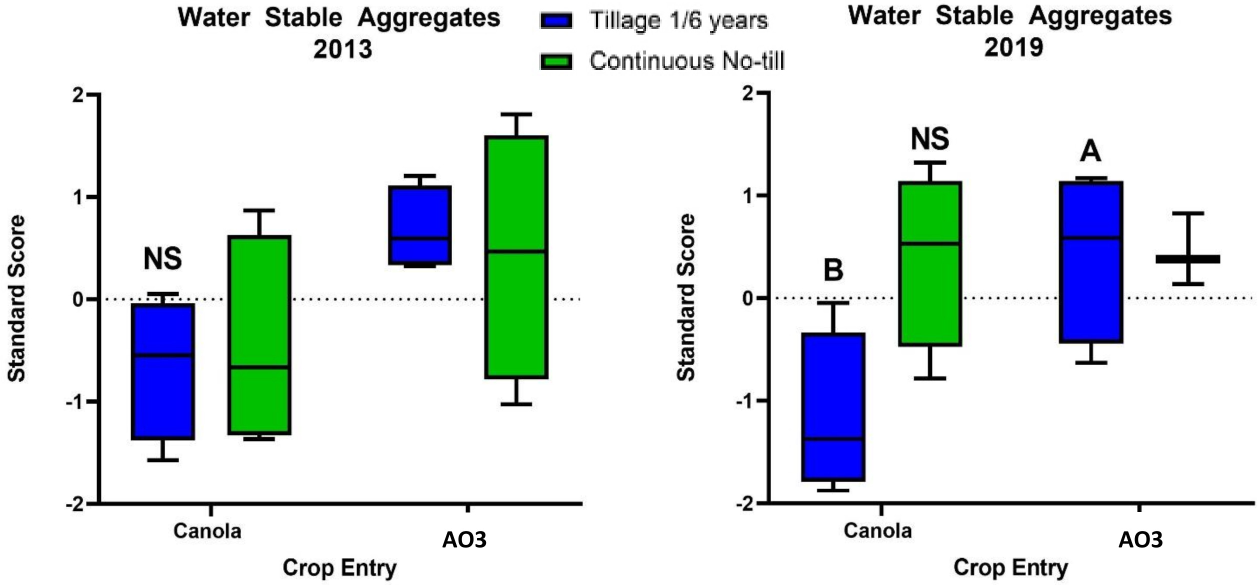


Figure 2.10 The water stable aggregate standard scores, calculated using the means and standard deviation of within-year scores, in canola (left graph) and AO3 (right graph). High standard score equates to higher aggregate stability—positive scores are higher than the year's mean, negative scores are lower than the year's mean. In 2013, there were no differences between systems in either crop entry. In 2019, the systems were not significantly different in either crop entry, but there was a 21% increase in aggregate stability between the canola and AO3 crop entries in the T1/6 system (different uppercase letters (A, B) indicate a significant difference between crop entries within a management system at the $p<0.05$ level).

Tables

Table 2.1. The main crops in the rotation sequence, the abbreviation used within the paper, as well as which analyses each crop was used in.

Main Crop Name	Abbreviation	Annual/ Perennial	Indicators Analyzed
Canola	Ca	Annual	All
Soybean	n/a	Annual	None
Corn grain/silage	n/a	Annual	None
Alfalfa-orchardgrass	AO (2 or 3)	Perennial	First year: None Second-year: TOC by volume Third year: All

Table 2.2 Labile carbon by POXC at 0-5 cm and total organic carbon percentage at 0-5 and 5-15 cm. Significant differences of main effects (management system, crop entry, and year) were determined by the SLICE statement in JMP to perform a partitioned F test of least square means (LSMeans) of their interaction.

	POXC (0-5 cm)	**TOC% (0-5 cm)**	**TOC% (5-15 cm)**
Main effect			
Crop	NS[†]	NS	NS
System	***	**	NS
Year	NS	NS	NS
System x Crop	NS	NS	NS
System x Year	NS	NS	NS
Crop x Year	NS	NS	NS
System x Crop x Year	NS	NS	NS
Crop			
Average of Ca	444a[a]	1.66a	1.37a
Average of AO3	464a	1.62a	1.24a
System			
Average of T1/6	383b	1.47b	1.35a
Average of NT	526a	1.80a	1.26a
Year			
Average of 2013	446a	1.64a	1.37a
Average of 2019	462a	1.64a	1.24a
Three-way effect combination			
Ca, T1/6, 2013	353B[b]**a**[c]**A**[d]	1.47B**aA**	1.52A**aA**
Ca, NT, 2013	506A**aA**	1.87A**aA**	1.35A**aA**
AO3, T1/6, 2013	440A**aA**	1.59A**aA**	1.34A**aA**
AO3, NT, 2013	486A**aA**	1.65A**aA**	1.25A**aA**
Ca, T1/6, 2019	342B**aA**	1.37B**aA**	1.35A**aA**
Ca, NT, 2019	574A**aA**	1.94A**aA**	1.26A**aA**
AO3, T1/6, 2019	395A**aA**	1.47A**aA**	1.17A**aA**
AO3, NT, 2019	537A**aA**	1.76A**aA**	1.19A**aA**
Slice tests			
Ca, T1/6, 2013 x Ca, NT, 2013	*	*	NS
Ca, T1/6, 2013 x AO3, T1/6, 2013	NS	NS	NS
Ca, NT, 2013 x AO3, NT, 2013	NS	NS	NS
AO3, T1/6, 2013 x AO3, NT, 2013	NS	NS	NS
Ca, T1/6, 2019 x Ca, NT, 2019	**	**	NS
Ca, T1/6, 2019 x AO3, T1/6, 2019	NS	NS	NS
Ca, NT, 2019 x AO3, NT, 2019	NS	NS	NS
AO3, T1/6, 2019 x AO3, NT, 2019	NS	NS	NS

[a] Lowercase letters (a, b) denote differences due to main effects at $p < .05$.
[b] Uppercase letters (A, B) denote systems that differ at $p < .05$ within the same crop entry and year via the ‘SLICE’ procedure.
[c] Bold lowercase letters (**a, b**) denote crop entries that differ at $p < .05$ within the same system and year via the ‘SLICE’ procedure.
[d] Bold uppercase letters (**A, B**) denote years that differ at $p < .05$ within the same system and crop entry via the ‘SLICE’ procedure
*Significant at the .05 probability level.
**Significant at the .01 probability level.
***Significant at the .001 probability level.
[†] NS, nonsignificant.

Table 2.3 Bulk density and TOC by volume averages at 0-5 and 5-15 cm depths for tillage once-in-six years (T1/6) and continuous no-till (NT) in canola (Ca), second-year alfalfa orchardgrass (AO2) and third-year alfalfa orchardgrass (AO3) crop entries. Significant differences of the main effects of management system (System) and crop entry (Crop) were determined by the SLICE function in JMP to conduct a partitioned F-test of LSMeans of the interaction of System x Crop.

	BD (0-5)	BD (5-15)	TOC Mg/ha (0-5 cm)	TOC Mg/ha (5-15cm)
System x Crop				
T1/6, Ca	1.17aA[a][b]	1.23aA	7.97aB	8.30aA
T1/6, AO2	1.13abA	1.26aA	9.09aB	8.01aA
T1/6, AO3	1.08bA	1.28aA	8.00aA	7.46aA
NT, Ca	1.15aA	1.28aA	11.18aA	8.06aA
NT, AO2	1.09bA	1.25aA	10.97aA	7.98aA
NT, AO3	1.03bA	1.27aA	9.07bA	7.48aA
System average				
T1/6	1.13A	1.26A	8.35B	7.92A
NT	1.09A	1.26A	10.41A	7.84A
Crop average				
Ca	1.16a	1.26a	9.58a	8.18a
AO2	1.11ab	1.26a	10.03a	8.00a
AO3	1.06b	1.27a	8.53a	7.47a
Factor				
Crop	**	NS	NS	NS
System	NS[†]	NS	***	NS
System x Crop	NS	NS	NS	NS
SLICE tests				
Effect of System				
T1/6, Ca x NT, Ca	NS	NS	***	NS
T1/6, AO2 x NT, AO2	NS	NS	**	NS
T1/6, AO3 x NT, AO3	NS	NS	NS	NS
Effect of Crop				
T1/6, Ca x T1/6, AO2	NS	NS	NS	NS
T1/6, Ca x T1/6, AO3	**	NS	NS	NS
T1/6, AO2 x T1/6 AO3	NS	NS	NS	NS
NT, Ca x NT, AO2	*	NS	NS	NS
NT, Ca x NT, AO3	***	NS	**	NS
NT, AO2 x NT, AO3	NS	NS	*	NS

*Significant at $p < .05$

** Signficant at $p < 0.01$

*** Significant at $p < 0.001$

[†] NS, not significant

[a] Different lowercase letters (a, b) indicate that crop entries differ at $p < .05$

[b] Different uppercase letters (A, B) indicate that systems differ at $p < .05$.

Table 2.4 2013 and 2019 water stable aggregate percentages and standard score averages of tillage once-in-six years (T1/6) and continuous no-till (NT) in both canola (Ca) and third-year alfalfa orchardgrass (AO3) crop entries. Significant differences of the main effects of management system (System) and crop entry (Crop) were determined by the SLICE function in JMP to conduct a partitioned F-test of LSMeans of the interaction of System x Crop.

	2013 WSA		**2019 WSA**	
	%	standard score	%	standard score
System x Crop				
T1/6, Ca	38a[a]A[b]	-0.654	48.0aB	-1.165
T1/6, AO3	43.5aA	0.681	58.2aA	0.429
NT, Ca	38.8aA	-0.456	58.1aA	0.4
NT, AO3	42.5aA	0.429	58.7aA	0.499
System average				
T1/6	40.8a	0.013	53.1a	-0.368
NT	40.7a	-0.013	58.4a	0.449
Crop average				
Ca	38.4A	-0.555	53.0B	-0.383
AO3	43A	0.555	58.5A	0.464
Factor				
Crop	NS[†]	NS	NS	NS
System	NS	NS	NS	NS
System x Crop	NS	NS	NS	NS
SLICE tests				
Effect of System				
T1/6, Ca x NT, Ca	NS	NS	NS	NS
T1/6, AO3 x NT, AO3	NS	NS	NS	NS
Effect of Crop				
T1/6, Ca x T1/6, AO3	NS	NS	*	*
NT, Ca x NT, AO3	NS	NS	NS	NS

*Significant at $p < .05$

[†] NS, not significant

[a] Different lowercase letters (a, b) indicate that systems differ at $p < .05$

[b] Different uppercase letters (A, B) indicate that crop entries differ at $p < .05$.

Chapter 3

Comparing Soil Health Indicators Under Four Fertilizer Application Strategies in a Corn-Soy Rotation

Introduction

No-till agriculture has become a common way to improve soil health, particularly soil structure (Stubbs et al., 2004). In a 2014 survey by the USDA, 66% of crop acreage was managed with no-till in Pennsylvania, evidence of its rising popularity. Because no-till usually includes leaving residue from terminated crops on the soil surface, it can increase organic matter accumulation which positively impacts macro-aggregate formation where organic matter is more protected from degradation (Batey, 2009). Despite its benefits to soil health, no-till relies heavily on herbicide use to terminate crops and control weeds. The presence of herbicide, fertilizer application, and crop residue without soil mixing can lead to nutrient stratification in no-till systems (Scheiner and Lavado, 2008). Though there is less nutrient runoff from no-till than conventional tillage systems, phosphorus build-up on surface layers is usually more severe in no-till systems (McDowell and McGregor, 1980; Grove et al., 2007; Scheiner and Lavado, 2008). As phosphorus runoff can easily lead to pollution and eutrophication of nearby waterways, it is an important concern for those managing water quality (Verbree et al., 2010). Conventional, full-inversion tillage is also well-documented as being detrimental to soil health (Bhardwaj et al., 2011; Cates et al., 2016; Nunes et al., 2020; Sprunger et al., 2021). These issues have led researchers to consider alternatives to no-till, such as reduced or conservation tillage options, which may provide a middle-ground between conventional tillage and no-till.

In the same 2014 survey where the USDA found 66% of Pennsylvania agricultural land being managed with no-till, the other 44% of land area was split nearly evenly between conservation tillage and conventional tillage (17.8% and 16.2% respectively). The USDA defines conservation tillage as, "tillage practices prior to planting which result in a minimum of 30 percent ground cover or residue being retained on the surface following planting". Conservation tillage includes ridge till, strip till, and mulch till" (USDA, 2014). Grass and weed control are accomplished primarily with herbicides. A 1992 study by Carter compared conventional tillage practices to shallow disking and chisel plowing—both of which are considered reduced tillage—and found that soil structural stability improved significantly in three to five years in the reduced tillage plots. Though it is clear that Pennsylvania farmers prefer no-till management to either reduced tillage or conventional tillage (USDA, 2014), it may be important for farmers to consider reduced tillage options.

The present study evaluates the effects of four fertilizer application strategies on soil health indicators in a conventional corn-soy rotation. The strategies included broadcasted commercial fertilizer, broadcasted manure, injected manure, and manure incorporated by chisel disking. Most no-till farmers are comfortable with the first three strategies. Although chisel disk incorporation of manure could fall under the category of reduced tillage, many no-till farmers would still be reluctant to use the practice. A long-term study by Duiker and Beegle (2006), compared the effects of no-till, chisel disking, and moldboard plow on soil organic matter content and did not find a significant difference between no-till and chisel-disking, though both were significantly different from moldboard plow.

A literature gap exists for studies examining the conversion from no-till to chisel disking without additions of cover crops, making it difficult to assess the direct impacts of chisel-plowing on previously no-till soils. Our investigation did not include a moldboard plow treatment and will therefore allow for a more targeted comparison between no-till fertilizer application strategies and manure incorporation with chisel disk. For soil health assessment, this investigation included measurements of total organic carbon (TOC), which is recognized as being slow to change in response to management. Therefore, additional measurements included soil bulk density, water stable aggregate percentage, and permanganate oxidizable carbon (POXC), which are considered to be more responsive to changes in management. The objective of this study was to compare soil health indicators in soils managed with contrasting fertilizer application strategies. Each “strategy” represented a combination of a fertilization practice and a tillage practice. Chisel-disking of manure was expected to be the strategy with greatest potential to reduce soil health, though because tillage typically reduces bulk density in the short term, we expected it to have a positive effect on bulk density (Logsdon and Karlen, 2004). Injection of manure into no-till soils, which was intended to introduce C and N to greater depth, was expected to have the next highest impact on soil health. Finally, no-till soils receiving surface applications of manure or synthetic fertilizer would have little or no disturbance and less negative impacts on soil health. We hypothesized that (1) bulk density would decrease in the chisel disk strategy; (2) carbon would be reduced at the 0-5 cm depth but increased in the 5-15 cm depth in the chisel disk strategy; (3) carbon would be increased in the 5-15 cm depth in the injected manure strategy compared to the other two no-till strategies, but otherwise no differences between no-till fertilizer application strategies, and (4) water stable aggregates and POXC would be reduced as a result of chisel disking.

Materials and Methods

The Dairy Cropping Systems (DCS) project was established in the spring of 2010 at the Pennsylvania State University Russell E. Larson Agronomy Research Farm near Pennsylvania Furnace, PA (40.72˚N, -77.92˚W). The study aims to simulate cropping operations of a 97-ha dairy farm at 1/20th the scale (about 5-ha) with the objective of providing forage and grain needed for a simulated 65-cow dairy herd, while minimizing off-farm inputs. A two-year corn-soy rotation with no cover crops and receiving conventional fertilizers and pesticide treatments was included in the farming systems experiment as an example of a common crop rotation in the region. Additionally, the system was evaluated for its ability to reduce nutrient loss, build soil health, and increase biodiversity while remaining energy efficient, productive, and both ecologically and economically sustainable (Busch et al., 2020).

The rotation system included a sequence of (1) corn grain (*Zea mays* L.) and (2) soybean [*Glycine max* (L.) Merr.] from 2010 to 2021 (**Figure 3.1**). Beginning in 2016, the system compared: i. continuous no-till with broadcast manure in corn years (NT-BM), ii. yearly chisel disk with broadcast manure in corn years (CD-BM), iii. continuous no-till with synthetic fertilizer application in corn years (NT-SF) and soybean years as needed, and iv. continuous no-till with injected manure in corn years (NT-IM). The predominant soil series at the experimental site is Murrill (Fine-loamy, mixed, semiactive, mesic Typic Hapludults), with Hagerstown, Buchanan, and Opequon soil series also present (**Figure 3.2**).

Experimental Design

The rotation was a full crop entry experiment, with both phases planted every year in a randomized complete block design, replicated four times. Crop phases were the main plot and tillage/nutrient amendment strategies were deployed in split-split-plots. Nutrient amendments included broadcast manure (BM), broadcast synthetic fertilizer (SF), and injected manure (IM).

Tillage practices were either no-till (NT) with inorganic fertilizer, injected or broadcast manure or chisel-disking (CD) of broadcast manure. Soil was sampled in the spring of 2010, prior to the start of the experiment and in spring prior to planting in 2013, 2016, and 2019, and 2021. Samples at 0-5 cm depth were tested for labile carbon, at 0-5 and 5-15 cm depths for total carbon, and in June after crop planting at 0-15 cm for water stable aggregates. Prior to 2016, chisel disking had not been employed at all, and both sides of the split plot had received synthetic fertilizer and either broadcast manure or injected manure. To evaluate the chisel disk strategy appropriately, 2016 is considered as time = 0 of this experiment.

In 2010 at the start of this experiment, the corn-soy rotation was intended to represent conventional practices comparing injected and broadcast manure fertilization with no-till. In 2012, the plots were split to receive synthetic fertilizer on one half of the plot and either broadcast manure or injected manure on the other half. In 2016, in the BM/Fert split, the BM plot began receiving chisel disking (CD-BM) and the Fert plot became broadcast manure (NT-BM). The injected manure and synthetic fertilizer plots for the IM/Fert split remained the same and were designated NT-IM and NT-SF respectively, creating the four separate strategies. Beginning in 2016, one strategy consisted of chisel disking every year in both corn and soy entries and broadcast manure in only the corn entry. The other three were continuously no-till throughout the experiment, with each of the three untilled strategies receiving the different fertilizer applications in the corn entry.

Soil sampling procedures

Soils were sampled in the spring, before planting and fertilization in 2010, 2013, 2016, 2019, and 2021. Composite samples were collected from plots receiving broadcast manure by mixing ten, 7.5 cm soil cores taken randomly throughout the plot with a soil probe. After 2010, soil sampling of plots receiving injected manure differed to account for manure distribution by injection. When sampling these plots, 18 cores were taken in three sets of six in lines perpendicular to the injection band (six inches apart) to obtain a more representative sample. Soil cores sampled for total organic carbon (TOC) and permanganate oxidizable carbon (POXC) were split into the 0-5 and 5-15 cm portions in the field and kept separate. Soil cores for aggregate stability analyses were sampled to 15 cm depth, with ten cores composited per plot and kept in cool, airtight containers prior to processing to minimize microbial activity

Bulk density

Due to the challenges presented by bulk density sampling in gravelly soils, 2019 is the only year that sufficient samples were taken to evaluate the four strategies. Bulk density was measured by the standard procedure described by Blake and Hartge (1986), which involves placing a 3-inch diameter metal cylinder on the sampling area and pounding it uniformly into the ground. To measure the bulk density from 0-15 cm, two cylinders were placed on top of each other and a large weight slightly larger than the size of the cylinders was dropped repeatedly onto the cylinders. Once the cylinder was fully in the ground, it was carefully removed, and any additional soil clinging to the top or bottom of the cylinder was sliced off using a knife. The two cylinders were then separated into 0-5 and 5-15 cm fractions again using a knife. The entire contents of the cylinders were then emptied into a labeled bag and sealed until further processing.

The weights of moist soil were recorded for each sample and then soils were wet sieved through a 2-mm sieve to exclude any large rocks. Rocks were then placed into a graduated cylinder with a known amount of water and the displacement of the water was measured to estimate the volume of the rocks. Moist soils were placed into a drying oven and reweighed after drying. The dry weight of the soil was used to calculate the bulk density using the formula: (oven dry weight of soil)/(volume of soil), where the volume of the soil is the known volume of the 3-inch diameter metal cylinder adjusted for the volume of any rocks or voids.

Total organic carbon

TOC was measured with a 2400 CHNS/O Series II Analyzer (Perkin Elmer, Waltham, MA, USA) in 2010, 2013, and 2016 and a Vario Max elemental analyzer (Elementar, Langenselbold, Hesse, Germany) in 2019 and 2021 and required 25 mg and 250-300 mg (respectively), ground soil samples. Carbon percentage was determined using high temperature combustion. The bulk density value was used to determine the total organic carbon by volume. The carbon in grams per cubic centimeter was calculated by multiplying the percent carbon by the bulk density and dividing by 100. This number was then converted to metric tons per hectare. The total organic carbon by volume was analyzed only for the year 2019.

Labile carbon (POXC)

Soils for carbon analysis (TOC and POXC) were air-dried and machine-ground. Soil POXC was measured using the Weil et al. (2003) method using a stock solution of 0.2 M potassium permanganate in 1 M calcium chloride (pH 7.2). A standard curve was created for each day samples were run. A handheld colorimeter (Hach, Loveland, CO) was used to measure absorbance at 550 nm. Absorbance values for each of the three standards were recorded and a standard curve was generated. The equation of the line was used to determine the constants for the equation for calculating POXC:

POXC (mg kg–1) = (0.02 Mol/L – (a+b*absorbance))*(9000 mg C/Mol)*(0.02 L soln./0.0025 kg soil)

In this equation, the constant a is the y-intercept and b is the slope of the line generated by the standards. Absorbance is the value measured for each soil sample.

To measure the POXC, 2.5 g of soil was mixed with 2 mL KMnO4 brought to 20 mL with water in a 50-mL conical tube and manually shaken at a rate of two shakes per second, timed for two minutes. The tube was uncapped then left undisturbed for ten minutes. Supernatant was diluted 1/10 in deionized water and mixed before measuring absorbance values used in the above equation.

Water stable aggregates

Field moist soils were sieved through 2-mm and 1-mm sieves, with material remaining on top of the 1-mm sieve retained and air-dried. To measure water stable aggregates (WSA), a modified version (Grover, 2008) of the Kemper-Rosenau (1986) method was used with a dispersing solution (2 g of sodium hexametaphosphate in 1 L of deionized water). Four grams of air-dried soils were added to sieve-bottom cups in an 8-cup wet-sieving apparatus (Eijkelkamp Soil & Water Giesbeek, Netherlands) (**Figure 2.3**). Tins containing deionized water were placed under the sieve-bottom cups, which were lowered to completely submerged the soils. After submersion without disturbance for five minutes, the cupholder was raised and lowered at a rate of 33 times per minute for another five minutes. Tins containing water were replaced with tins containing dispersing solution, and the process was repeated.

Once raised out of the solution, samples in the sieve-bottom cups were gently rubbed with a rubber-tipped rod for 20 seconds each. Samples were lowered again into dispersing solution and raised and lowered for a final five minutes. The two sets of tins, one containing water and unstable soil fraction and the other containing dispersing solution and the stable fraction, were removed and placed in a drying oven at 110℃ for two days. Any sand, rock, or particulate organic matter remaining in the sieve bottom cup was discarded.

WSA percentage was measured using the equation:

[Stable Aggregate/(Stable Aggregate + Unstable Aggregate)]*100

where stable aggregate refers to the fraction of the soil slaked off in dispersing solution and unstable aggregate refers to the fraction of the soil that slaked off in water. Two replicates were performed for each plot and a percent difference between replicates was determined using the equation:

[(Rep 1 – Rep 2)/Average(Rep1,Rep2)]*100

If the percent difference between replicates was greater than 29%, a third replicate was performed. This was only necessary for one sample and a fourth replicate was never necessary. Results from this WSA procedure, which was carried out by different individuals in 2010, 2013, 2016, and 2019, can be subject to variation due to technique and handling practices. To account for this variation, standardized scores were generated for water stable aggregate data within each year. Standardized scores were calculated by subtracting the population mean (all blocks and both strategies) from each WSA percentage and dividing this value by the population standard deviation.

Statistical analysis

Statistical analyses were performed using the Standard Least Squares personality procedure in JMP Pro 15 by SAS (SAS Institute, Inc. Cary, North Carolina). For all indicators, strategy was the main fixed effect, with block included as a random effect. WSA percentages were analyzed in individual years separately due to a sensitivity in one step of the procedure making year to year comparisons unreliable with just the raw percentages. Additionally, the change in individual plot values between 2016 and 2021 were calculated for labile C and TOC percentage because there were no differences between strategies in 2016 at $p<0.05$. Bulk density and TOC by volume data were only sufficient for analysis in 2019 so year was not a factor in analysis. Tukey's HSD test was used to separate LSmeans and differences were considered significant at $p<0.05$.

Standardized scores were calculated for WSA by subtracting the population mean (all blocks and strategies) from each WSA percentage and dividing this value by the population standard deviation for each year. This allowed us to include year as a variable in our model when analyzing WSA results. TOC percentage was also analyzed with year included as a variable. Only these two indicators had sufficient data to include in this two-factor analysis. The model for these two indicators included strategy, year, and strategy x year as fixed effects and block and block x year as random effects. The SLICE test, which analyzes simple effects to separate LSmeans within an interaction, was used to test the pre-planned hypotheses even if fixed effects were not considered significant. In the model used for WSA and TOC percentage, the SLICE test was used on the two-way interaction term between strategy and year. Means were considered significantly different at $p<0.05$.

Results and Discussion

Total organic carbon percentage

TOC percentage at the 0-5 cm depth decreased between 2016 and 2021 in the CD-BM strategy compared to the three no-till strategies (**Figure 3.3**). TOC percentage at 0-5 cm was reduced in the CD-BM strategy by 66% compared to the NT-BM strategy, 63% compared to the NT-IM strategy and, 55% compared to the NT-SF strategy (**Table 3.1**). When chisel disk was excluded from the analysis, there still were no significant differences between the three continuous no-till strategies.

When the TOC percentages from 2019 were added and year was considered as an effect, strategy and strategy x year were considered significant effects (p= 0.04141 and p= 0.02069 respectively) at the 0-5 cm depth (**Table 3.2**). There were no differences between strategies in 2016, the year the chisel disk strategy was initiated. By 2019, there was a significant difference between the CD-BM strategy and all three no-till strategies (**Figure 3.4**). TOC percentage at 0-5 cm was reduced in the CD-BM strategy by 24% compared to the NT-BM strategy (p= 0.0067), 22% compared to the NT-SF strategy (p=0.0129), and 20% compared to the NT-IM strategy (p= 0.0427) (**Table 3.2**). Similarly, in 2021 TOC percentage at 0-5 cm was reduced in the CD-BM strategy by 26% compared to the NT-BM strategy (0.0022), 21% compared to the NT-SF strategy (0.0171), and 19% compared to the NT-IM strategy (0.0268) (**Table 3.2**). Additionally, within the NT-BM strategy, TOC increased by 42% between 2016 and 2019 (p= 0.0057) and by 48% between 2016 and 2021 (p= 0.0019).

There were no differences in TOC percentage at the 5-15 cm depth between the four strategies when analyzing the difference between 2016 and 2021, **(Figure 3.3)** but when 2019 was added and year was considered as an effect (**Figure 3.4**), year was significant (p= 0.04285).

Though there were no differences at this depth between strategies, within the NT-BM strategy, TOC increased by 38% between 2016 and 2019 (p= 0.003) and by 31% between 2016 and 2021 (p= 0.0116; though this is a decrease between 2019 and 2021, this decrease was not significant). Similarly, within the NT-SF strategy, TOC increased by 25% between 2016 and 2019 (p= 0.0235) and by 29% between 2016 and 2021 (p= 0.0105) (**Table 3.2**). These results are interesting as they did not consistently behave in the ways we expected. We expected there to be increased soil carbon at the 5-15 cm depth in the CD-BM and NT-IM strategies as a result of the soil mixing in the CD-BM strategy and the injection directly into the soil in NT-IM strategy. Though there were no differences between any of the strategies at this depth, there were increases within the NT-BM and NT-SF strategy between years. One possible explanation for the increase is the fact that the NT-BM strategy had previously received synthetic fertilizer prior to 2016, so the increase may be due to transition to receiving manure. The reason for the increase in the NT-SF is less clear, as this management strategy was employed prior to 2016.

Though we hypothesized that soil carbon would be increased in the CD-BM and NT-IM strategies at the 5-15 cm depth, most research comparing fertilizer application strategies focus on nitrogen dynamics rather than carbon dynamics which may account for the discrepancies between our expected and actual results (Duncan et al., 2017; Miner et al., 2020). Another possible explanation for the lack of increase at 5-15 cm in the NT-IM strategy is that the manure slurry applied was only about 10-15% solids and those solids were only about 40% carbon. Therefore, a single manure application may not have added enough carbon to cause a significant increase. It is also possible, due to the rocky conditions of the soils, that the manure injector was being forced off of its normal trajectory and not applying the manure to the depth expected. An additional factor in the carbon dynamics of our study is the fact that corn stover is left on the soil surface as the corn was only harvested for grain. This additional source of organic matter may have resulted in higher carbon present in our system overall than in a system that harvests the corn for forage. Had the forage also been harvested from the system, it is possible that differences between the four strategies would have been more profound.

Bulk density and total organic carbon by volume

There were no differences in bulk density between the four strategies at either depth (**Figure 3.5; Table 3.3**). TOC by volume at the 0-5 cm depth in the CD-BM strategy was reduced by 22% compared to the NT-SF strategy only (**Figure 3.5; Table 3.3**). The CD-BM and NT-SF strategies were not significantly different from the NT-BM strategy or the NT-IM strategy. When chisel disk was excluded from the analysis, there were no significant differences between the three no-till strategies.

There were no differences in TOC by volume at the 5-15 cm depth in 2019 (**Figure 3.5**) between the four strategies. When chisel disk was excluded from the analysis, there still were no significant differences between the three no-till strategies. It is interesting and perhaps encouraging that there were no significant differences in bulk density between the strategies, as bulk density is expected to decrease in the short term with tillage, but over time, increase with increased soil disturbance (Logsdon and Karlen, 2004; Nunes et al., 2020). It is possible, however, that the bulk density would show differences between years, had bulk density been measured in 2021. Otherwise, these results did not differ from the TOC percentage results, which also showed no differences between strategies. These results did not support Hypothesis (1) that bulk density would decrease as a result of chisel disk compared to the three no-till strategies. Results partially supported Hypothesis (2) that soil carbon would decrease at the 0-5 cm depth in the CD-BM strategy, although this was seen only when compared to the NT-SF strategy; also, carbon was not increased at the 5-15 cm depth in CD-BM strategy. Results partially supported Hypothesis (3) though soil carbon did not increase in the NT-IM strategy at the 5-15 cm depth, there were otherwise no differences in soil health indicators between the three no-till strategies.

Labile carbon

Labile carbon results followed the same trends as TOC percentage. Labile carbon by POXC decreased between 2016 and 2021 in the CD-BM strategy compared to the three no-till strategies (**Figure 3.6**). POXC in the CD-BM strategy was reduced by 47% compared to the NT-SF strategy, 41% compared to the NT-IM strategy and, 40% compared to the NT-BM strategy (**Table 3.1**). When chisel disk was excluded from the analysis, there still were no significant differences between the three continuous no-till strategies. Due to gaps in data, 2019 was not analyzed and year was not considered as a factor in analysis for POXC.

Because labile carbon was only measured at the 0-5 cm depth, we cannot assess the possible redistribution of labile carbon at deeper depths with chisel disk, though we were able to assess this possibility for total carbon. These results support Hypothesis (3) there would be no significant differences in indicators between no-till strategies and Hypothesis (4) that POXC would be reduced at the 0-5 cm depth in the CD-BM strategy compared to the three no-till strategies.

Water stable aggregates

WSA did not differ between strategies, either in 2016, the year the CD-BM strategy was initiated, or after three years (2019). After five years (2021), WSA in the CD-BM strategy showed a significant reduction compared to the other three strategies (**Figure 3.7**). WSA in the CD-BM strategy was reduced by 32% compared to the NT-IM strategy (p=0.0027), 30% compared to the NT-BM strategy (p=0.0052), and 29% compared to the NT-SF strategy (p=0.0059) (**Table 3.4**). When chisel disk was excluded from the analysis, there still were no significant differences between the three continuous no-till strategies. These results indicate that water stable aggregates were negatively impacted by the addition of chisel disk, but this impact was not significant until at least three years after the initiation of the CD-BM strategy. These results also indicate that WSA was slightly less sensitive than TOC percentage to chisel disking as there was already a significant difference in TOC between the CD-BM strategy and the three no-till strategies after three years (**Figure 3.4**), whereas only after five years was the difference significant in the WSA.

The WSA standard scores were also analyzed between years, though none of the main effects were considered significant. However, within the CD-BM strategy, WSA was significantly reduced between 2016 and 2021 (p=0.0085). By 2021, five years after the initiation of the chisel-disking, the WSA in the CD-BM strategy was reduced by 85% compared to 2016 (**Table 3.4**) The differences between 2019 and the other two years were not significant but do imply that the decrease happened gradually over time (**Figure 3.8**). These results show the same trend observed when WSA percentages were analyzed within year, with the addition of seeing the downward trend over time in the CD-BM strategy.

WSA showed similar sensitivity to chisel disking as labile C, though the lack of 2019 labile C data does not allow us to note how quickly the reduction in POXC occurred over the five years. A study by Quincke et al. (2007) that looked at the effects of chisel disking compared to no-till directly following a tillage event, did not find a significant difference between the two strategies, so it is likely that labile carbon in our study behaved similarly to WSA and decreased over time rather than immediately following the first tillage event.

The 2006 study by Duiker and Beegle did not find a difference in soil organic matter between chisel disk and no-till strategies in a corn rotation—the study also included moldboard plow tillage treatment in the comparison and the inclusion of this much higher disturbance treatment may have made the smaller differences between chisel disk and no-till insignificant. However, in our shorter-term study without a moldboard plow treatment, the negative impact of chisel disking on previously no-till soil was significant. Additionally, the study by Duiker and Beegle measured soil organic matter, which though found to be highly correlated with aggregate stability (McVay et al., 2006), is not a direct measurement of soil structure. Our findings also highlight the importance of regular sampling as there were no differences after only three years, but there were significant differences between the CD-BM strategy and the three no-till strategies after five years. These results support Hypothesis (3) that there would be no significant differences in indicators between the three no-till strategies and (4) that water stable aggregates would be reduced as a result of tillage, though it did take more than three years for a significant reduction to occur.

Conclusions

All four of the soil health indicators analyzed showed decreased soil health in the CD-BM strategy compared to the three no-till strategies at the 0-5 cm depth (or 0-15 cm for WSA), with the exception of the TOC by volume where only the CD-BM and NT-SF strategies were significantly different. Hypothesis (1) was not supported as bulk density did not differ between strategies and therefore was not decreased as a result of chisel disking. However, our findings did support Hypotheses (2) and (4) that chisel disking broadcast manure as a fertilizer application strategy would reduce soil health compared to no-till strategies, though Hypothesis (2) was only partially supported as the CD-BM strategy did not increase soil carbon at the 5-15 cm depth.

Additionally, when only the no-till strategies were analyzed, there were no differences among the three methods of nutrient management. This supports Hypothesis (3) that the three types of nutrient management would not differ, though only partially as this hypothesis also expected that there would be an increase in soil carbon at the 5-15 cm depth in the NT-IM strategy which was not observed. Overall, the chisel disk tillage negatively affected soil health as expected, though no positive benefit to soil carbon was observed as a result of chisel-disk at the 5-15 cm depth. The lack of significant differences in any soil health indicators between the three no-till practices, indicated that, in our study system, the choice of nutrient amendment did not have a significant impact on soil carbon or structure after 5 years.

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Figures

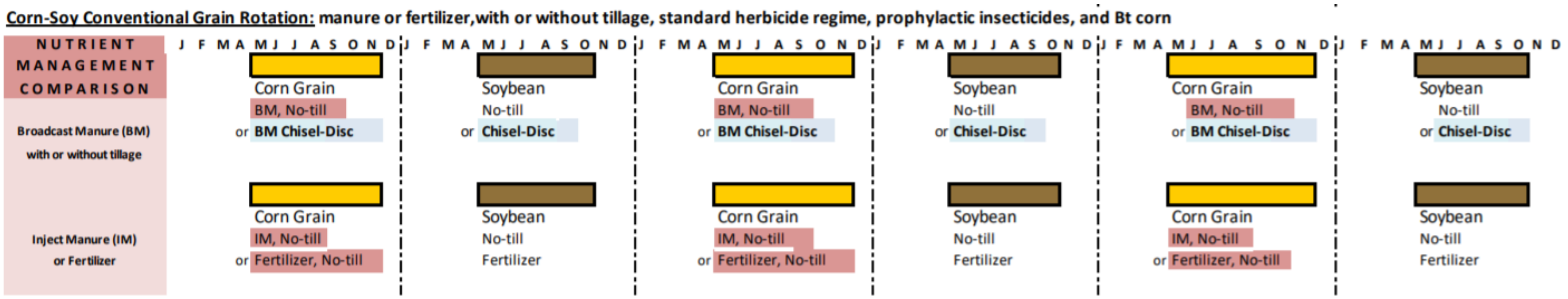
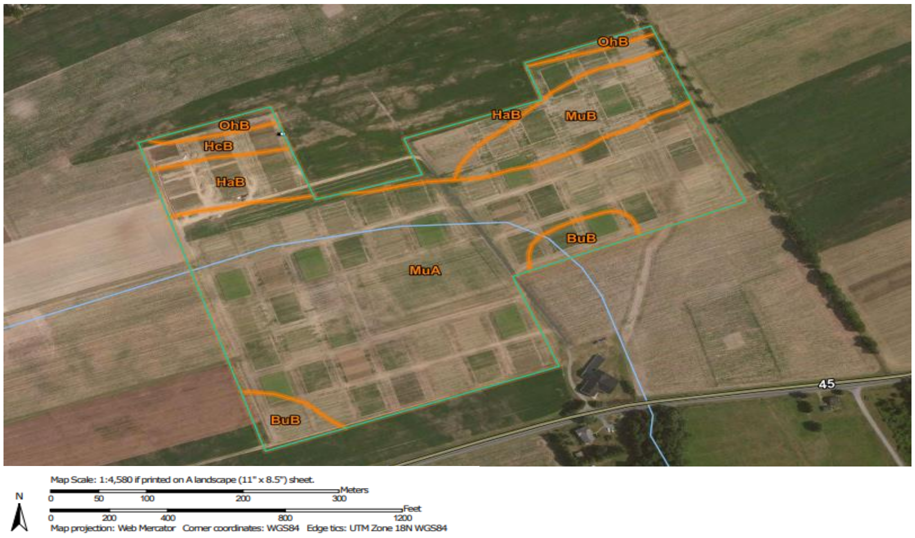


Figure 3.1 Rotation sequence and management for a conventional corn grain-soybean rotation with broadcast manure with or without tillage and injected manure or fertilizer as split-split plots.



MuA: Murrill channery silt loam, 0 to 3 percent slopes

HaB: Hagerstown silt loam, 3 to 8 percent slopes

MuB: Murrill channery silt loam, 3 to 8 percent slopes

BuB: Buchanan channery loam, 3 to 8 percent slopes

HcB: Hagerstown silty clay loam, 3 to 8 percent slopes

OhB: Opequon-Hagerstown complex, 3 to 8 percent slopes

Figure 3.2 Soil map (NRCS SoilWeb) of the study site showing the soil types present. The primary soil type is the Murrill soil series.

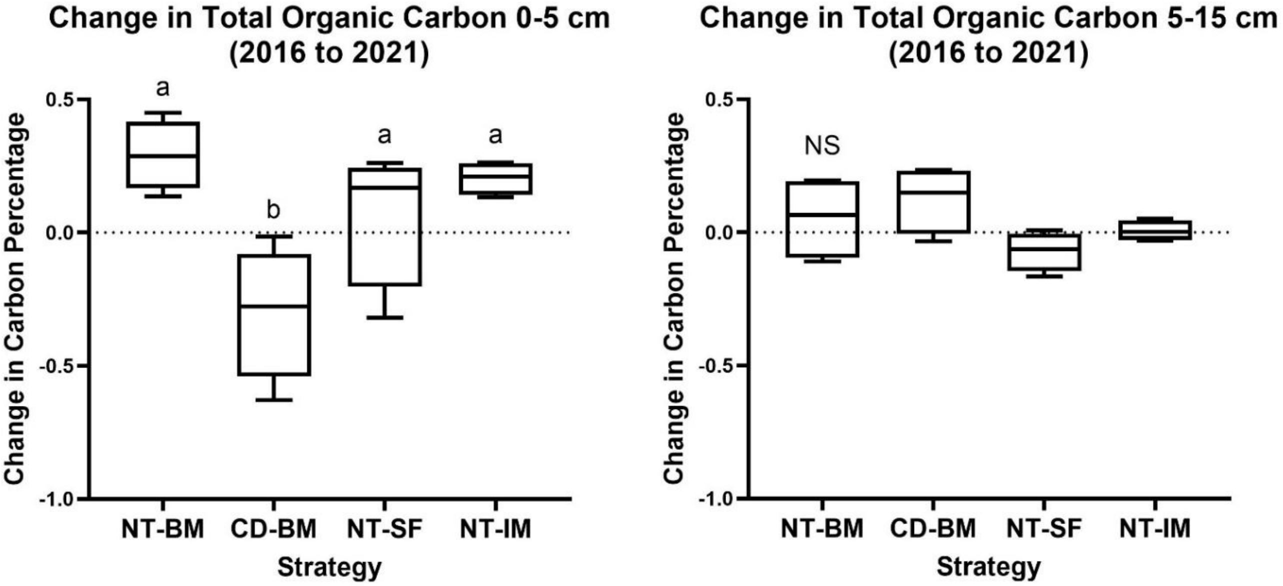


Figure 3.3 The difference between TOC percentage at the 0-5 cm depth (left graph) and 5-15 cm depth (right graph) in 2021 and 2016. Positive values represent an increase in TOC between 2016 and 2021 and negative values represent a decrease in TOC between 2016 and 2021. TOC percentage in the CD-BM strategy was significantly reduced compared to the NT-BM, NT-IM, and NT-SF strategies at the 0-5 cm depth. There were no differences between strategies at the 5-15 cm depth. Different letters indicate strategies differed significantly at $p<0.05$ via Tukey's HSD test.

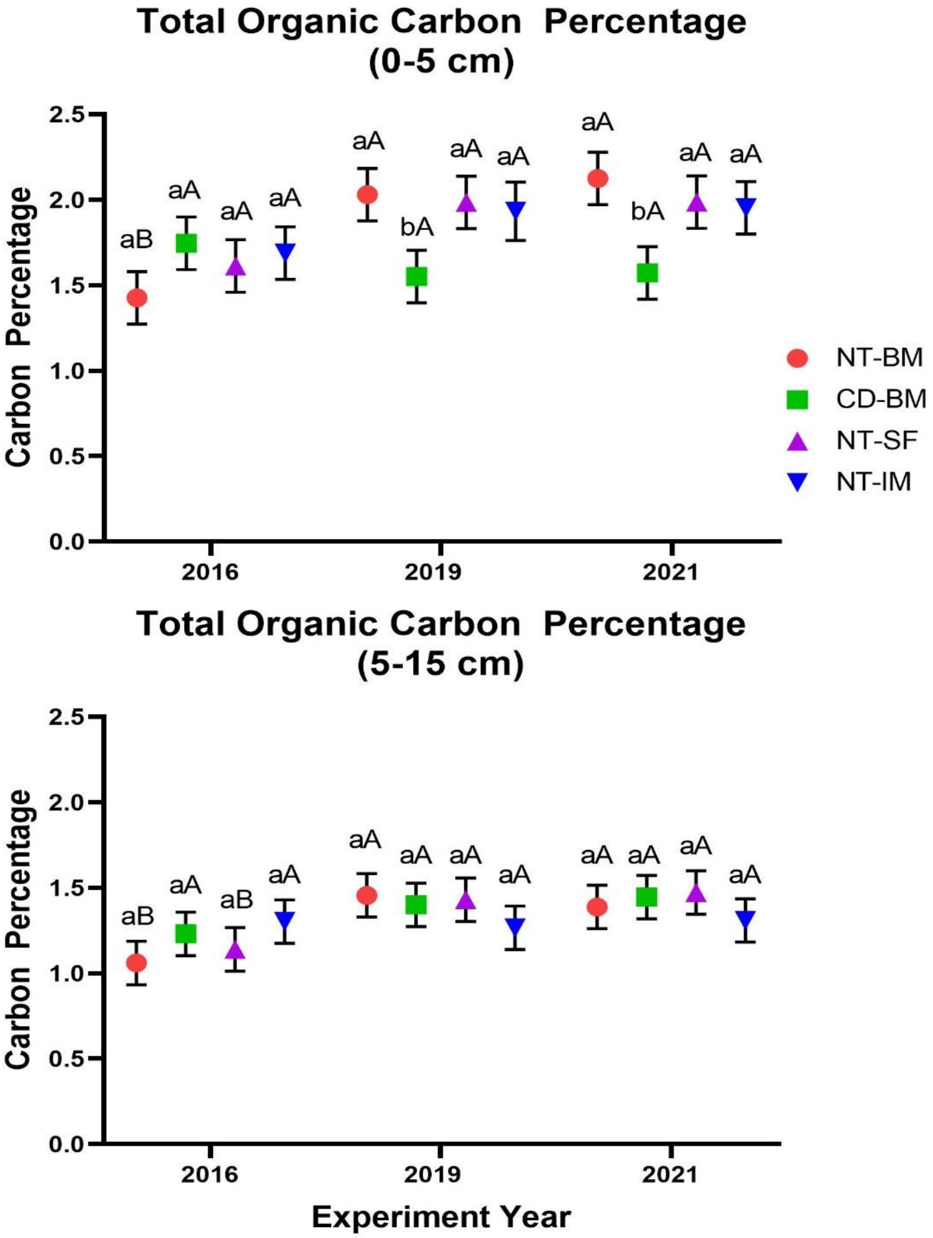


Figure 3.4 Total organic carbon percentage by year at 0-5 cm (top) and 5-15 cm (bottom). In 2016 there were no differences between strategies at either depth. In 2019 and 2021, TOC in the CD-BM strategy was significantly reduced compared to the NT-IM, NT-BM, and NT-SF strategies. Within the NT-BM strategy, TOC increased significantly between 2016 and 2019 at both depths and increased between 2016 and 2021 within the NT-SF strategy at 5-15 cm. Lowercase letters (a, b) denote strategies that differ at $p < .05$ within the same year via the SLICE procedure. Uppercase letters (A, B) denote years that differ at $p < .05$ within a strategy via the SLICE procedure.

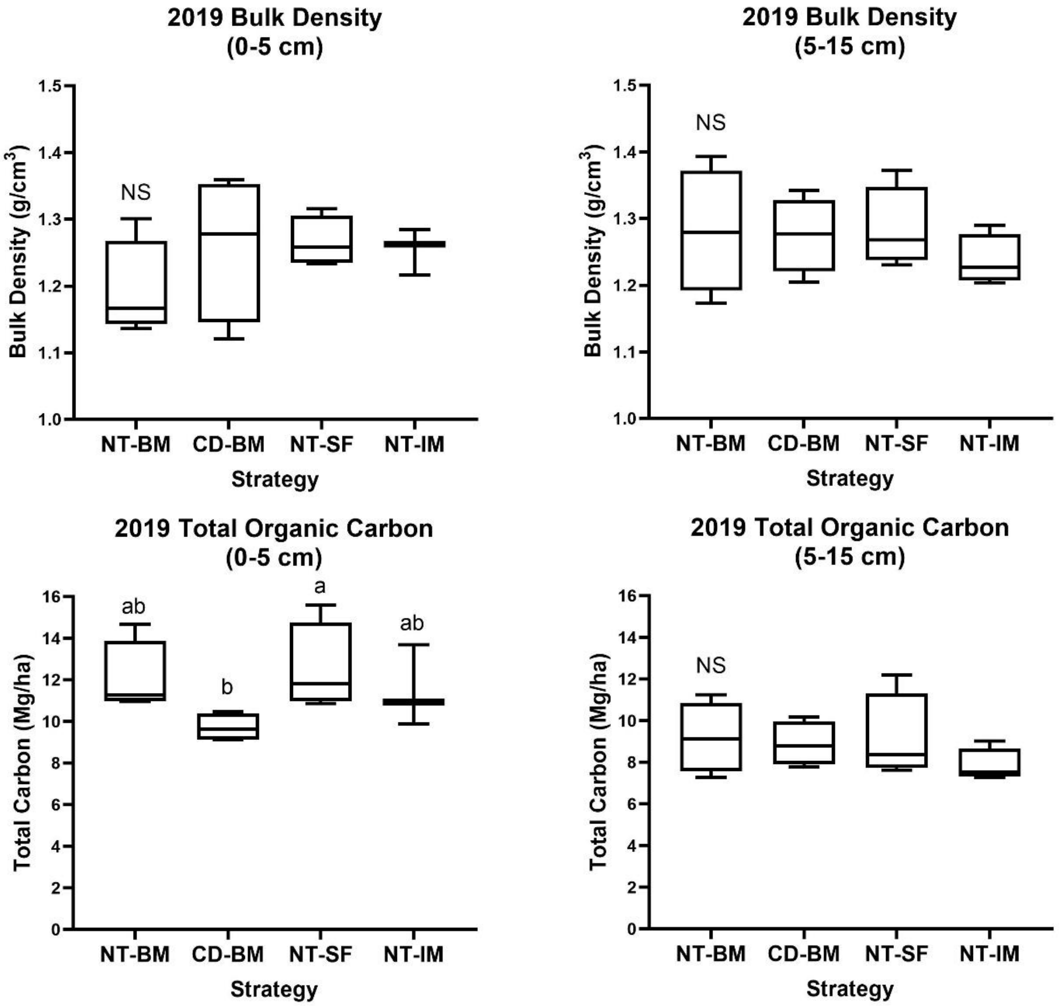


Figure 3.5 Bulk density at the 0-5 cm depth (top left) and 5-15 cm graph (top right) and TOC by volume at the 0-5 cm depth (bottom left) and 5-15 cm depth (bottom right) in 2019. There were no significant differences in bulk density between any of the strategies. TOC by volume in the CD-BM strategy was significantly reduced compared to the NT-SF strategy at the 0-5 cm depth. The difference between the NT-BM and NT-IM strategies and the CD-BM and NT-SF strategies were not significant at 0-5 cm. There were no differences between strategies at the 5-15 cm depth. Different letters indicate strategies differed significantly at $p<0.05$ via Tukey's HSD test.

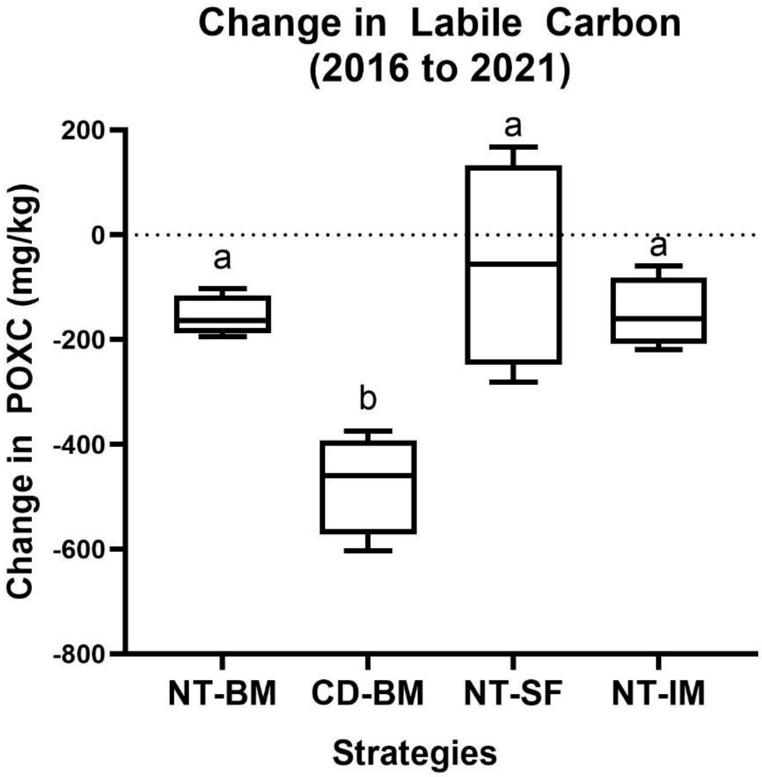
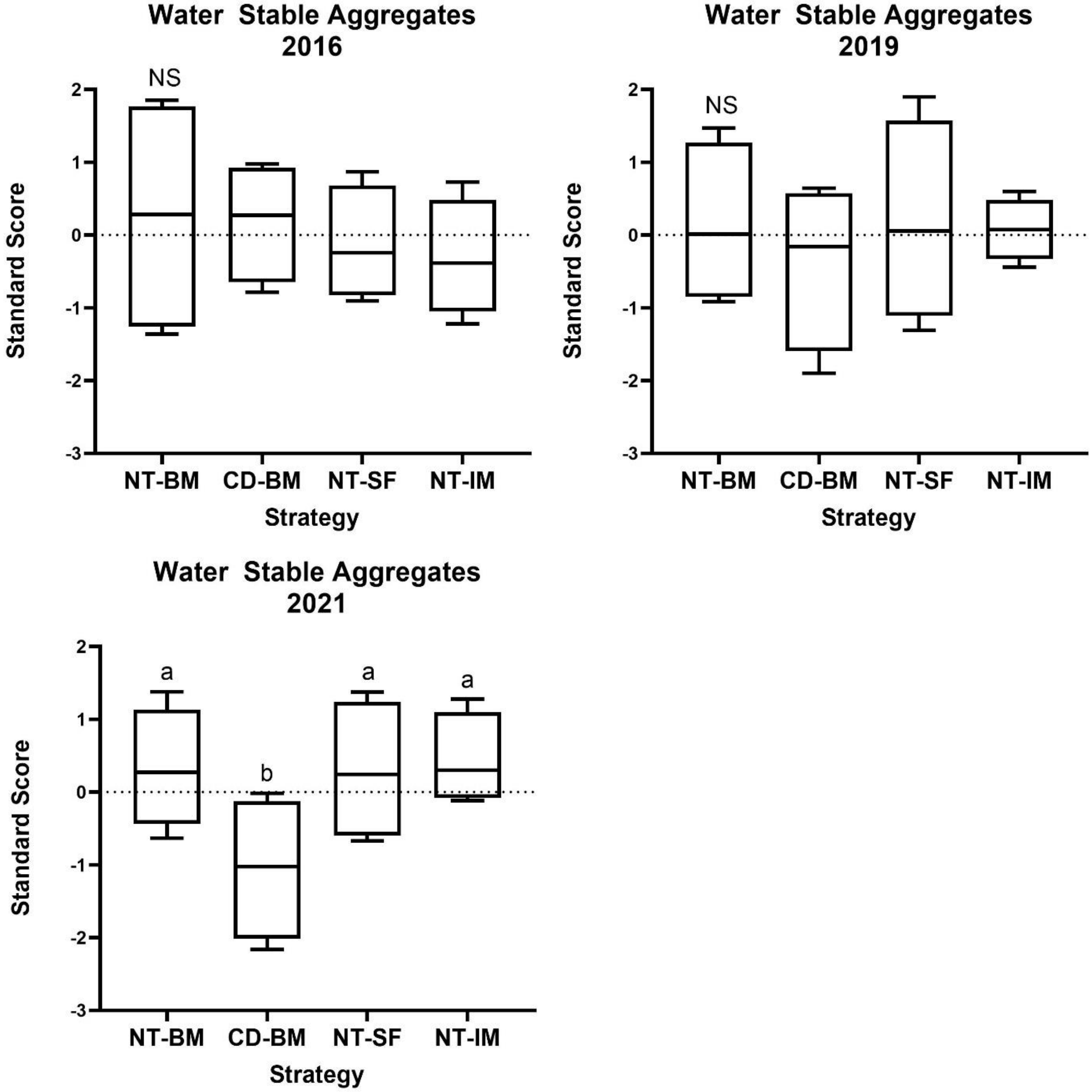


Figure 3.6 The difference between POXC measurement (mg/kg) in 2021 and 2016. Positive values represent an increase in labile carbon between 2016 and 2021 and negative values represent a decrease in labile carbon between 2016 and 2021. Labile C in the CD-BM strategy was significantly reduced compared to the NT-SF, -NT-IM, and NT-BM strategies. Different letters indicate strategies differed significantly at $p<0.05$ via Tukey’s HSD test.


Water Stable Aggregates 2016

Water Stable Aggregates 2019

Water Stable Aggregates 2021

Figure 3.7 The water stable aggregate standard scores, calculated using the means and standard deviation of within-year scores, plotted by strategy each year. High standard score equates to higher aggregate stability—positive scores are higher than the year's mean, negative scores are lower than the year's mean. In 2016 (top left) and 2019 (top right), there were no differences between strategies. In 2021 (bottom left), WSA in the CD-BM strategy were significantly reduced compared to the NT-IM, NT-BM, and NT-SF strategy. Different letters indicate strategies differed significantly at $p<0.05$ via Tukey's HSD test.

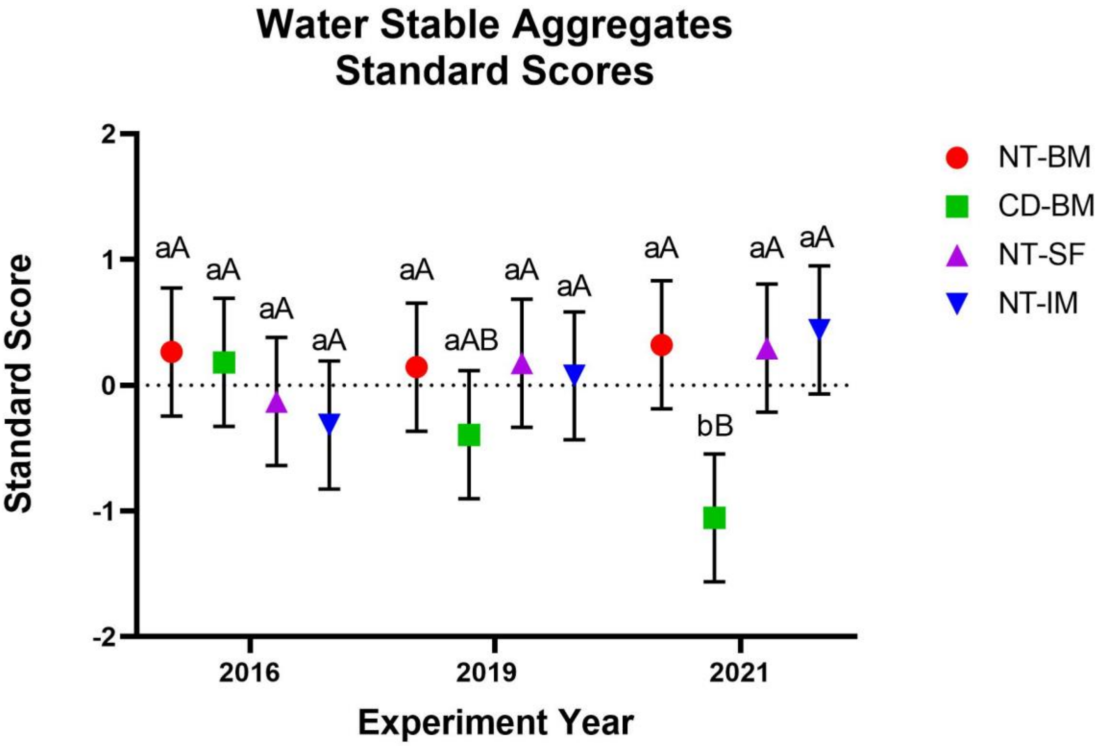


Figure 3.8 The water stable aggregate standard scores, calculated using the means and standard deviation of within-year scores, plotted by year. High standard score equates to higher aggregate stability—positive scores are higher than the year's mean, negative scores are lower than the year's mean. In 2016 and 2019, there were no differences between strategies. In 2021, WSA in the CD-BM strategy were significantly reduced compared to the NT-IM, NT-BM, and NT-SF strategy. Within the CD-BM strategy, WSA was significantly reduced between 2016 and 2021. Lowercase letters (a, b) denote strategies that differ at $p < .05$ within the same year via the SLICE procedure. Uppercase letters (A, B) denote years that differ at $p < .05$ within a strategy via the SLICE procedure.

Tables

Table 3.1 The change in POXC at 0-5 cm and TOC percentage at 0-5 and 5-15 cm depths between 2016 and 2021 for the NT-BM, CD-BM, NT-SF, and NT-IM strategies. Significant differences between strategies were determined by the Tukey's HSD test in JMP.

	TOC % 0-5 cm	**TOC % 5-15 cm**	**POXC**
BM	0.29a	0.05a	-156a
Ch	-0.3b	0.13a	-475a
Fert	0.07a	-0.07a	-57b
IM	0.20a	0.006a	-150a

[a]Different lowercase letters (a, b) indicate that strategies differ at p < .05 via Tukey's HSD test

Table 3.2 Water stable aggregate average standard scores at 0-15 cm and average TOC percentage at 0-5 and 5-15 cm depths for the NT-BM, CD-BM, NT-SF, and NT-IM strategies in 2016, 2019, and 2021. Significant differences of the main effects of strategy and year were determined by the SLICE function in JMP to conduct a partitioned F-test of LSMeans of the interaction of Strategy x Year.

	TOC % (0-5 cm)	**TOC % (5-15 cm)**	**WSA Standard Score**
Strategy x Year			
BM, 2016	1.43a[a]B[b]	1.06aB	0.26aA
Ch, 2016	1.74aA	1.23aA	0.18aA
Fert, 2016	1.61aA	1.14aB	-0.13aA
IM, 2016	1.69aA	1.30aA	-0.32aA
BM, 2019	2.03aA	1.46aA	0.14aA
Ch, 2019	1.55bA	1.40aA	-0.39aAB
Fert, 2019	1.98aA	1.43aA	0.17aA
IM, 2019	1.93aA	1.27aA	0.07aA
BM, 2021	2.12aA	1.39aA	0.32aA
Ch, 2021	1.57bA	1.45aA	-1.05bB
Fert, 2021	1.99aA	1.47aA	0.29aA
IM, 2021	1.95aA	1.31aA	0.44aA
Factor			
Strategy	*	NS	NS
Year	NS[†]	*	NS
Strat x Year	*	NS	NS
SLICE tests			
Effect of Strategy			
2016 BM x 2016 Ch	NS	NS	NS
2016 Fert x 2016 Ch	NS	NS	NS
2016 IM x 2016 Ch	NS	NS	NS
2019 BM x 2019 Ch	**	NS	NS
2019 Fert x 2019 Ch	*	NS	NS
2019 IM x 2019 Ch	*	NS	NS
2021 BM x 2021 Ch	**	NS	**
2021 Fert x 2021 Ch	*	NS	**
2021 IM x 2021 Ch	*	NS	**
Effect of Year			
2016 Ch x 2019 Ch	NS	NS	NS
2016 Ch x 2021 Ch	NS	NS	**
2019 Ch x 2021 Ch	NS	NS	NS
2016 BM x 2019 BM	**	**	NS
2016 BM x 2021 BM	**	*	NS
2019 BM x 2021 BM	NS	NS	NS

2016 Fert x 2019 Fert	NS	*	NS
2016 Fert x 2021 Fert	NS	*	NS
2019 Fert x 2021 Fert	NS	NS	NS
2016 IM x 2019 IM	NS	NS	NS
2016 IM x 2021 IM	NS	NS	NS
2019 IM x 2021 IM	NS	NS	NS

[a] Lowercase letters (a, b) denote strategies that differ at $p < .05$ within the same year via the SLICE procedure

[b] Uppercase letters (A, B) denote years that differ at $p < .05$ within a strategy via the SLICE procedure.

*Significant at the .05 probability level.

**Significant at the .01 probability level.

[†] NS, nonsignificant.

Table 3.3 Bulk density and TOC by volume at 0-5 and 5-15 cm depths in 2019 for the NT-BM, CD-BM, NT-SF, and NT-IM strategies. Significant differences between strategies were determined by the Tukey's HSD test in JMP.

	BD 0-5cm (g/cm^3)	**TOC 0-5 cm (Mg/ha)**	**BD 5-15cm (g/cm^3)**	**TOC 5-15cm (Mg/ha)**
BM	1.19a[a]	12.0ab	1.28a	9.2a
Ch	1.26a	9.7b	1.28a	8.9a
Fert	1.27a	12.5a	1.28a	9.1a
IM	1.27a	12.1ab	1.24a	7.8a

[a] Different lowercase letters (a, b) indicate that strategies differ at $p < .05$ via Tukey's HSD test

Table 3.4 Water stable aggregate average percentages and standard scores for the NT-BM, CD-BM, NT-SF, and NT-IM strategies in 2016, 2019, and 2021. Significant differences between strategies were determined by the Tukey's HSD test in JMP.

	2016	**WSA**	**2019**	**WSA**	**2021**	**WSA**
	%	**Standard Score**	**%**	**Standard Score**	**%**	**Standard Score**
NT-BM	36.3a[a]	0.26a	55.7a	0.14a	48.4a	0.32a
CD-BM	35.7a	0.18a	50.5a	-0.39a	34.0b	-1.05b
NT-SF	33.6a	-0.13a	60.0a	0.18a	48.2a	0.29a
NT-IM	32.3a	-0.31a	55.0a	0.07a	49.7a	0.44a

[a] Different lowercase letters (a, b) indicate that strategies differ at $p < .05$ via Tukey's HSD test

Chapter 4

Conclusions and Recommendations

This thesis research was conducted in response to growers' concerns that occasional soil disturbance, which can be beneficial for such purposes as herbicide reduction or nutrient re-distribution, will be detrimental to soil health. The first experiment described in Chapter Two of this thesis documented the impacts of one-time tillage as an alternative to herbicide use as the method of perennial termination. In this management system employing a six-year rotation, the immediate impacts of tillage were observed by a reduction in total organic carbon by percentage and by volume at 0-5 cm and labile carbon at 0-5 cm in the year following tillage. Water stable aggregates and bulk density did not show differences following tillage, and significantly improved between the canola crop entry, the year directly following tillage and the third-year alfalfa-orchardgrass crop entry, five years following tillage and after two years of perennials. The benefits of a diverse system with cover crops and perennials in rotation were evidenced by a lack of significant difference between the T1/6 and NT systems in any of the indicators surveyed following two full years in perennials. Although no effects of tillage were observed in the soil carbon at the 5-15 cm range, because the plow depth is closer to 30 cm, it is possible that some of the carbon lost from the 0-5 cm range following tillage, is redistributed within the 15-30 cm range and warrants further investigation in future studies.

The second experiment, documented in Chapter Three of this thesis, focused on a traditional corn-soy rotation which lacks the system diversity explored in the first experiment but is more representative of crop rotations commonly found on cash crop Pennsylvania farms. Four fertilizer application strategies, which coupled tillage and fertilizer types, were examined. The CD-BM strategy performed as expected, showing significantly reduced soil health in all indicators surveyed compared to the three no-till strategies at the 0-5 cm depth (or 0-15 cm for WSA), except in the case of the TOC by volume where only the NT-SF strategy differed significantly from the CD-BM. There were no differences attributed to tillage at the 5-15 cm depth in the soil carbon measurements. Though mostly in line with our expectations, the three different fertility practices did not behave differently when coupled with no-till, indicating that tillage practice and not fertilizer type is the primary driver of differences in our experiment. There was no increase to soil carbon in the NT-IM strategy, which shows that contrary to our expectations, injected manure does not appear to add soil carbon at deeper depths compared to broadcast manure or synthetic fertilizer. There were no clear advantages or disadvantages to injected manure over other fertilizer application strategies in terms of soil carbon or aggregate stability, however its other benefits, acknowledged in other studies, may still encourage its use.

This project is of particular relevance to Pennsylvania farmers as the system replicates a dairy farm at 1/20th of the scale and uses manure from a neighboring farm for its manure application. Pennsylvania plays a major role in global agriculture, the Pennsylvania Department of Agriculture (2019) calling its farmers worldwide leaders in agriculture and naming dairy production as one of the fastest growing sectors in Pennsylvania. According to the most recent data from the Center for Dairy Excellence (2016), Pennsylvania represents 15.9% of all dairy farms in the United States which equates to about 6,200 farms and the demand for raw and organic milk in the region is increasing. The recommendations generated by this project could be influential in the decision making of a very important region for dairy production.

Based on our findings in chapter two, we recommend that one-time tillage can be used in diverse rotations, particularly when two or more years of perennials are included, as a means to reduce herbicide use. Our findings indicate that one-time tillage does not negatively affect the benefits derived from no-till, but can be used to mitigate problems such as herbicide reliance and resistance. The tillage type used for the one-time tillage event in our experiment was full inversion moldboard plow, which is on the extreme end of soil disturbance. Because this type of tillage is considered necessary for terminating perennial crops, it was chosen for our study, but if farmers are using one-time tillage for incorporation of manure or mixing of stratified nutrients, a less invasive form of tillage may be possible and would likely have even less impact on soil health.

Our findings in chapter three do not encourage the use of chisel disk in a traditional corn-soy rotation. There were clear detrimental impacts to the soil over time when using chisel disk yearly in this type of rotation. However, it did appear to take several years for soil structure to be negatively impacted and soil carbon by volume was only significantly different in the CD-BM from one of the no-till strategies. Therefore, if chisel disk is used only occasionally to mix stratified nutrients, the detrimental effects to soil health may be negligible, though this possibility should be investigated further. Our findings did not find any differences in soil carbon or water stable aggregates based on the type of fertilizer applied and therefore we cannot make a recommendation as to which type of fertilizer application strategy from the three no-till strategies is most effective at managing soil health, which may be positive news to farmers who prefer a certain application strategy.

For future investigations of soil health in this experimental system or similar studies, we recommend that the metric of soil carbon by volume continue to be considered when long term data is available, particularly if the study is aiming to provide information on carbon sequestration over time, which we expect to be a growing aspect of agricultural studies. Additionally, we would recommend that future studies consider a more microbially mediated soil biological health indicator, such as soil respiration, as the impact of disturbance on microbial populations may not be as clear in soil carbon pools as a more direct measure (Lehmann et al., 2020).

As the dairy cropping system studied is now funded by the USDA Agricultural Research Service's Long-Term Agro-Ecosystem Research (LTAR) program, we hope these recommendations will be useful towards advancing the LTAR goals of understanding how agro-ecosystems function at multiple scales as well as maximizing the return for producers while minimizing the damage to the surrounding ecosystem (Walbridge and Shafer, 2011). Overall, our findings imply that frequent reduced-tillage events in a rotation lacking diversity have more severe and lasting impacts on soil health than a single, severe disturbance event properly mitigated with cover crops and perennials. These findings should encourage no-till farmers to experiment with occasional disturbance in managing their fields, especially if herbicide resistance or overuse is a concern. Additionally, we hope that our findings inspire future LTAR studies to consider the effects of disturbance in the context of other management practices such as perennial crops in rotation and fertilizer application strategies.

Caveats and limitations

Although the findings of this study have potential implications for management in the important dairy regions of Pennsylvania, it is important to note that these recommendations are limited by soil type, climate, and geography. As emphasized by Sprunger et al. (2021), soil texture plays a large role in soils' response to management—our study was conducted on very deep, well drained fine-loamy, mixed, semiactive, mesic Typic Hapludults soils and the applicability of our findings may vary in finer or coarser soils. Additionally, the soil health indicators selected for this study focus on soil carbon and stability. Though soil carbon is highly correlated with soil microbial activity (Culman et al., 2012), our study did not include any direct measures of microbial activity and our recommendations are limited to the effects of management on soil carbon dynamics and aggregate stability.

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