IMPACTS OF LONG-TERM MANAGEMENT PRACTICES ON SOIL HEALTH IN CENTRAL MISSOURI

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Dylan J. Sutton

Morgan P. Davis & Kristen S. Veum

Thesis Supervisors

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The undersigned, appointed by the Dean of the Graduate School, have examined

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Presented by

Dylan J. Sutton

A Candidate for the Degree of

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And hereby certify that, in their opinion, it is worthy of acceptance.

Dr. Morgan P. Davis

Dr. Kristen S. Veum

Dr. Kerry M. Clark

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Abstract

Soil health, defined as the capacity of a soil to function as a vital living system, is critical for agricultural sustainability, especially in inherently vulnerable soils. Historical land use and agricultural intensification have led to significant soil degradation across the U.S., particularly in the Central Claypan Region of Missouri. This thesis investigates the role of alternative cropping systems in maintaining or improving soil health within this region. Alternative cropping systems relative to annual corn-soybean practices emphasize the four soil health principles: maximizing soil cover, minimizing disturbance, maintaining continuous living roots, and diversifying plant species. Two long-term experiments at the Soil Productivity Assessment for Renewable Energy and Conservation (SPARC) field and three sites within the USDA-ARS's Long-Term Agroecosystem Research (LTAR) Network in Central Missouri served as the basis for evaluating soil health responses among varying land management practices. Given the inherent vulnerability of soils in this region, maintaining or improving soil health is inherent to long-term agricultural sustainability, productivity, and security. Both studies evaluate the differences of soil health indicators between alternative cropping systems and annual corn-soybean rotations. The first study compared the use of perennial biofuel crops with an annual corn-soybean rotation on varying depth to claypan, with results showing that perennials have a greater potential to have soil health improvements on shallow soils compared to a corn-soybean rotation. The second study compared a business-as-usual tilled annual corn-soybean rotation without the use of cover-crops to a no-till aspirational field with a complex crop rotation using cover-crops, along with a remnant prairie serving as a reference state of soil health in the area. Results of this study showed that there were no differences in soil organic carbon or total nitrogen, but differences did exist in more complex soil health indicators. Results of these studies show the effects of varying management practices on soil health indicators in the Central Claypan Area. These results can be applied to fit a wide range of scenarios for farmers in the region, applicable to aspiring perennial biofuel growers, and to corn-soybean growers concerned about the long-term sustainability of their soil health.

1. Background and Literature Review

In the US, soil quality has been a topic of interest for farmers and researchers for about a century, while the concept of soil health as we know it today only gained significant traction in the 1990s. Soil health has many definitions but is most wholistically defined as the capacity of soil to function as a vital living system, within ecosystem and land-use boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and promote plant and animal health (Doran & Zeiss, 2000). Anthropogenic activities have degraded nearly 40% of the world's agricultural land, with leading causes including soil erosion, atmospheric pollution, extensive soil cultivation, over-grazing, land clearing, salinization, and desertification (Oldeman, 1994; Lal, 2001; Blaikie & Brookfield, 2015). These quantifications of loss have given urgency to movements in sustainable agriculture and regenerative agriculture within the US and across the world to try and maintain or even recover soil fertility around the world (Intergovernmental Panel on Climate Change [IPCC], 2019). Global efforts in sustainability lag behind degradation from land use change. In 2018, the European Union's Joint Research Center estimated that 75% of all land in the world is now degraded and is expected to rise to 90% by 2050 if no change is made to our agricultural systems (European Commission, Joint Research Centre, 2018).

The southwestern Great Plains region of the US was once a semi-arid grassland. When Congress passed the Homestead Act in 1862, thousands of settlers migrated from the eastern U.S. and began farming and raising cattle on the land. Grassland ecosystems were tilled primarily for wheat production. When the drought of the 1930s began, the dry, bare soil began to blow away (Schubert et al., 2004). Farmers had over-tilled the soil to maximize crop yields, destroying the organic matter which held the soil together (Lee & Gill, 2015). The absence of cover crops along with over-tillage caused what we know today as the Dust Bowl. While this was a tragedy, some positive change came from it. The event sparked more development of soil conservation practices such as contour plowing, strip cropping, and increased implementation of cover crops. It also aided in establishing governmental conservation programs such as the Soil Conservation Service, now the Natural Resources Conservation Service (NRCS) in 1935, and the Shelterbelts Program of 1934 to plant trees and shrubs in the Great Plains to prevent soil erosion and dust storms. Many more advancements in soil science were spurred by the Dust Bowl, specifically research into soil properties, erosion processes, and sustainable farming methods.

The Dust Bowl led into what is known as the Green Revolution (1940s-1960s), which focused on increasing agricultural productivity. It brought widespread adoption of new ideas, such as synthetic nitrogen, phosphorus, and potassium fertilizers to enhance crop yields. Because of this, researchers gained more insight into nutrient cycling and soil chemistry (Lodge, 1951; Bennett et al., 1953). While this period was a time of innovation, it brought many challenges and cause for concern. One of which is the transition to synthetic fertilizers, reducing acres in complex crop rotations that improved soil health. Tractor use and increased mechanization caused soil compaction, limiting water infiltration and root growth (Shah et al., 2017). In the 1970s, US grain was in high demand globally, and in 1973, President Nixon’s Secretary of Agriculture called for farmers to plant “fencerow to fencerow”, further increasing agricultural intensification. This also led to an increase in monocropping, depleting soil nutrients (Gebru, 2015). The impacts of the Dust Bowl and the Green Revolution both brought change to the agriculture industry and led to a greater knowledge and goal of sustainable agriculture in the US.

While sustainable agriculture is a general description that can have many management strategies, its purpose is clear. It is a site-specific approach that attempts to meet human production needs, enhance environmental quality, make efficient use of resources using natural biological cycles, while also maintaining economic viability (USDA, n.d.). The future of human civilization and advancement relies on the premise that we will continue to be able to feed the growing population of the world. To achieve this, there is still a lot of work to be done in successfully conserving our soil health for future generations to be able to have the same opportunities that we have currently.

Conserving soil health comes with a unique set of principles, which can be applied to most systems. The five core principles of soil health are to maximize soil cover, minimize soil disturbance, maintain continuous living roots, diversify plant species, and the integration of livestock. For the studies in this thesis, we will only focus on the first four principles of soil health. Soil cover, or soil armor, can be maximized in an agricultural site by leaving residue on the field after harvesting. This can have many benefits to soil health, such as increased soil water content, soil porosity, aggregate stability, organic carbon, and more (Fu et al., 2021). Soil cover can also be maximized by utilizing cover crops, which can improve soil fertility, prevent soil erosion, and enhance nutrient and water availability (Sharma et al., 2018). Soil disturbance is minimized from reducing or removing tillage out of the system, which reduces carbon mineralization, increases soil health, and can even improve crop yields (Nunes et al., 2018;

Wulanningtyas et al., 2021). Cover crops can also be applied to the next two principles of soil health, which is maintaining continuous living roots, and diversifying plant species. Diversification of plant species can improve soil health via increased nutrient availability, aggregate stability, organic matter, and more (Congreves et al., 2015; Agomoh et al., 2021). These outlined principles demonstrate the importance of soil health in maximizing the physical, chemical, and biological components that make up healthy soil. While these soil health principles can be applied broadly, their relevance becomes especially critical in regions with inherently vulnerable soils, such as the Central Claypan Area of Missouri.

The Central Claypan Area, which is the host of this thesis' research sites, is located in the Dissected Till Plains of Missouri and classified as Major Land Resource Area (MLRA) 113. The soil in this region is loess-dominated with deep Alfisols and high subsoil clay content (NRCS, 2022). Conventional row crop practices have resulted in degraded soil and water resources (USDA, 1981) due in part to high runoff and erosion potential of these soils (Lerch and Blanchard, 2003). Erosion directly results in loss of topsoil and removes nutrients and organic matter while negatively impacting productivity (Moraru & Rusu, 2010; Kimble et al., 2001) and surface runoff water quality (Lerch et al., 2005). Additionally, soil disturbance from tillage increases susceptibility to erosion and mineralization of soil organic matter (Morris et al., 2004).

In soil with shallow topsoil, depth to claypan (DTC) is an important factor that constrains the effects of management on soil characteristics and production outcomes. For example, increased DTC is associated with greater plant available water and transpiration which can increase plant biomass and grain yield (Schreiner-McGraw & Baffaut, 2023). As soil erosion continues to strip topsoil across the Central Claypan Area, shallow soils are becoming more common (Baffaut et al., 2020; Thaler et al., 2021) leading to increased susceptibility to drought due to less water holding capacity and increased runoff (Gautam et al., 2021).

In 2008, with oil prices skyrocketing, part of the Food, Conservation, and Energy Act outlined the creation of the Biomass Crop Assistance Program to support the establishment and production of biomass crops. The major downside in the economic viability of biofuel crops is that they are mostly dependent on oil prices (Lipsky, 2008). Biofuel markets are often inversely related to oil prices, and the lack of value of cellulosic feedstocks had led to a lack of widespread development of cellulosic ethanol production facilities. When biofuels are desired again, we are left with the same problems as before, so other incentives such as improving soil health are needed to buffer the economic viability of growing perennial biofuels.

Perennial cropping systems consistently demonstrate greater soil health properties compared with annual row crop systems (Augarten et al., 2023; Ledo et al., 2020; Veum et al., 2015). Improved soil health has been linked to improved yield, but not always consistently (Van Es and Karlen, 2019; Crookston et al., 2022). A long-term study of perennial cropping systems, such as hay, pasture, or perennial biofuel crops, shows that yields can be maintained or even increased over decades (Glover et al., 2010), adding to the incentives of growing perennials on the agricultural landscape. Results of these studies demonstrate the potential for perennial grasses to improve soil health and water quality, while offsetting some impact of climate change by sequestering more carbon into the soil.

Perennial biofuel grass crops such as switchgrass (*Panicum virgatum*) and miscanthus (*Miscanthus x giganteus*) are high yielding and can be grown on marginal land (Lewandowski et al., 2000; Wang et al., 2015; Hudiburg et al., 2016). This is beneficial because farmers likely wouldn't implement perennial biofuel crops on their entire farm, so it can be a win-win for use on low-quality land that doesn't have the return on investment needed to make traditional cropping systems viable. It can also help buffer agricultural runoff into nearby water bodies, especially since marginal land is often the areas with steepest slope and therefore the highest runoff potential. One study showed switchgrass being capable of removing between 28 and 42% of nitrate runoff, and another showed miscanthus removing between 63 and 80% of nitrate runoff (Lee et al., 1998; Ferrarini et al., 2017). Results of these studies show benefits of perennial switchgrass and miscanthus exist outside of biofuel production.

Since 1983, extensive research has been undertaken in northern Europe on miscanthus production as a renewable energy source. Since then, many European programs have been funded to investigate things such as the biomass potential and effective management practices of miscanthus production (Lewandowski et al., 2000). Results of studies have shown that miscanthus has the largest energy yield and highest energy-use efficiency of all potential bioenergy crops (Hastings et al., 2009; Sims et al., 2006). One study even set a societal economic value of miscanthus between roughly $530 and $1850 per acre after converting to USD, largely from the value of raw material, floodplain management, climate regulation, groundwater buffering, nutrient cycling, and erosion prevention (Von Cossel et al., 2020). While miscanthus research in Europe has been relatively extensive, US miscanthus research is still limited in comparison.

The study site of the first study chapter takes place at the University of Missouri South Farm Research Center – Soil Productivity Assessment for Renewable Energy and Conservation (SPARC) field. The study site was set up with the goal of assessing soil productivity across different claypan depths so that results can be applied to fit a wide range of scenarios for farmers in the region. The soil series is a Mexico silt loam (fine, smectitic, mesic, aeric, vertic, epiaqualf) located within the Central Claypan Area (MLRA 113). The site was established in 1982 consisting of 32 artificially constructed blocks with varying DTC. The site was originally established with tilled continuous corn and soybeans lasting for 10 years (Gantzer & McCarty, 1987). The site was then fallowed and annually mowed until 2009 when the site was burned in preparation for the current study. SPARC field's plot design allows for unique research opportunities in scalable agricultural practices by having the ability to match a variety of baseline conditions of farmers in the region. The Mexico silt loam soil series which the site is dominated by is a very common series in Missouri (Miller, 1918). With a common soil type, research here can be easily adapted to fit many farmers' goals. Coupled with plot designs containing varying DTC's, SPARC research can apply to an even greater range of Missouri farms.

Figure 1.1 An aerial photo showing the Soil Productivity Assessment for Renewable Energy and Conservation (SPARC) field used as the study site for Chapter 2 of this thesis.

The second study of this thesis was conducted at one of many USDA-ARS Long-Term Agroecosystem Research (LTAR) sites across the US. The LTAR network contains 18 research sites within the US, covering most of the continental US. The LTAR network was developed with a mission of “Food for the future: Understanding and enhancing the sustainability of agriculture”, with the vision of developing national strategies for the sustainable intensification of agriculture production (USDA-ARS, n.d.). The sites used in this study were located at Tucker Prairie near Kingdom City Missouri and Centralia Missouri, both within the Central Claypan Region (MLRA 113) of the US. LTAR network research is important for the national scale by incorporating datasets to the publicly available USDA data repositories, allowing for collaborative science that can be utilized across the country, to fit different regions due to the number and range of LTAR network sites (Bean et al., 2021).

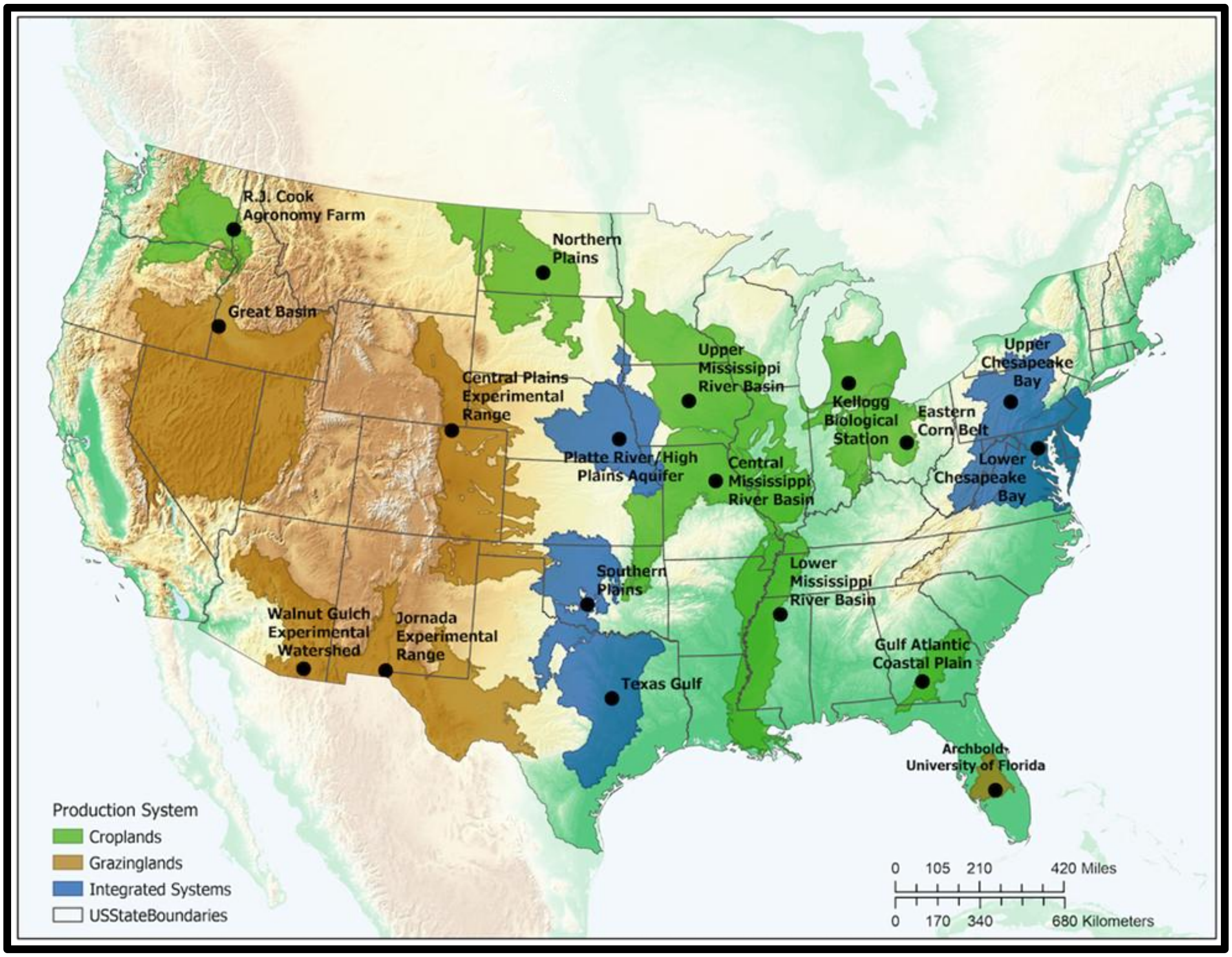

Figure 1.2 A map showing Long-Term Agroecosystem Research (LTAR) Network sites within the United States. The study site used in Chapter 3 of this thesis took place in the Central Mississippi River Basin LTAR site.

Improving or maintaining soil health in the region is extremely important to support the sustainability and security of American agriculture for centuries to come.

With increased responsibility put on the farmer, consumers priorities and governmental policies need to reflect the goals of nationwide sustainability and food security, aid in long-term agricultural solutions, and offer support and increased market value for sustainable practices. This thesis aims to highlight alternative practices to annual corn-soybean rotations in the lens of soil health, which is an important aspect to support future soil productivity, sustainability, and food security.

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2. Soil Health Benefits of Perennial Biofuel Crops on Claypan Soils

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Dylan J. Sutton[1], Kristen Veum[2], Morgan Davis[1], Samuel Lord[1], Curtis Ransom[2], Kenneth Sudduth[2]

[1]University of Missouri, School of Natural Resources, Columbia, MO 65211, USA

[2]USDA-ARS, Cropping Systems and Water Quality Research Unit, Columbia, MO 65211, USA

2.1 Abstract

Claypan depth is one of the most significant drivers in varying soil productivity across different agricultural cropping systems in claypan soils, and the benefits of perennial biofuel cropping systems have been a topic of interest for decades. In general, perennial systems consistently exhibit higher soil health status than annual row crop systems due to greater above and belowground organic inputs and year-round soil cover. In this study, we evaluated the effect of long-term (14 yr) cropping systems (i.e., corn-soybean rotation, switchgrass, and miscanthus) and claypan depth (i.e., < 15 cm, 15-30 cm, and 30+ cm) on soil health indicators at the Soil Productivity Assessment for Renewable Energy and Conservation site located in the Central Claypan Area of Missouri, USA. The cropping systems were sampled at 0-15 cm and a suite of 12 soil health indicators were measured. Analysis of variance models were used to examine the effect of cropping system and claypan depth on soil health indicators. Results showed that switchgrass and miscanthus systems had consistently higher soil health status than the corn-soybean system, with switchgrass demonstrating larger response ratios than miscanthus. Further, differences were enhanced in soils with shallow (< 15 cm) depth to claypan, including soil organic carbon, total nitrogen, acid phosphatase activity, arylsulfatase activity, β-glucosaminidase activity, permanganate oxidizable carbon, soil respiration, total protein, potentially mineralizable nitrogen, and aggregate stability. These results demonstrate the potential soil health benefits of long-term, perennial biofuel cropping systems on shallow claypan soils.

2.2 Introduction

The Central Claypan Area is located in the Dissected Till Plains of Missouri and classified as Major Land Resource Area (MLRA) 113. The soil in this region is loess-dominated with deep Alfisols and high subsoil clay content, and conventional row crop practices in this region have resulted in degraded soil and water resources (USDA, 2022) due in part to high runoff and erosion potential of these soils (Lerch and Blanchard, 2003). Erosion directly results in loss of topsoil and removes nutrients and organic matter, which negatively impacts productivity (Moraru and Rusu, 2010; Kimble et al., 2001) and water quality (Lerch et al., 2005). Additionally, soil disturbance from tillage, a common practice in annual row crop systems, increases susceptibility to erosion and mineralization of soil organic matter (Morris et al., 2004).

In soil with shallow topsoil, depth to claypan (DTC) is an important factor that constrains the effects of management on soil characteristics and impacts production outcomes. For example, increased DTC is associated with greater plant available water and transpiration which can increase plant biomass and grain yield (Schreiner-McGraw & Baffaut, 2023). As soil erosion continues to strip topsoil across the Central Claypan Area, shallow soils are becoming more common (Baffaut et al., 2020; Thaler et al., 2021), leading to increased susceptibility to drought from reduced water holding capacity and increased runoff (Gautam et al., 2021). Long-term studies of perennial cropping systems, such as hay, pasture, or biofuel crops, show that yields can be maintained or even increased over decades (e.g., Glover et al., 2010), and in the Central Claypan Area, a comparison of a corn [*Zea mays L.*] – soybean [*Glycine max (L.)* Merr.] rotation and switchgrass (*Panicum virgatum*) demonstrated that shallow DTC reduced relative yield %, especially during dry years, and increased yield variation, especially for corn. In contrast, switchgrass yield was less variable than corn or soybean yield on shallow (< 15 cm) DTC soil; however, switchgrass was economically better than corn or soybean only on very shallow (< 3 cm) DTC soil (Conway et al., 2017).

As a result of the long-term degradation and loss of productivity in this region, an initiative funded in part by the USDA through the former Missouri Farmers Association (MFA) Oil Biomass venture was implemented in 2002 to support production of perennial biofuel crops (Scrivner et al., 1985, Kitchen et al., 1999, USDA-NRCS, 2006). Soil health is defined as the capacity of soil to function and serve multiple outcomes, including productivity (Doran & Zeiss, 2000). Perennial cropping systems consistently demonstrate higher soil health status compared with annual row crop systems (Augarten et al., 2023; Ledo et al., 2020; Veum et al., 2015), and improved soil health has been linked to improved yield (e.g., Crookston et al., 2022, van Es and Karlen, 2019), but not consistently. Therefore, research is needed to understand the soil health benefits of implementing perennial biofuel systems in the Central Claypan Area.

Soil health characteristics can be categorized as physical, chemical, and biological. Soil physical properties determine how water is partitioned (Cosby et al., 1984), and how resistant soil is to wind and water erosion (Barthès & Roose, 2002). Soil organic carbon (SOC), the primary component of soil organic matter, promotes the microbial environment below ground and indirectly facilitates the uptake of nutrients by plants (Lord, et al., 2024; Ontl, & Schulte, 2012). SOC has also been found to increase water holding capacity, a major contributor to drought resilience and root growth (Ontl & Schulte, 2012). Permanganate oxidizable carbon (POXC), is a carbon fraction that is often sensitive to short and long-term management changes in cropping systems (Weil et al., 2003; Culman et al., 2013), and has been correlated with SOC, microbial biomass carbon (Culman et al., 2012), and various other C and N fractions (Morrow et al., 2016). Soil respiration, a measure of microbial carbon mineralization, is also an early indicator of soil health improvements in agricultural systems (Crookston et al., 2023).

Organic matter is also a source of organic nitrogen that can be converted to plant-available forms. The capacity of soil microorganisms to convert organic nitrogen to plant available forms is measured as potentially mineralizable nitrogen (PMN). Similarly, total protein reflects a labile pool of organically bound nitrogen in the soil and provides insight into the carbon and nitrogen cycling ability of the soil (Hurisso et al., 2018) and has been shown to also be responsive to tillage (Moebius-Clune et al., 2008; Nunes et al., 2020).

Microorganisms also produce a wide array of extracellular enzymes that play important roles in soil nutrient cycling, including β-glucosidase, β-glucosaminidase, phosphatase, and arylsulfatase, that serve as useful soil health indicators (Dick, 2011). Soil pH, an important factor in plant nutrient availability (Alam, S. M., 1999; Barrow & Hartemink, 2023), is also a driver of microbial enzyme activity (Sinsabaugh et al., 2008) and microbial community structure and composition (Zhalnina et al., 2015) in the soil.

Despite extensive research on soil health in annual and perennial cropping systems in the Central Claypan Area (e.g., Veum et al., 2012; 2015), there remains limited understanding of how varying DTC influences the ability to improve soil health on degraded soils. Therefore, the primary objective of this study was to compare soil health under two perennial biofuel crops [switchgrass and miscanthus (*Miscanthus* x *giganteus*)] with an annual corn-soybean rotation at a long-term experimental site with variable DTC located in the Central Claypan Area of Missouri. Understanding the influence of claypan depth on soil health in annual and perennial cropping systems is crucial for developing sustainable agricultural practices that can rebuild and enhance soil health and productivity in this region.

2.3 Methods

Site Description & Sampling

This experiment was conducted at the University of Missouri South Farm Research Center in Columbia, Missouri (38°54′ N, 92°16′ W) on a Mexico silt loam (fine, smectitic, mesic Aeric Vertic Epiaqualf) located within Major Land Resource Area 113 named the Central Claypan Area (USDA-NRCS, 2006). The site was established in 1982 consisting of 32 artificially constructed blocks with varying DTC. The topsoil was scraped from the field and reapplied to create four DTC categories for each block (shallow 4-9 cm, moderate 9-14 cm, deep 18-27 cm, and very deep 28-86 cm). Specific methods for plot construction can be found in Gantzer and McCarty (1987). The site was originally established with tilled continuous corn and soybean lasting for 10 years. The site was then fallowed and annually mowed until 2009 when the site was burned in preparation for the current study. The experimental design consists of 16 randomized complete blocks each containing 4 plots (5.5m wide x 9.4m long) of unique cropping systems (corn-soybean, soybean-corn, switchgrass, and miscanthus). For the purposes of this study and to achieve a balanced design, only the corn phase of the corn-soybean treatments was sampled in each block. Composite soil samples from 0-15 cm were collected totaling 48 samples (3 plots x 16 blocks; n = 48). Three DTC categories were used for this study: 15 (0-15 cm), 30 (15-30 cm), and 30+ (30+ cm), and DTC was confirmed in a three-point transect across each plot. All samples were collected from 0 to 15 cm, reflecting typical the soil health and soil fertility testing sampling depth. Due to the fixed DTC categories, the claypan layer was present in some DTC 15 samples. Samples were then air-dried and sieved (2 mm) for lab analyses.

Lab Analysis

All lab analyses were conducted at the USDA-ARS Soil & Water Quality Laboratory in the Cropping Systems and Water Quality Research Unit in Columbia, Missouri.

Soil organic carbon (SOC) and total nitrogen (TN) were analyzed by dry combustion on a Leco TruMac C/N (St. Joseph, MI). A pre-weighed sample was placed into a ceramic weigh boat and loaded into a purge chamber where atmospheric gasses are removed from the sample and then loaded into the furnace chamber which is at a temperature of 950 °C. After combustion, the gasses were moved into a thermoelectric cooler. For carbon detection, the sample gases are put through an infrared detector and the subsequent gas was sent through copper reagents to remove oxygen and reduce nitrogen species into N_2 gas for detection via thermal conductivity (Nelson & Sommers, 1996).

Permanganate oxidizable carbon was analyzed with 2.5 g air dried, powdered soil being placed in a tube with 18 ml of deionized (DI) water, then 2 ml of 0.2 M potassium permanganate ($KMnO_4$) is added to the tube at pH 7.2 to begin the reaction. The mixture is shaken for 2 minutes at 120 oscillations per minute and then placed under a box for 5 minutes and left to settle. Then, 0.5 ml of the reacted supernatant is pipetted into a separate tube filled with 49.5 ml of DI water to dilute the sample for a spectrophotometry reading at 550nm (Weil et al., 2003).

Soil respiration was analyzed following Moebius-Clune (2016), where 20 g of 2mm sieved, air-dried soil was placed into an aluminum weigh boat inside a mason jar. A 10 ml beaker glued to a three-pronged stand is set on top of the weigh boat containing 9 ml of 0.5 M potassium hydroxide (KOH). Prior to dispensing, the electrical conductivity (EC) of the KOH was measured using an EC meter probe. Once KOH is dispensed into the beaker, 7.5 ml of Ultrapure DI water is added to the soil to wake the microbes up. Immediately following the water addition, the lids were sealed and incubated at room temperature for 4 days. Following incubation, the jar is opened and the KOH is read on the EC meter probe. This process uses the reaction of KOH and CO_2 which lowers the EC of the KOH. Three readings are required to average the EC of each sample.

Autoclaved-citrate extractable total protein was analyzed following Moebius-Clune (2016), where 3 g of air-dried, 2mm sieved soil was added to a 50 ml glass tube. Then, 24 ml of 0.02 M sodium citrate is added to the tubes to dispense soil aggregates and placed on the shaker for 5 minutes at 180 oscillations per minute. Next, the tubes are transferred to a rack and placed into an autoclave on the liquid cycle at 121 °C. When complete, the samples will rest for 5 minutes then be placed on the shaker for 1 minute at 180 oscillations per minute. Then, 1.75 ml of extractant solution is pipetted into a micro-centrifuge tube. When all samples have been transferred, they were placed into the centrifuge at 10,000 rpm for 5 minutes. Next, 1 ml of supernatant is transferred from each tube into a 96 well plate and stored at 4 °C overnight. The 96 well plate block heaters will be preheated to 61.5 °C. A 10 µL aliquot of sample and standard are dispensed into the plates along with 200 µL of bicinchoninic acid (BCA) working reagent dispensed into each well. Next, the plates are transferred to the heater block to incubate for 60 minutes at 61.5 °C. Finally, samples are removed from the block and left to rest for 5 minutes and then read at an absorbance of 560 nm.

β-glucosidase was conducted with 1 g of 2mm sieved soil placed into a 50 ml Erlenmeyer flask. Then, 4 ml of Modified Universal Buffer (MUB) at pH 6 is dispensed into the flask with 1 ml of p-nitrophenyl-β-d-glucoside (PNG) substrate to begin the reaction. The flask is then placed into an incubator at 37 °C for one hour. To stop the reaction, 4 ml of 0.1 tris(hydroxymethyl)aminomethane (THAM) buffer at pH 12 and 1 ml of 0.5 $MCaCl_2$ are dispensed into the flask. After stopping the reaction, the flask is poured through a filter with a vacuum pump. 3 ml of the result is dispensed into a cuvette and read on the spectrophotometer at 405 nm (Dick, 2011).

β-glucosaminidase was measured with 1 g of 2mm sieved soil placed into a 50 ml Erlenmeyer flask. Then, 4 ml of acetate buffer at pH 5.5 is dispensed into the flask with 1 ml of P-NNAG substrate to begin the reaction. The flask is then placed into an incubator at 37 °C for one hour. To stop the reaction, 4 ml of 0.1 tris(hydroxymethyl)aminomethane (THAM) buffer at pH 12 and 1 ml of 0.5 M$CaCl_2$ are dispensed into the flask. After stopping the reaction, the flask is poured through a filter with a vacuum pump. 3 ml of the result is dispensed into a cuvette and read on the spectrophotometer at 405 nm (Dick, 2011).

Phosphatase was measured with 1 g of 2mm sieved soil placed into a 50 ml Erlenmeyer flask. Then, 4 ml of MUB at pH 6.5 is dispensed into the flask with 1 ml of phosphate substrate to begin the reaction. The flask is then placed into an incubator at 37 °C for one hour. To stop the reaction, 4 ml of 0.5 M sodium hydroxide (NaOH) and 1 ml of 0.5 M $CaCl_2$ are dispensed into the flask. After stopping the reaction, the flask is poured through a filter with a vacuum pump. 3 ml of the result is dispensed into a cuvette and read on the spectrophotometer at 405 nm (Dick, 2011).

Arylsulfatase was measured with 1 g of 2mm sieved soil placed into a 50 ml Erlenmeyer flask. Then, 4 ml of acetate buffer at pH 5.8 is dispensed into the flask with 1 ml of p-nitrophenyl sulfate (PNS) substrate to begin the reaction. The flask is then placed into an incubator at 37 °C for one hour. To stop the reaction, 4 ml of 0.5 M sodium hydroxide (NaOH) and 1 ml of 0.5 M $CaCl_2$ are dispensed into the flask. After stopping the reaction, the flask is poured through a filter with a vacuum pump. 3 ml of the result is dispensed into a cuvette and read on the spectrophotometer at 405 nm (Dick, 2011).

Wet aggregate stability was conducted by isolating soil particles from 1mm-2mm using a sieve on top of a Retsch Vibratory Sieve Shaker. The retained soil is then weighed at 3 grams into aluminum foil boats. Next, plastic bowls are filled with 2 liters of DI water with 0.5 mm sieves placed inside. The 3-gram samples are evenly scattered on the sieve and left to soak overnight. On day two, the samples are agitated by raising and lowering the sieve in the bowl 20 times in 40 seconds which drains and refills the sieves. Then, sieves are removed from water bowls, placed on a metal plate, and dried in the oven for 2 hours at 110 °C. Samples are then removed from oven and weighed with sieve + plate + dry sample. Another plastic bowl is filled with 2 liters of DI water and 25 ml of 5% Sodium hexametaphosphate. The 0.5 mm sieves with samples are placed into the bowl one at a time. For each sample, the soil particles are pressed with fingers through the sieve until no more will pass through, leaving only >0.05 mm particles left in the sieve. After all sieves are completed and rinsed, they are dried again in the oven for 2 hours at 110 °C. Next, samples are removed from the oven and weighed sieve + plate + sand. Finally, sieves and plates are wiped clean and removed of any particles left on them and weighed sieve + plate. The other two weights are subtracted from the final weight to get aggregate stability data. (Kellogg, 2014).

Potentially mineralizable nitrogen was conducted with 4 g of air-dried soil placed in a falcon tube. Then 40 ml of 1 M KCl is dispensed into each sample tube and placed on the shaker at 200 oscillations/min for 30 minutes. Once complete, samples are filtered via a vacuum manifold to collect filtrate into culture tubes. After collection occurs, samples are transferred into plastic microtubes and refrigerated for one week or frozen in a -80 °C cryogenic freezer until analysis. The incubation period for the NH_4 extraction portion takes place over 7 days. To begin, we weigh 4.0 g air dried soil into a plastic 50 ml falcon tube. Then 20 ml of DI water is dispensed into the falcon tubes and capped tightly. Once prepared, those samples are incubated at 40 °C for one week. The time and date are recorded to ensure the samples are removed at the same time exactly one week apart. Upon completion of the 7-day incubation period, 20 ml of 2.0 M KCl is dispensed into the falcon tubes and manually shaken. It is then put onto the shaker at 200 oscillations per minute for 30 minutes. Then the sample is filtered via vacuum manifold with a P2 Fisher filter. The resulting filtrate is collected into culture tubes and further into plastic microtubes. The filtrate is then placed in refrigeration until the colorimetric reaction to quantify NH_4 is conducted. The colorimetric reaction process is as follows. The samples removed from refrigeration must be allowed to reach room temperature before starting the reaction. Once the samples have reached sufficient temperature, 70 µl of each sample and appropriate standards are distributed into a deep 96 well plate. Two replicates of each sample and standard are analyzed. Then 50 µl of citrate reagent is added to each sample and standard well using a multichannel pipette. The resulting solution is allowed to react for 1 minute before proceeding. After 1 minute has passed, 50 µl of PPN reagent is dispensed into each well, again with a multichannel pipette. Next, 25 µl of buffered sodium hypochlorite solution is dispensed into each well and finally, 50 µl of ultrapure DI water is dispensed into each well. The resulting solution is swirled manually for 30 seconds to allow for mixing of the reagents and then allowed to react for 2 hours under dark, room temperature conditions allowing color development to occur. The absorbance of each well is then read at 660 nm using a GloMax plate reader.

Soil pH was measured in a 2:1 water to soil ratio following Soil Survey Staff (2014), where 10 g of powdered soil is mixed with 20 ml of DI water and left to set for 30 minutes before reading pH via a probe.

Statistical Analysis

All statistical analyses were conducted in R Studio (version 4.4.0). Analysis of variance (ANOVA) was conducted first with cropping system as a fixed factor, and second with DTC and cropping system as fixed factors and using Tukey's honestly significant difference (HSD) test for pairwise comparisons ($\alpha = 0.05$). Box plots were formulated in R Studio using "ggplot2" box model (Wickham, 2016). Principal component analysis (PCA) model was created using "factoextra" PCA package (Kassambara & Mundt, 2020), and significance was tested using "PCAtest" (Camargo, 2024).

Response ratios, which are a measure of effect size (Hedges et al., 1999), were calculated by the equation below where x_1 is either the switchgrass or miscanthus variable, and x_2 is the corn-soybean variable. This was calculated at the block level (n=16) for switchgrass and miscanthus relative to corn-soybean (i.e., corn-soybean served as the control within each block).

$$\ln\left(\frac{x_1}{x_2}\right)$$

Response ratio values were included in statistical t-tests from the base R package to derive p-values for the effect of cropping system (switchgrass or miscanthus) relative to the control (corn-soybean). Response ratios were calculated to examine the effect of switchgrass and miscanthus compared to the corn-soybean control. This data was evaluated utilizing a one-sample t-test to determine if response ratios were significantly different from 0 (base R package).

2.4 Results

Management effects on soil health

Cropping system significantly influenced several soil health indicators. Seven indicators were significantly different ($P \leq 0.05$) in perennial systems compared to the corn-soybean system, with no observed difference between switchgrass and miscanthus (Table 2.1). Of these seven indicators, five were notably higher ($P < 0.001$). Specifically, miscanthus and switchgrass exhibited greater levels of SOC (24%), arylsulfatase activity (46 to 67%), β-glucosaminidase (58 to 80%), soil respiration (122 to 139%), and total protein (23 to 37%), compared to the corn-soybean system. Miscanthus and switchgrass exhibited greater levels of PMN than the corn-soybean system (33 to 51%) but was not as significant as other indicators ($P < 0.002$). The remaining soil health indicators (i.e., TN, β-glucosidase activity, acid phosphatase activity, POXC, aggregate stability, and soil pH), exhibited the general trend of being greater under perennial systems compared to corn-soybean systems, but results were variable or did not follow the same trend (Table 2.1).

Table 2.1 Means with standard errors in parentheses for soil health indicators by management practice (0-15 cm sampling depth). Lowercase letters within a row correspond to Tukey HSD groupings (α = 0.05).

Soil Health Indicator	Corn-Soybean	Miscanthus	Switchgrass
Soil Organic C (%)	1.44 (0.058)b	1.78 (0.040)a	1.78 (0.072)a
Total N (%)	0.155 (0.0058)b	0.165 (0.0045)ab	0.173 (0.0058)a
β-glucosidase (μg PNP g^{-1} soil hr^{-1})	81.4 (5.46)a	92.4 (4.48)a	83.1 (3.19)a

Acid Phosphatase (μg PNP g^{-1} soil hr^{-1})	203 (8.3)a	235 (10.1)a	235 (12.3)a
Arylsulfatase (μg PNP g^{-1} soil hr^{-1})	56.0 (3.43)b	81.7 (4.57)a	93.5 (3.44)a
β-glucosaminidase (μg PNP g^{-1} soil hr^{-1})	23.3 (0.99)b	36.9 (1.60)a	42.0 (2.90)a
POXC (g C kg^{-1} soil)	0.409 (0.0303)b	0.520 (0.0214)a	0.438 (0.0271)ab
Soil Respiration (mg CO_2 g^{-1})	0.416 (0.0481)b	0.924 (0.0626)a	0.996 (0.0804)a
Total Protein (mg g^{-1})	4.50 (0.174)b	5.53 (0.241)a	6.15 (0.330)a
Potentially Mineralizable N (mg kg^{-1} NH_4-N)	32.4 (3.51)b	43.0 (2.64)a	48.9 (2.93)a
Aggregate Stability (%)	34.1 (4.72)b	50.5 (6.81)ab	68.5 (4.03)a
Soil pH	6.73 (0.044)b	6.29 (0.032)a	6.29 (0.029)a

Principal component analysis was used to compare cropping systems and soil health indicators. Figure 2.1 illustrates strong separation of the perennial cropping systems from the corn-soybean cropping system when all soil health indicators are considered together. Dimension 1 accounted for 42.5% of the variability associated with cropping systems and soil health indicators. The only soil health indicator that was not significant on dimension 1 was aggregate stability, but was significant on dimension 2, which accounted for 18.7% of the variability. The separation was driven primarily by SOC, TN, arylsulfatase, total protein, and PMN, as demonstrated by the arrows showing the magnitude of the positive relationships between perennial management and higher soil health metrics.

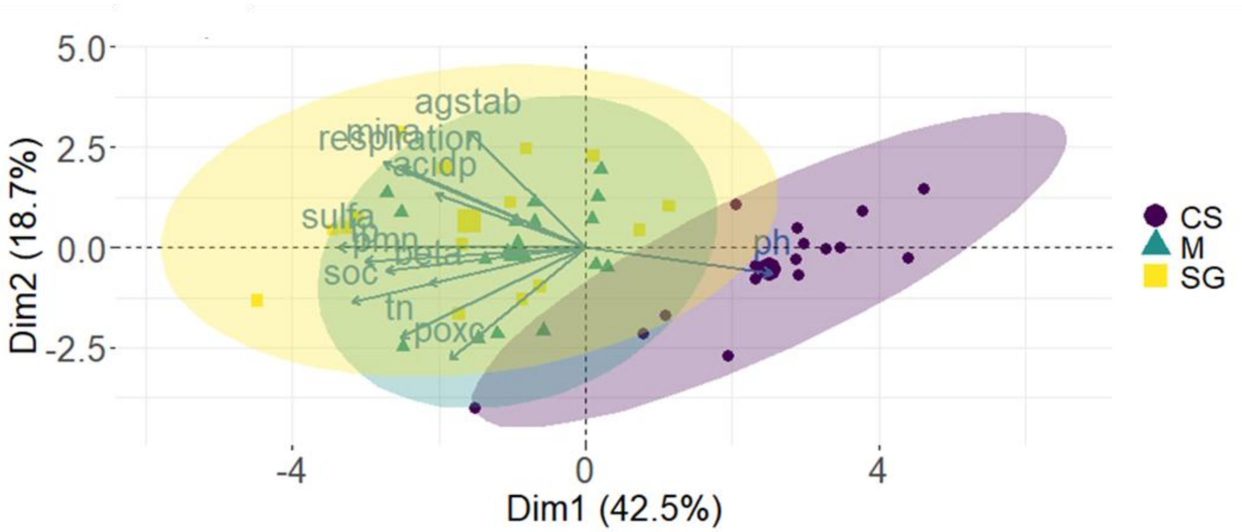

Figure 2.1 Principal component analysis (PCA) biplot of soil health metrics for switchgrass (SG), miscanthus (M), and corn-soybean (CS) systems. Arrows indicate the direction and strength of the correlation between soil health metrics and the ordination axes. Arrow labels from top to bottom: agstab = aggregate stability, mina = β-glucosaminidase, respiration = soil respiration, acidp = acid phosphatase, sulfa = arylsulfatase, pmn = potentially mineralizable N, beta = β-glucosidase, ph = soil pH, soc = soil organic carbon, tn = total N, poxc = permanganate oxidizable C.

Depth to claypan effects on soil health

The DTC effects on soil health indicators were further assessed at three depth categories: 0-15 cm, 15-30 cm, and 30+ cm (DTC 15, 30, and 30+). In the shallow DTC 15 class, SOC was 36% higher under miscanthus and switchgrass compared to the corn-soybean system. Arylsulfatase activity was 77% higher for miscanthus and 98% higher for switchgrass compared to the corn-soybean system. β-glucosaminidase activity was 73% higher under miscanthus and 111% higher under switchgrass compared to the corn-soybean system. In addition, soil respiration was 181% higher for miscanthus and 193% higher for switchgrass compared to the corn-soybean system. Finally, PMN was 76% higher for miscanthus and 89% higher for switchgrass compared to the corn-soybean system. Of the remaining soil health indicators in the shallow DTC 15 class, many exhibited the same trend of perennial systems exhibiting greater soil health metrics than corn-soybean, but the differences were not significant (Table 2.2).

In the moderate DTC 30 class, β-glucosaminidase was 47% higher under miscanthus and 53% higher under switchgrass compared to the corn-soybean system. Soil respiration and aggregate stability were 91% and 127% higher, respectively, under switchgrass compared to the corn-soybean system. Soil pH was significantly lower under miscanthus and switchgrass compared to the corn-soybean system. The remaining soil health indicators were not significantly different across cropping systems in the DTC 30 class. In the deep DTC 30+ class, PMN was 25% higher under miscanthus and 54% higher under switchgrass compared to the corn-soybean system (Table 2.2).

Table 2.2 Means and standard errors in parentheses for soil health indicators (0-15 cm sampling depth) by management and DTC class. Lowercase letters within a row correspond to Tukey HSD groupings (α = 0.05).

Soil Health Indicator	DTC Class	Corn-Soybean	Miscanthus	Switchgrass
Soil Organic C (%)	15 cm	1.31 (0.042)b	1.79 (0.020)a	1.78 (0.120)a
	30 cm	1.54 (0.130)a	1.68 (0.099)a	1.74 (0.168)a
	30+ cm	1.61 (0.134)a	1.95 (0.073)a	1.86 (0.058)a
Total N (%)	15 cm	0.145 (0.0041)b	0.158 (0.0034)ab	0.169 (0.0094)a
	30 cm	0.164 (0.0162)a	0.160 (0.0067)a	0.172 (0.0107)a
	30+ cm	0.166 (0.0079)a	0.192 (0.0097)a	0.188 (0.0041)a
β-glucosidase (μg PNP g^{-1} soil hr^{-1})	15 cm	73.8 (8.74)a	94.7 (8.49)a	89.3 (3.25)a
	30 cm	89.3 (9.45)a	83.9 (1.80)a	71.0 (6.09)a
	30+ cm	88.6 (5.32)a	100.3 (4.26)a	86.8 (2.49)a
Acid Phosphatase (μg PNP g^{-1} soil hr^{-1})	15 cm	206 (15.6)b	240 (18.2)ab	263 (13.2)a
	30 cm	198 (3.8)a	223 (15.5)a	192 (21.0)a
	30+ cm	201 (19.9)a	241 (5.5)a	230 (18.2)a
Arylsulfatase (μg PNP g^{-1} soil hr^{-1})	15 cm	47.6 (3.64)b	84.3 (8.01)a	94.2 (3.44)a
	30 cm	67.0 (6.14)a	76.4 (3.90)a	88.3 (9.62)a
	30+ cm	60.2 (4.16)b	83.4 (12.30)ab	100.6 (2.61)a
β-glucosaminidase (μg PNP g^{-1} soil hr^{-1})	15 cm	24.0 (1.58)c	41.4 (1.59)b	50.6 (3.38)a
	30 cm	23.0 (2.01)b	33.9 (1.35)a	35.2 (2.26)a
	30+ cm	21.7 (0.36)a	29.6 (3.28)a	30.3 (2.54)a

Permanganate Oxidizable C (g C kg^{-1} soil)	15 cm	0.331 (0.0214)b	0.473 (0.0113)a	0.392 (0.0216)b
	30 cm	0.471 (0.0588)a	0.511 (0.0344)a	0.484 (0.0557)a
	30+ cm	0.514 (0.0644)a	0.661 (0.0152)a	0.483 (0.0953)a
Soil Respiration (mg CO_2 g^{-1})	15 cm	0.387 (0.0717)b	1.090 (0.0567)a	1.136 (0.0970)a
	30 cm	0.491 (0.0775)b	0.820 (0.0793)ab	0.938 (0.128)a
	30+ cm	0.367 (0.132)a	0.655 (0.155)a	0.720 (0.221)a
Total Protein mg g^{-1})	15 cm	4.10 (0.188)b	5.23 (0.389)ab	6.35 (0.565)a
	30 cm	4.78 (0.282)a	5.56 (0.293)a	6.09 (0.543)a
	30+ cm	5.10 (0.403)a	6.28 (0.474)a	5.72 (0.491)a
Potentially Mineralizable N (mg kg^{-1} NH_4-N)	15 cm	25.0 (4.78)b	44.1 (4.94)a	47.1 (3.95)a
	30 cm	41.0 (6.09)a	39.2 (2.96)a	46.2 (6.64)a
	30+ cm	37.5 (2.71)c	46.8 (1.36)b	57.8 (1.03)a
Aggregate Stability (%)	15 cm	45.3 (7.19)b	69.0 (9.58)ab	80.9 (2.39)a
	30 cm	26.8 (3.54)b	35.3 (2.28)b	60.9 (4.84)a
	30+ cm	16.7 (2.57)b	26.4 (6.43)ab	48.3 (7.84)a
Soil pH	15 cm	6.72 (0.064)a	6.36 (0.032)b	6.32 (0.037)b
	30 cm	6.77 (0.061)a	6.24 (0.070)b	6.21 (0.060)b
	30+ cm	6.69 (0.155)a	6.20 (0.047)b	6.35 (0.015)ab

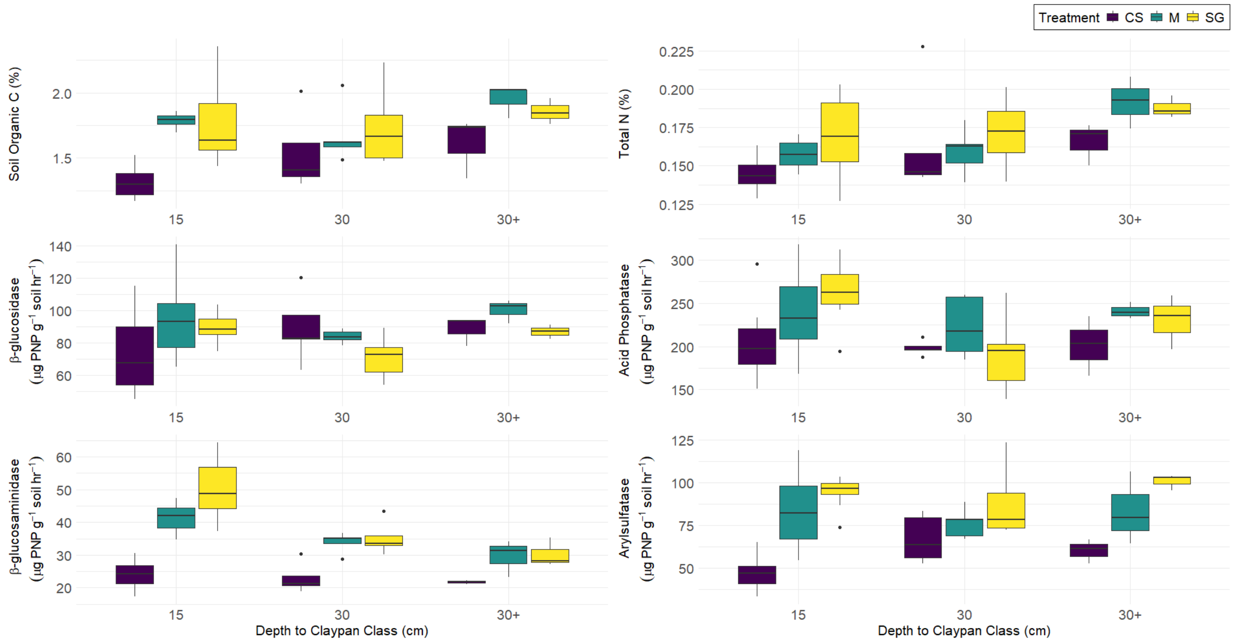

Figure 2.2 Box plots showing soil health indicators by depth to claypan (DTC) class. Management categories are CS = corn-soybean, M = miscanthus, and SG = switchgrass. DTC classes are 0-15 cm, 15-30 cm, 30+ cm.

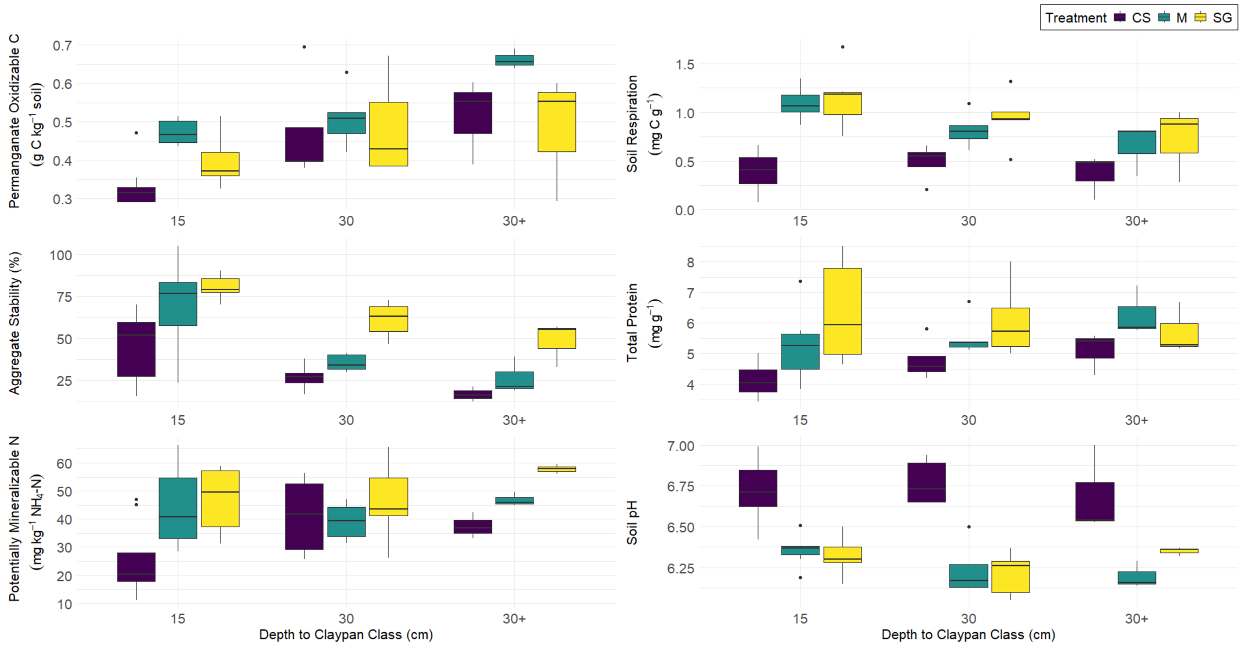

Figure 2.3 Box plots of soil health indicators by depth to claypan (DTC) class. Management variables are CS = corn-soybean, M = miscanthus, and SG = switchgrass. DTC classes are 0-15 cm, 15-30 cm, 30+ cm.

Response Ratios

Overall, switchgrass soil health indicators consistently showed higher positive response ratios ($P < 0.05$) than miscanthus (where corn-soybean served as the control). For the DTC 15 class, switchgrass response ratios were significant and positive for nine soil health indicators: SOC (P < 0.001), TN (P = 0.02), acid phosphatase activity ($P = 0.007$), β-glucosaminidase activity ($P < 0.001$), arylsulfatase activity ($P < 0.001$), soil respiration ($P = 0.002$), aggregate stability ($P = 0.006$), total protein ($P = 0.002$), and PMN ($P = 0.005$). Switchgrass response ratios for the DTC 30 class were significant and positive for four soil health indicators: β-glucosaminidase activity ($P = 0.008$), arylsulfatase activity ($P = 0.037$), soil respiration ($P = 0.004$), and aggregate stability ($P = 0.002$). For the DTC 30+ class, switchgrass response ratios were significant and positive for only two soil health indicators: soil respiration ($P = 0.046$), and aggregate stability ($P = 0.008$).

Interestingly, a significant negative response ratio was observed for β-glucosidase activity under switchgrass in the DTC 30+ class (*P = 0.05*). The pH response ratio was also negative in the DTC 15 and 30 classes (*P < 0.001* and *P = 0.006,* respectively; Fig. 2.4).

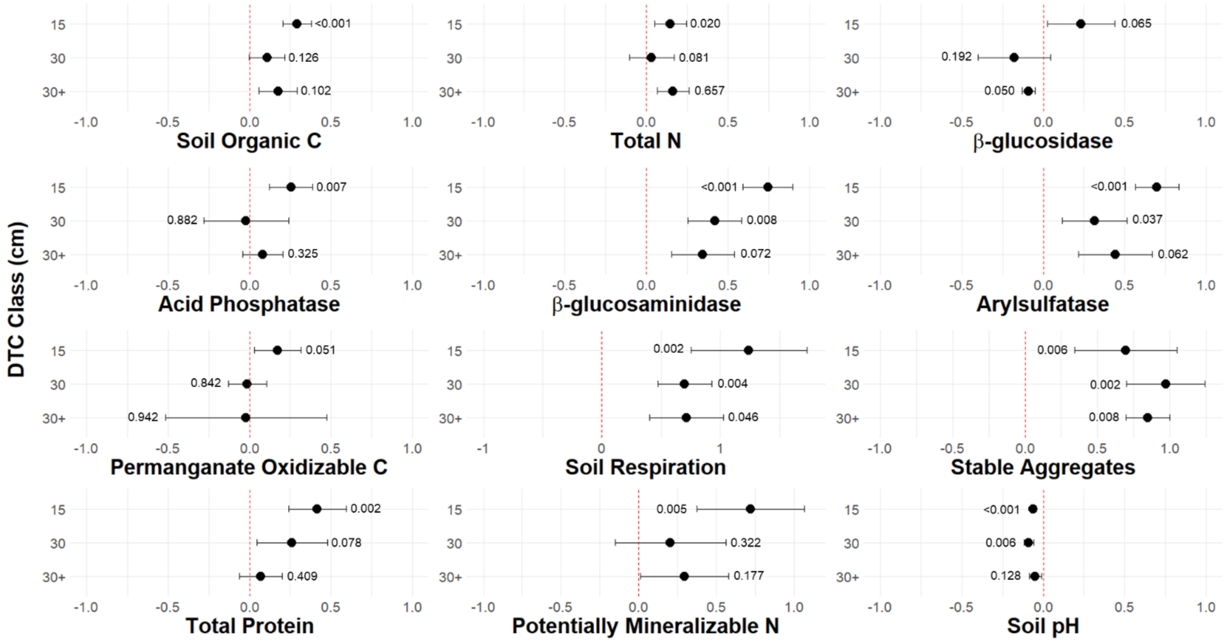

Figure 2.4 Box plots of soil health indicator response ratios across depth to claypan (DTC) classes (0-15 cm, 15-30 cm, 30+ cm) for switchgrass relative to corn-soybean. P-values are displayed next to their corresponding response ratio.

Miscanthus demonstrated high and significant positive response ratios (*P < 0.05*) for many soil health indicators. In the DTC 15 class, the miscanthus response ratios were significant and positive for 10 soil health indicators: SOC (*P < 0.001*), TN (*P = 0.025*), β-glucosidase activity (*P = 0.029*), β-glucosaminidase activity (*P < 0.001*), arylsulfatase activity (*P < 0.001*), POXC (*P < 0.001*), soil respiration (*P = 0.002*), aggregate stability (*P < 0.001*), total protein (*P = 0.003*), and PMN (*P < 0.001*). For the DTC 30 class, the miscanthus response ratios were significant and positive for only two soil health indicators: β-glucosaminidase (*P = 0.004*) and soil respiration (*P = 0.031*). In the DTC 30+ class, the miscanthus response ratios were only significant and positive for PMN (*P*

= *0.037*). In addition, significant negative response ratios for soil pH were observed in the DTC 15 and 30 classes (*P < 0.001* and *P = 0.001,* respectively) for miscanthus (Fig. 2.5).

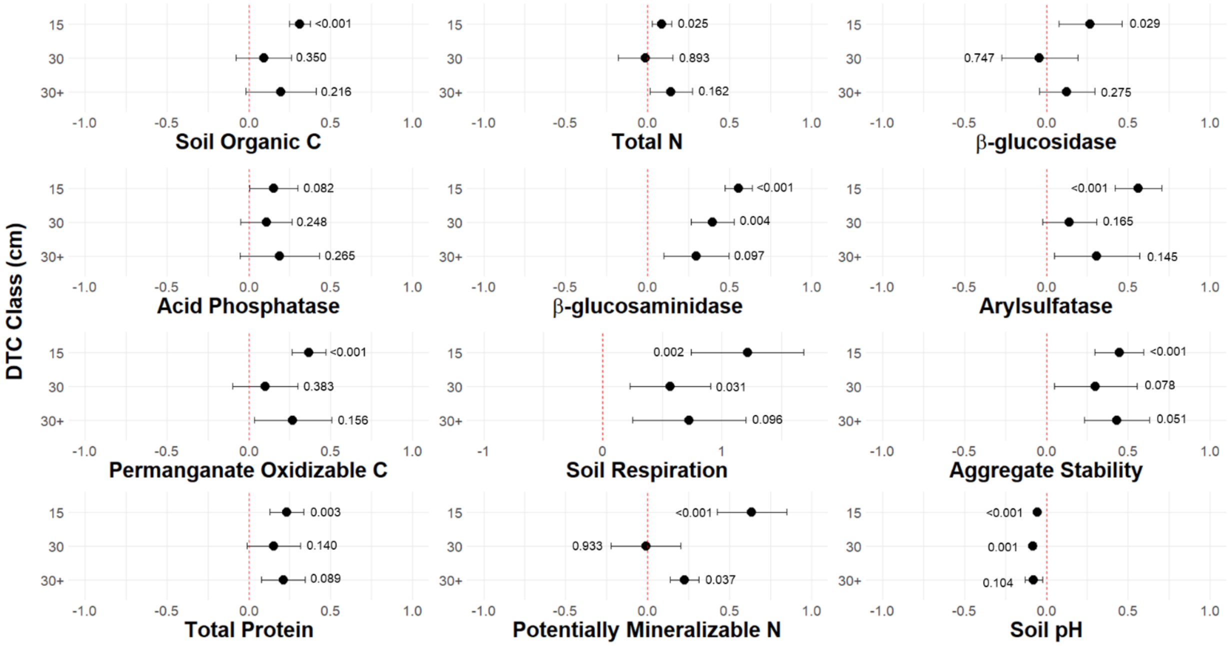

Figure 2.5 Box plots of soil health indicator response ratios across depth to claypan (DTC) classes (0-15 cm, 15-30 cm, 30+ cm) for miscanthus relative to corn-soybean. P-values are displayed next to their corresponding response ratio.

2.5 Discussion

Our results are consistent with other research that has shown perennial systems have greater soil health than annual corn-soybean systems (Augarten et al., 2023; Ledo et al., 2020, Lemus and Lal, 2005; Veum et al., 2015). Furthermore, these findings confirm soil health benefits from converting marginal claypan farmland under annual row crop management to perennial biofuel cropping systems. Across all DTC classes, six of the soil health indicators (SOC, soil respiration, total protein, PMN, β-glucosaminidase activity, and arylsulfatase activity) were significantly greater under perennial switchgrass and miscanthus relative to the corn-soybean system, but not significantly different between switchgrass and miscanthus. These changes to soil health metrics are likely due to the greater above- and below-ground biomass and greater soil cover of the perennial systems relative to the annual corn-soybean rotation. Perennial crops invest significant resources belowground (Bolinder et al., 2002), and their extensive root system makes them an effective tool for increasing soil carbon, drought resilience, and water efficiency (Ojija, 2024), while annual plants focus on shoot development (Ordóñez et al., 2020). The increased organic matter contributions from root exudates and crop residue in turn support increased microbial activity, as demonstrated by the greater enzyme activity and soil respiration.

Additionally, DTC played a role in the magnitude of change observed. Many of the soil health indicators measured in this study demonstrated a greater response, measured via response ratios, in shallower DTC systems relative to deeper DTC systems (Fig. 2.4 & Fig. 2.5). This suggests there is a greater potential to increase soil health in claypan soils with eroded A soil horizons. Perennial systems provide soil protection and organic inputs, enhancing stratification and concentration of soil constituents in shallow topsoil layers where plant roots and root exudates are confined above the claypan. These results highlight soil health benefits from perennial biofuel crops in areas with shallow DTC and limited topsoil, including increased SOC and microbial enzymatic activity. Further, SOC values for switchgrass and miscanthus systems only marginally increased as DTC increased, while SOC in the corn-soybean system increased drastically with greater DTC (Table 2.4.2).

Permanganate oxidizable carbon generally increased with deeper DTC; however, only miscanthus on the shallow DTC (15) soils contained significantly more POXC than the corn-soybean system (Table 2.2). This result suggests that miscanthus may contain or release more POXC-reactive organic compounds. Miscanthus contains higher percentages of lignin than switchgrass, and even many hardwood species (Ussiri & Lal, 2014), and studies have found that POXC may be more influenced by more stable forms of carbon, such as lignin, than by compounds traditionally considered biologically 'labile' (Woodings & Margenot, 2023).

Soil respiration quantifies the microbial mineralization of organic matter to gain insight into the potential microbial activity and carbon turnover in the soil. In this study, soil respiration values surprisingly decreased with deeper DTC, with the highest values for the perennial systems found in the shallow DTC (15) class, and the highest values for the corn-soybean system found in the moderate DTC (30) class. This was surprising given that soils with deeper topsoil layers tend to have higher organic matter content and thus support more microbial respiration. For example, a recent study of eroding versus non-eroding soils found that soil respiration on non-eroding soils with deeper topsoil contained higher soil respiration values than an eroding soil with shallower topsoil (Li et al., 2019). In this study, both switchgrass and miscanthus systems exhibited significantly higher soil respiration than the corn-soybean system in the shallow DTC (15) class, while in the moderate DTC (30) class, only switchgrass had significantly higher soil respiration values than the corn-soybean system. Overall, soil respiration in the perennial systems were double that observed in the corn-soybean system in the shallow DTC (15) class, and soil respiration was the most sensitive to perennial versus annual cropping systems.

Total nitrogen values were significantly different between perennial and annual systems only in the shallow DTC (15) class (Table 2.2). Given that TN includes inorganic nitrogen and the pool of organic nitrogen available for transformation into plant-available forms, higher TN has been associated with improved fertility (Mukhametov et al., 2024). In this study, PMN was consistent across DTC classes for switchgrass and miscanthus, while the corn-soybean system had the lowest values of PMN in the shallow DTC (15) class, reflecting only half of the PMN available in the perennial systems (Table 2.2). These findings suggest that in shallower DTC soils, more nitrogen inputs will be needed to support corn-soybean production relative to deeper DTC soils. Again, switchgrass and miscanthus systems exhibited very little difference in PMN across DTC categories, pointing to enhanced sustainability of the perennial biofuel systems.

Soil enzymes represent microbial activity and cycling of nutrients through the soil. β-glucosaminidase, an enzyme involved in N mineralization, was consistently higher in the shallow DTC (15) class across all three cropping systems, suggesting that organic substrates may be more concentrated in the shallow DTC soils (Table 2.2). Overall, switchgrass had significantly greater β-glucosaminidase activity than the miscanthus and corn-soybean systems, and acid phosphatase was greater under switchgrass relative to the corn-soybean rotation only in the shallow DTC (15) class. One study linked increased clay content to increased β-glucosaminidase and acid phosphatase activity (Hsiao et al., 2018), but more research is necessary to understand this link. Arylsulfatase, an enzyme that catalyzes the hydrolysis of organic sulfate, was relatively consistent across DTC classes but increased with increasing DTC in the corn-soybean system. In the shallow

DTC (15) class, both switchgrass and miscanthus systems exhibited significantly higher arylsulfatase activity, reflecting a 2-fold increase, over the corn-soybean system.

Conway et al. (2017) reported that switchgrass yields exhibited less variability than corn and soybean yields in shallow claypan soil. This aligns with our findings that switchgrass (and, to a slightly lesser degree, miscanthus) fosters superior soil biological functioning—which can, in turn, buffer against environmental stressors. The combined findings highlight the significant role of perennial root systems in supporting soil health and ensuring more stable and potentially more profitable crop production on marginal claypan lands. Future research to confirm these findings over time (beyond 14 years) and at the field scale, are needed, especially due to the nature of the artificially constructed DTC soil profiles.

2.6 Conclusion

Our study highlights that perennial biomass crops, specifically switchgrass and miscanthus, contribute significantly to improved soil health compared with conventional corn-soybean systems, especially on the fragile, shallow (< 15 cm) DTC soils in the Central Claypan Area of Missouri, USA. These results illustrate that perennial biofuel systems may be better suited to marginal land than annual corn-soybean rotations. In addition to reducing erosion and runoff, perennial biofuel systems have the potential to improve degraded soils, and in turn, improve the productivity, profitability, and sustainability of marginal lands. Thus, perennial biofuel crops, or extended rotations that include biofuel crops, may offer an alternative to non-profitable annual crop production by maintaining or improving long-term soil health and sustainability on claypan soils.

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3. Sensitivity of Dynamic Soil Properties to Long-Term Agricultural Management on Claypan Soils

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Dylan J. Sutton[1], Kristen Veum[2], Morgan Davis[1], Samuel Lord[1], Kenneth Sudduth[2]

[1]University of Missouri, School of Natural Resources, Columbia, MO 65211, USA

[2]USDA-ARS, Cropping Systems and Water Quality Research Unit, Columbia, MO 65211, USA

3.1 Abstract

Conservation management practices have the potential to improve soil health status, especially in regions with degraded soils. To assess the soil health benefits of conservation management in the Central Claypan Area of Missouri, two long-term (30-yr) agronomic production systems and an ecological reference site were compared. Study systems included a corn (*Zea mays*) - soybean (*Glycine max*) - wheat (*Triticum aestivum*) rotation under no-till with cover-crops (ASP), a corn-soybean rotation with tillage and no cover-crops (BAU), and a remnant native prairie which served as an ecological reference site (TP). A suite of soil health indicators were measured from soil samples collected to a depth of 0-15 cm. Indicators included physical, chemical, and biological properties. Overall, soil health indicators were greatest at TP followed by ASP and BAU. Soil organic carbon and total nitrogen were less sensitive to management than the more dynamic soil health indicators, such as total protein, permanganate oxidizable carbon,

alkali-absorbed soil respiration, and fresh soil respiration. Tucker Prairie served as an ecological reference site, highlighting the divergence from a claypan summit prairie into cropland, which was common in the region. This study aims to quantify differences in two unique cropping systems, with a reference state comparison.

3.2 Introduction

Conventional row crop practices in the Central Claypan Area of Missouri (MLRA 113) have resulted in degraded soil and water resources (USDA, 1981) due in part to the high runoff and erosion potential of these soils (Lerch and Blanchard, 2003; Baffaut et al., 2020). Erosion directly results in loss of topsoil and removes nutrients and organic matter while negatively impacting productivity (Moraru & Rusu, 2010; Kimble et al., 2001) and surface runoff water quality (Lerch et al., 2005). Additionally, soil disturbance from tillage increases susceptibility to erosion and mineralization of soil organic matter (Morris et al., 2004). The soil in this region is loess-dominated with deep Alfisols and high subsoil clay content (NRCS, 2022), and when coupled with erosion, increases runoff and limits root growth and water infiltration.

In addition, management practices have been shown to impact biological, physical, and chemical indicators of soil health in this region (Veum et al., 2014; Veum et al., 2015; Zuber et al., 2020). A wide range of soil health indicators can be used to represent important soil functions (Doran and Parkin, 1996). Soil physical properties determine how water is partitioned (Cosby et al., 1984), and how resistant soil is to wind and water erosion (Barthès & Roose, 2002). Tillage disturbs soil and studies have shown that greater tillage corresponds to reduced aggregate stability (Six et al., 1998). In addition, aggregate stability typically has a positive correlation to organic matter storage in the soil (Liu et al., 2019).

Multiple biological soil health indicators provide insight into soil function. Soil organic carbon (SOC), the primary component of soil organic matter, promotes the microbial environment below ground and indirectly facilitates the uptake of nutrients by plants (Lord, et al., 2024; Ontl, & Schulte, 2012). Soil organic carbon (SOC) has also been found to be a major contributor to drought resilience and root growth, by increasing water holding capacity (Ontl & Schulte, 2012). Overall, SOC is a key soil health indicator due to its role in multiple soil processes and functions. Organic matter is also the source of organic nitrogen, a pool of nitrogen with the potential to be converted to inorganic, plant-available forms to support plant growth in agricultural and natural systems (Schulten & Schnitzer, 1997). Permanganate oxidizable carbon (POXC), also called active carbon, is a rapid and inexpensive oxidation reaction that is considered a reflection of the labile or microbially available fraction of carbon in the soil (Weil et al., 2003). POXC is correlated with more expensive and labor-intensive methods such as particulate organic carbon, SOC, and microbial biomass carbon (Culman et al., 2012) and various other C and N fractions (Morrow et al., 2016). Therefore, POXC is frequently used to detect short and long-term management changes in corn-based cropping systems (Culman et al., 2013). Similarly, total protein reflects a labile pool of organically bound nitrogen in the soil and provides insight into the carbon and nitrogen cycling ability of the soil (Hurisso et al., 2018) and has been shown to also be responsive to tillage (Moebius-Clune et al., 2008; Nunes et al., 2020). Soil respiration is the measure of microbial mineralization of organic carbon to carbon dioxide. Soil respiration is primarily associated with heterotrophic microbial digestion of soil organic matter as an energy source (Trumbore, 2000; Giardina et al., 2004). Concurrently, soil respiration has shown to be a helpful early indicator of soil health improvements in agricultural systems (Crookston et al., 2023). Soil respiration methods vary based on desired study objectives, with many opting to use a dried-rewetted method in a laboratory setting. For this study, a dried-rewetted method was used in conjunction with a fresh soil respiration method aimed at capturing a more realistic metric of what might be happening at the field level. Dried-rewetted methods have shown to catalyze a more extreme event of soil respiration, often outputting more CO_2 than fresh methods (Thomson et al., 2010).

The goal of this study was to compare soil health among three contrasting management systems on the same soil type: 1) a remnant prairie serving as a reference site, 2) an aspirational, extended corn (*Zea mays*) – soybean (*Glycine max*) – wheat (*Triticum aestivum*) – hay (variable) rotation with cover crops under no-till (ASP) system for 30 years, and 3) a business-as-usual (BAU) corn-soybean rotation under annual tillage and without cover crops for 30 years. The remnant prairie serves as a reference site, given that this region was dominated by prairie prior to modern agronomic development, and the two corn-soybean production systems represent two contrasting row cropping management approaches in this region. The overall objectives of the study were to analyze temporal variation of soil health indicators across production systems, compare overall soil health among production systems, specifically ASP and BAU, and quantify fresh soil respiration differences between production systems.

3.3 Methods

Site Description & Sampling

This experiment was conducted at three Long-Term Agroecosystem Research (LTAR) study sites located in Missouri, USA. Each of the three sites are independently managed from one another in divergent systems. Tucker Prairie (TP) is a remnant prairie used as a baseline of soil health located in McCredie Township, Missouri, USA (38.95042, -91.99184). The second, an aspirational (ASP) agricultural system using conservative agricultural practices such as no-till and conservative chemical and fertilizer management with a complex crop rotation (corn (*Zea mays*) - soybean (*Glycine max*) - wheat (*Triticum aestivum*) -hay (variable)), located in Centralia, Missouri, USA (39.22954, -92.11705). The third, a business-as-usual (BAU) agricultural system utilizing tillage and conventional chemical and fertilizer management without the use of cover crops in a typical annual corn-soybean rotation, located in Centralia, Missouri, USA (39.23175, -92.14953). All three sites are dominated by the Mexico Silt loam soil series (fine, smectitic, mesic Vertic Epiaqualfs) and have a mean annual temperature of 12.2 °C, with a mean annual precipitation of 108.2 cm (PRISM Climate Group, n.d.). The three sites were broken up into plots to capture landscape variability (3 sites x 3 plots = 9 plots) and sampled in transects biweekly for 20 weeks (10 weeks of samples). The temporal aspect of this research was done to capture variability during the growing season. Five samples were taken from each Tucker Prairie plot (n=15) per sampling week, and ten samples were taken from each agriculture plot (5 in-row, 5 out-of-row, n=30 per agriculture site) for a total of 75 samples per sampling week ($n_{total} = 750$). All samples were collected from 0 to 15 cm, reflecting the typical soil health and soil fertility testing sampling depth.

Lab Analysis

All lab analyses were conducted at the USDA-ARS Soil & Water Quality Laboratory within the Cropping Systems and Water Quality Research Unit in Columbia, Missouri. Samples were air-dried for a minimum of 96 hours, then each sample was sieved at 2 mm to prepare for lab analysis. A subsample was taken from weeks 4, 6, 7, 9, and 10 to be used for fresh soil respiration. For the subsample, four samples were taken from each of the nine total plots (n = 36), with ASP and BAU plots having two samples from in-row and two samples from out-of-row (n_{total} = 180).

Permanganate oxidizable carbon was analyzed with 2.5 g air dried, powdered soil being placed in a tube with 18 ml of deionized (DI) water, then 2 ml of 0.2 M potassium permanganate ($KMnO_4$) is added to the tube at pH 7.2 to begin the reaction. The mixture is shaken for 2 minutes at 120 oscillations per minute and then placed under a box for 5 minutes and left to settle. Then, 0.5 ml of the reacted supernatant is pipetted into a separate tube filled with 49.5 ml of DI water to dilute the sample for a spectrophotometry reading at 550nm (Weil et al., 2003).

Soil organic carbon (SOC) and total nitrogen (TN) were analyzed by dry combustion on a Leco TruMac C/N (St. Joseph, MI). A pre-weighed sample was placed into a ceramic weigh boat and loaded into a purge chamber where atmospheric gasses are removed from the sample and then loaded into the furnace chamber which is at a temperature of 950 °C. After combustion, the gasses were moved into a thermoelectric cooler. For carbon detection, the sample gases are put through an infrared detector and the subsequent gas was sent through copper reagents to remove oxygen and reduce nitrogen species into N_2 gas for detection via thermal conductivity (Nelson & Sommers, 1996).

Alkali-absorption (AA) soil respiration was analyzed following Moebius-Clune (2016), where 20 g of 2mm sieved, air-dried soil was placed into an aluminum weigh boat inside a mason jar. A 10 ml beaker glued to a three-pronged stand is set on top of the weigh boat containing 9 ml of 0.5 M potassium hydroxide (KOH). Prior to dispensing, the electrical conductivity (EC) of the KOH was measured using an EC meter probe. Once KOH is dispensed into the beaker, 7.5 ml of Ultrapure DI water is added to the soil to wake the microbes up. Immediately following the water addition, the lids were sealed and incubated at room temperature for 4 days. Following incubation, the jar is opened and the KOH is read on the EC meter probe. This process uses the reaction of KOH and CO_2 which lowers the EC of the KOH. Three readings are required to average the EC of each sample.

Autoclaved-citrate extractable total protein was analyzed following Moebius-Clune (2016), where 3 g of air-dried, 2mm sieved soil was added to a 50 ml glass tube. Then, 24 ml of 0.02 M sodium citrate is added to the tubes to dispense soil aggregates and placed on the shaker for 5 minutes at 180 oscillations per minute. Next, the tubes are transferred to a rack and placed into an autoclave on the liquid cycle at 121 °C. When complete, the samples will rest for 5 minutes then be placed on the shaker for 1 minute at 180 oscillations per minute. Then, 1.75 ml of extractant solution is pipetted into a micro-centrifuge tube. When all samples have been transferred, they were placed into the centrifuge at 10,000 rpm for 5 minutes. Next, 1 ml of supernatant is transferred from each tube into a 96 well plate and stored at 4 °C overnight. The 96 well plate block heaters will be preheated to 61.5 °C. A 10 μL aliquot of sample and standard are dispensed into the plates along with 200 μL of bicinchoninic acid (BCA) working reagent dispensed into each well. Next, the plates are transferred to the heater block to incubate for 60 minutes at 61.5 °C. Finally, samples are removed from the block and left to rest for 5 minutes and then read at an absorbance of 560 nm.

Fresh soil respiration was conducted with a Licor Gas Analyzer (LI-830) using 20 g of fresh, field-moist soil analyzed over 96 hours the day following sample collection, adapted from Moebius Clune (2016), Parkin et al. (2015), and Franzluebbers (2018). After Licor analysis, the samples were oven dried at 110°C for 48 hours and weighed to calculate the gravimetric water content and dry weight to be used in the fresh soil respiration equation (Gardner, 1986).

Statistical Analysis

All statistical analyses were conducted in R Studio (R version 4.4.3). Significant differences were interpreted using analysis of variance (ANOVA) with production system as a fixed factor, and also with production system and sampling week as fixed factors. Tukey’s honestly significant difference (HSD) was used for production system comparisons and groupings, specifically between ASP and BAU, since TP only served as a reference state ($\alpha = 0.05$). Analysis of variance was conducted for in-row versus out-of-row samples to see if there were significant differences for the sampling rows. Tukey’s HSD was then used to see specific differences between production systems based on in-row or out-of-row ($\alpha = 0.05$). Linear models were constructed to assess the influence of soil health metrics on one another and to derive r-squared values using the base R package. Individual sample data points within each production system were used as replicates, and plots were used for in-field replication. Box plots were formulated in R Studio using “ggplot2” box model (Wickham, 2016).

3.4 Results

Week by Week Comparison

To account for any temporal variability, samples were collected throughout the growing season to evaluate differences among management strategies. The comparison of focus was between ASP and BAU, with TP only serving as a reference state. Soil organic carbon ranged from 0.91% to 2.68% for ASP, 0.86% to 2.13% for BAU, and 1.83% to 5.08% for TP, but exhibited no significant differences between ASP and BAU for any sampling week. Total nitrogen ranged from 0.109% to 0.248% for ASP, 0.111% to 0.227% for BAU, and 0.189% to 0.409% for TP, but exhibited no significant differences between ASP and BAU for any sampling week except for week 10 ($p = 0.02$). Permanganate oxidizable carbon ranged from 0.068 to 0.941 g C kg^{-1} soil for ASP, 0.113 to 1.06 g C kg^{-1} soil for BAU, and 0.230 to 1.11 g C kg^{-1} soil for TP, but showed no significant differences between ASP and BAU for any sampling week except for week 8 ($p < 0.001$). Total protein ranged from 3.00 to 9.30 mg g^{-1} for ASP, 2.63 to 6.49 mg g^{-1} for BAU, and 5.17 to 15.5 mg g^{-1} for TP, and exhibited significant differences between ASP and BAU in week 3 and week 10 ($p < 0.001$), but not for any other week. Alkali absorption soil respiration ranged from 0.185 to 1.71 mg CO_2 g^{-1} for ASP, 0.180 to 1.19 mg CO_2 g^{-1} for BAU, and 0.835 to 3.48 mg CO_2 g^{-1} for TP, and exhibited significant differences between ASP and BAU in week 1 ($p = 0.01$), week 3 ($p < 0.001$), week 8 ($p = 0.005$), week 9 ($p < 0.001$), and week 10 ($p < 0.001$). For the fresh soil respiration analyzed on a subset of the full sample size (only weeks 4, 6, 7, 9, and 10), the data ranged from 0.004 to 0.322 mg CO_2 g^{-1} for ASP, 0.008 to 0.465 mg CO_2 g^{-1} for BAU, and 0.038 to 0.520 mg CO_2 g^{-1} for TP, but showed no significant differences between ASP and BAU for any week. In field gravimetric water content on the sample subset ranged from 0.049 to 0.433 for ASP, 0.094 to 0.300 for BAU, and 0.115 to 0.507 for TP, and exhibited significant differences between ASP and BAU in week 6 ($p < 0.001$), week 9 ($p = 0.02$), and week 10 ($p < 0.001$)(Figure 3.1).

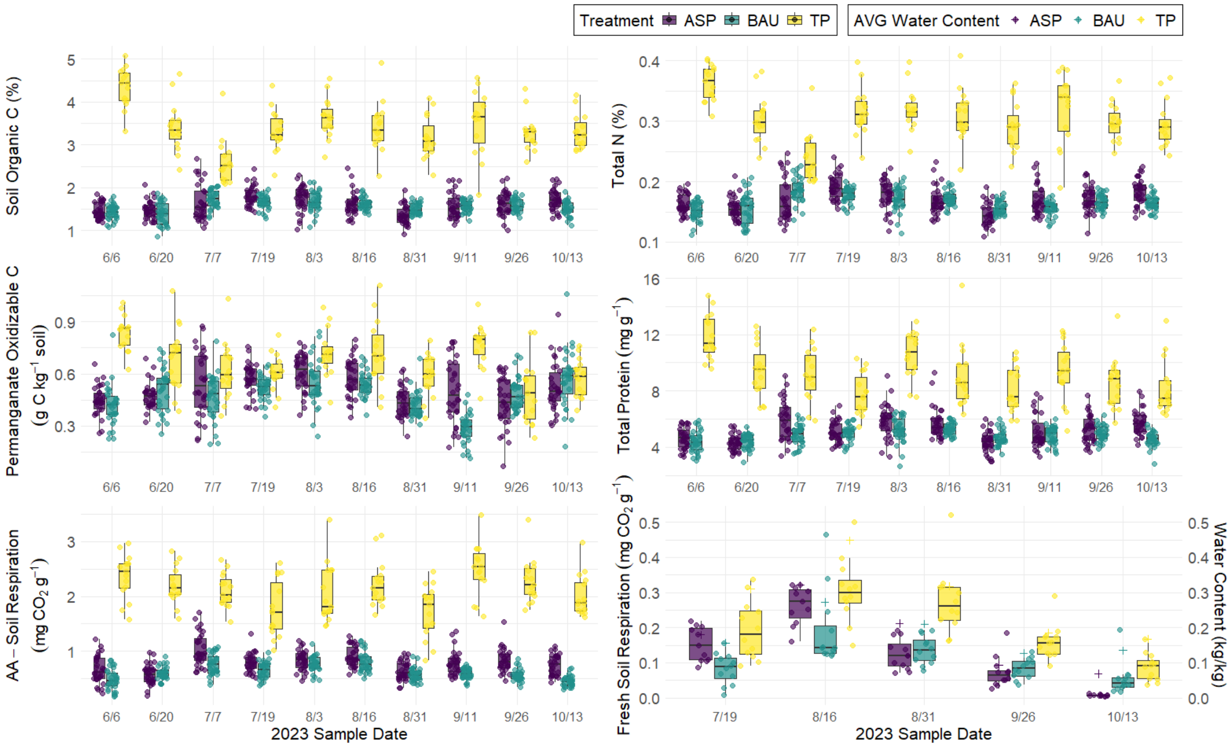

Figure 3.1 Box Plots of soil health indicators by sampling date for each system. ASP = Aspirational, BAU = Business-as-Usual, and TP = Tucker Prairie.

Overall Comparison

The overall model without a temporal aspect was analyzed comparing ASP, BAU, and TP, but for the purposes of this study, the comparison of focus was only between ASP and BAU. Results from the output showed that ASP and BAU were significantly different from each other for POXC ($p = 0.008$), total protein ($p < 0.001$), AA-soil respiration ($p < 0.001$), and fresh soil respiration ($p < 0.001$). Specifically, ASP was 7.4% higher than

BAU for POXC, 7.9% higher than BAU for total protein, 25.1% higher than BAU for AA-soil respiration, and 12.2% higher than BAU for fresh soil respiration (Table 3.1). In field gravimetric water content and effective precipitation were not significantly different from each other between ASP and BAU, so fresh soil respiration differences were not caused by different field conditions within the two systems. There existed a moderate to strong correlation between fresh soil respiration and in field gravimetric water content where 66% of the variation in fresh soil respiration was explained by gravimetric water content, even though the respiration calculations were corrected for differences in water content within each sample. This indicates that there was greater respiration where soils can hold onto more water. Soil organic carbon and total nitrogen were not significantly different between ASP and BAU (Table 3.1). When TP was added to the model, it showed significant differences from both ASP and BAU for soil organic carbon, total nitrogen, permanganate oxidizable carbon, total protein, AA-soil respiration, fresh soil respiration, and gravimetric water content ($p < 0.001$). Specifically, TP was 116.6% higher than BAU for soil organic carbon, 84.5% higher than BAU for total nitrogen, 38.8% higher than BAU for POXC, 90.8% higher than BAU for total protein, 238.9% higher than BAU for AA-soil respiration, 86.4% higher than BAU for fresh soil respiration, and 58.8% higher than BAU for gravimetric water content. There were no significant differences of effective precipitation between any of the three systems, so the large difference in gravimetric water content indicates increased water holding capacity within the prairie system. Analysis of variance was conducted to see if sampling in-row or out-of-row was a significant predictor of any soil health indicator and only showed significance for AA-soil respiration ($p = 0.031$), however Tukey's HSD showed that sampling in-row and sampling out-of-row did not have a significant effect on AA-soil respiration for either ASP or BAU.

Table 3.1 Means with standard errors in parentheses for soil health indicators compared across production systems (0-15 cm sampling depth). Lowercase letters within a row correspond to Tukey's groupings (α = 0.05). ASP = Aspirational, BAU = Business-As-Usual, TP = Tucker Prairie, AA-Soil Respiration = Alkali Absorption Soil Respiration, Effective Precipitation = precipitation from previous 6 days prior to sampling.

Soil Health Indicator	ASP	BAU	TP
Soil Organic C (%)	1.60 (0.018)b	1.57 (0.012)b	3.40 (0.054)a
Total N (%)	0.170 (0.0015)b	0.166 (0.0011)b	0.306 (0.0040)a
Permanganate Oxidizable C (g C kg^{-1} soil)	0.512 (0.0080)b	0.477 (0.0079)c	0.662 (0.0140)a
Total Protein (mg g^{-1})	5.20 (0.066)b	4.82 (0.041)c	9.20 (0.175)a
AA-Soil Respiration (mg CO_2 g^{-1})	0.783 (0.0140)b	0.626 (0.0106)c	2.120 (0.0394)a
Fresh Soil Respiration (mg CO_2 g^{-1})	0.123 (0.0125)b	0.110 (0.0099)c	0.205 (0.0139)a
In field Gravimetric Water Content (kg kg^{-1})	0.174 (0.0123)b	0.180 (0.0076)b	0.286 (0.0140)a
Effective Precipitation (mm)	21.3 (1.27)a	21.3 (1.27)a	24.3 (2.06)a
Temperature C	23.7 (0.20)a	23.7 (0.20)a	23.7 (0.28)a

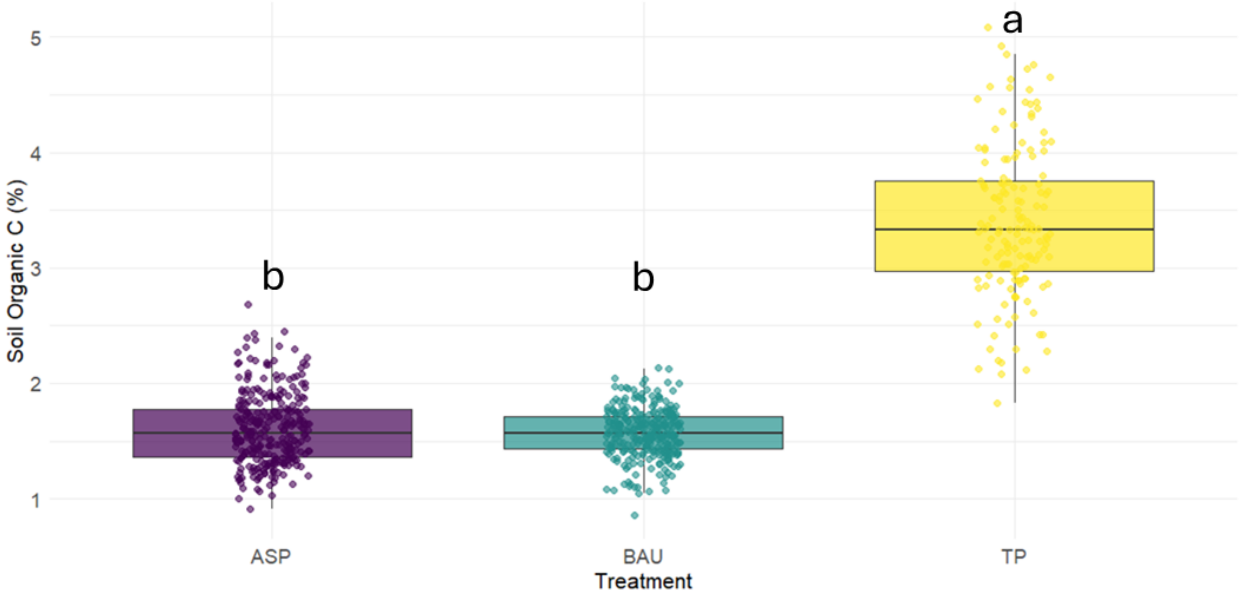

Figure 3.2 A box and whisker plot showing soil organic carbon percentage by production system. ASP = Aspirational, BAU = Business-as-Usual, TP = Tucker Prairie.

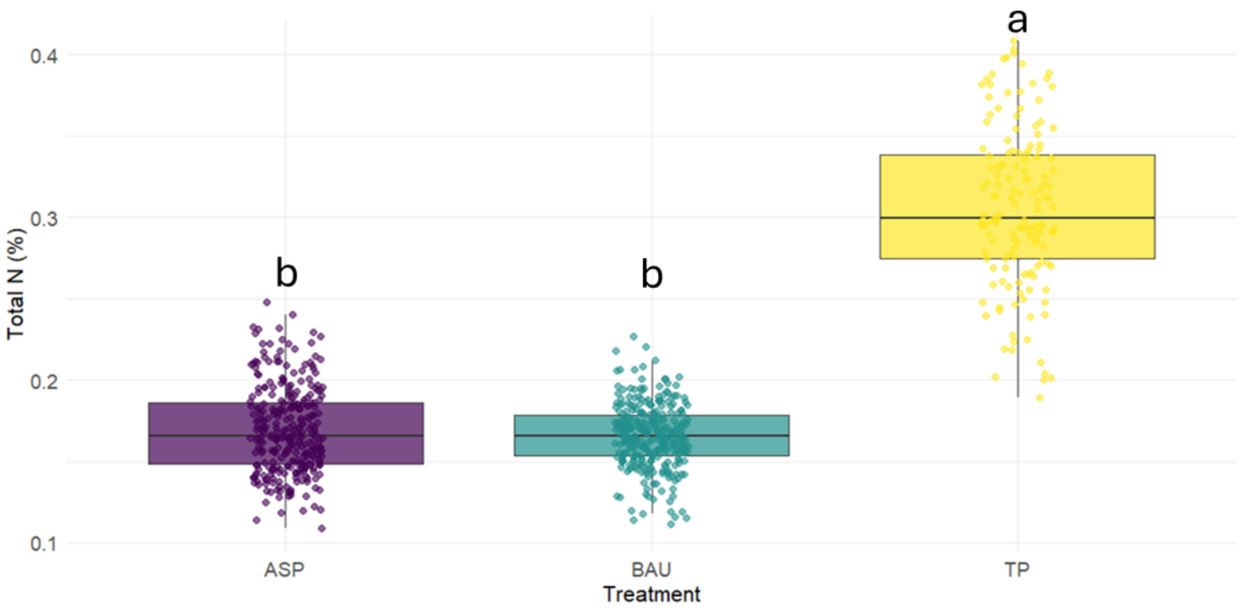

Figure 3.3 A box and whisker plot showing soil total nitrogen percentage by production system. ASP = Aspirational, BAU = Business-as-Usual, TP = Tucker Prairie.

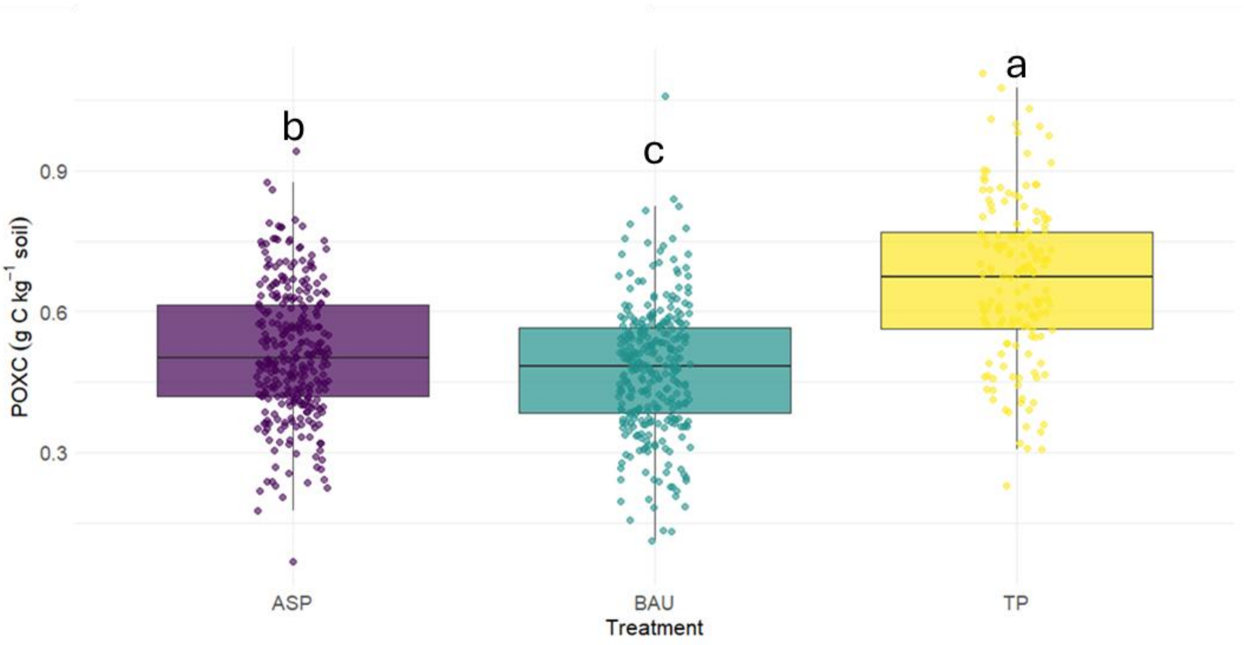

Figure 3.4 A box and whisker plot showing permanganate oxidizable carbon (POXC) content by production system. ASP = Aspirational, BAU = Business-as-Usual, TP = Tucker Prairie.

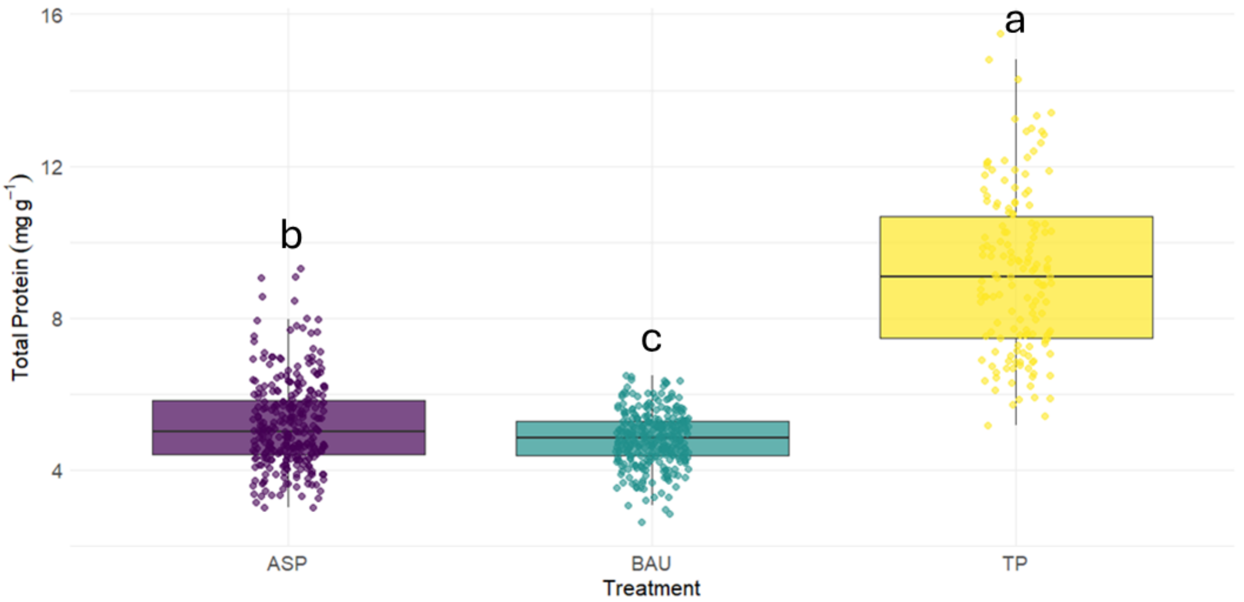

Figure 3.5 A box and whisker plot showing total soil protein content by production system. ASP = Aspirational, BAU = Business-as-Usual, TP = Tucker Prairie.

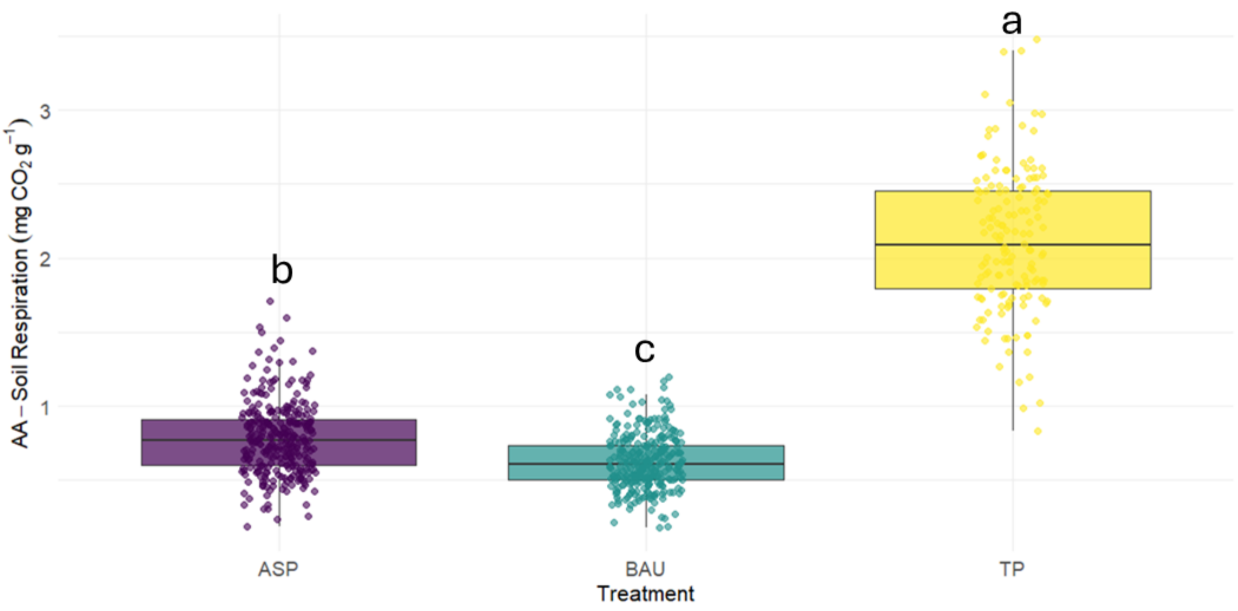

Figure 3.6 A box and whisker plot showing alkali-absorbed (AA) soil respiration by production system. ASP = Aspirational, BAU = Business-as-Usual, TP = Tucker Prairie.

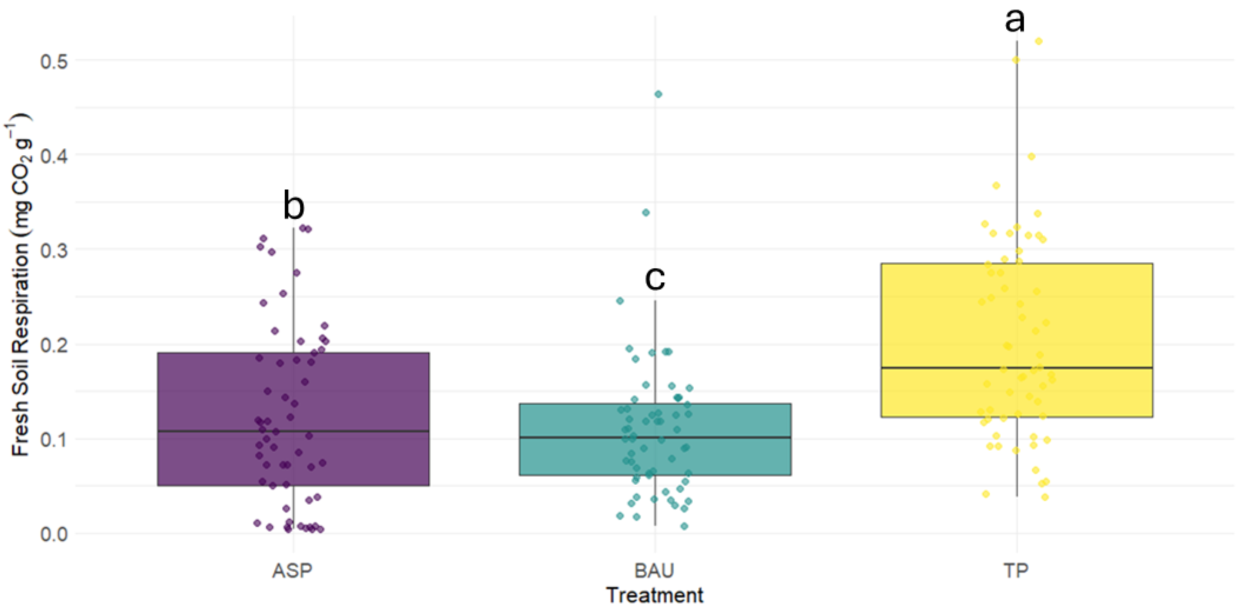

Figure 3.7 A box and whisker plot showing fresh soil respiration by production system. ASP = Aspirational, BAU = Business-as-Usual, TP = Tucker Prairie.

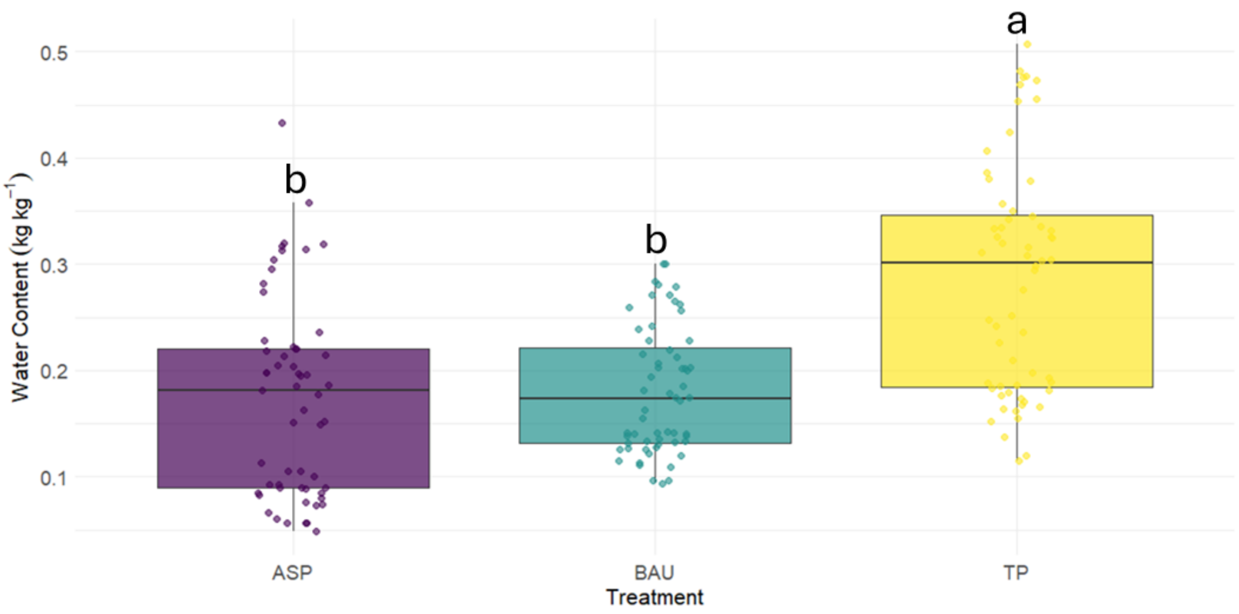

Figure 3.8 A box and whisker plot showing gravimetric water content by production system. ASP = Aspirational, BAU = Business-as-Usual, TP = Tucker Prairie.

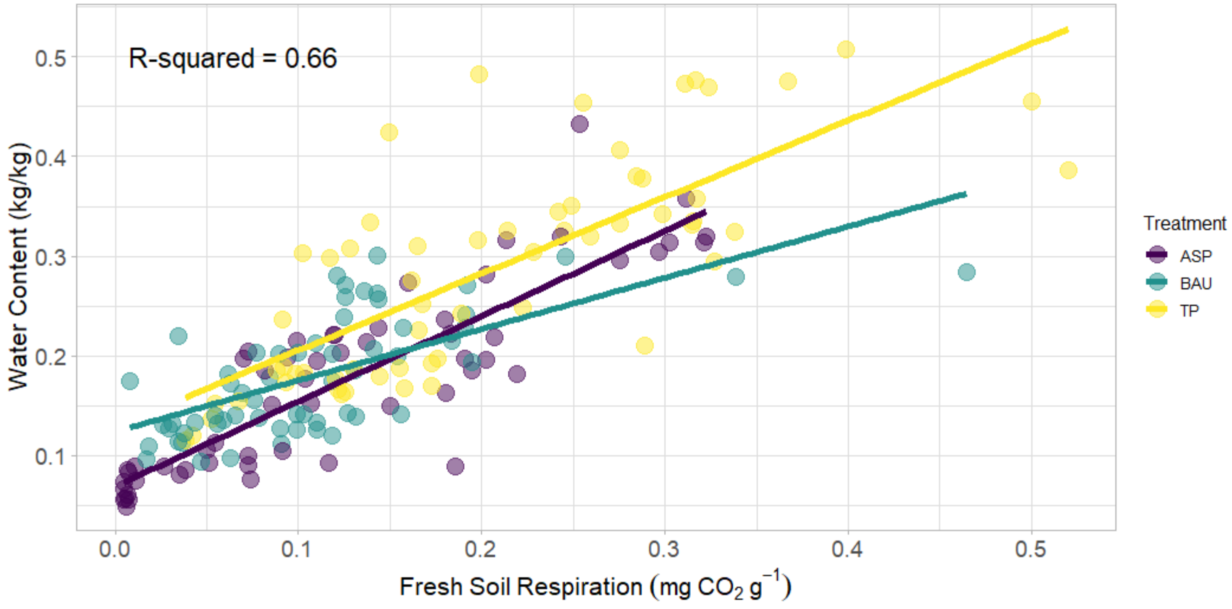

Figure 3.9 A linear regression model showing the relationship between gravimetric water content and fresh soil respiration ASP = Aspirational, BAU = Business-as-Usual, TP = Tucker Prairie.

3.5 Discussion

Results demonstrate that between the two agricultural production systems, there were no differences in SOC or TN, but differences were shown in more complex soil health indicators such as POXC, total protein, AA-soil respiration, and fresh soil respiration. Total soil nitrogen is often measured via combustion analysis, which converts all forms of nitrogen into a measurable gas. More stable forms of nitrogen that are included in the totals are too stable to reliably reflect quick management induced changes (Hurisso et al., 2018), but in theory should reflect changes over the time scale of this study (Yan et al., 2012). Soil organic carbon was also measured via combustion analysis, where organic matter is burned and the resulting carbon within it is quantified. Organic carbon is less sensitive to management changes than other soil health indicators, but like TN, should reflect changes over the timescale of this study (Yan et al., 2012). Many studies have shown that implementing no-till corresponds to an increase in SOC and TN (Franzluebbers et al., 1999; Six et al., 1999; Al-Kaisi et al., 2005), but that was not the case in this long-term study. A response of SOC and TN to management may not have been detectable due to sampling from 0-15 cm instead of quantifying the near surface layer where more dynamic organic carbon cycling exists (Ellert et al., 2001). A previous comparative carbon study between ASP and BAU found significant ΔSOC from 2016 to 2022, showing that ASP had a positive ΔSOC in a 1 m soil core, but when restricted to the top 30 cm, ASP had almost 5x less change in SOC, while ΔSOC at BAU was largely the same in the top 30 cm as in the 1 m cores (Schreiner-McGraw et al., 2024). Tucker prairie served as the reference state for the agricultural production systems, since the sites were likely similar prior to European settlement. In theory, this is the equilibrium state of soil health at which the aspirational and business-as-usual sites *could* exist, specifically for SOC and TN. In reality, the degradation of both sites prior to the project being set up would make soil health recovery more difficult (Jensen et al., 2020).

Another reason for the lack of SOC and TN differences could lie in the prior selection pressures on the agricultural sites' microbial communities from decades of agricultural soil degradation (Schmidt et al., 2019; Porter & Sachs, 2020). These anthropogenic selection pressures favor more generalist microbial communities such as actinobacteria (Liu et al., 2021; Lord et al., 2024) whose main effect in the soil is carbon and nitrogen cycling and can break down more complex carbon structures (Zhang et al., 2019; Barka et al., 2016). When the degraded microbial community of the aspirational site implemented better management practices for the purpose of the study, such as no-till and cover crops, carbon inputs increased, continuing to "feed" the generalist microbial communities. This could result in little SOC and TN accumulation, because organic matter is being mineralized at the same rate at which it is being added, even with a more diverse crop rotation. For the case of the business-as-usual site, it is likely working with a similar microbial composition but may have less microbial abundance than the aspirational site due to less organic inputs. Total protein was 7.6% higher, and POXC was 7.1% higher in ASP compared to BAU, supporting the theory of carbon and nitrogen turnover differences in the two systems. Higher total protein is related to the ability of a soil to mineralize nitrogen (Hurisso et al., 2018), and POXC aims to measure the labile carbon within the soil, which serves as an energy source for microorganisms (Weil et al., 2003). Both analyses show that ASP has a higher ability to cycle carbon and nitrogen than the BAU site but lacks the ability to increase SOC and TN.

Fresh soil respiration methods highlight the in-field microbial dynamics arguably better than dried and rewetted respiration methods, with rewetted methods often turning over more CO_2 than fresh methods (Thomson et al., 2010). This may be caused by a soil priming effect, where generalist microbes are reawakened and begin breaking down more carbon than normal, and often they are breaking down more stable forms of carbon, that likely would’ve remained unbroken in the field setting (Liu et al., 2015; West et al., 1992; Denef et al., 2001; Guenet et al., 2012), but other studies show that dried and rewetted soils can reduce mineralization of carbon (Mikha et al., 2005).

3.6 Conclusions

Effective management practices in agriculture are always being learned from and expanded. This study demonstrates that in thirty years of divergent management practices, SOC and TN concentrations are no different between a no-till field with cover-crops and a tilled field without cover-crops to a depth of 15 cm. However other soil health indicators including total protein, POXC, AA-soil respiration, and fresh soil respiration all showed significant differences between agricultural management practices. This indicates that more dynamic soil health properties have been maintained or improved over thirty years of divergent management, especially in a previously degraded site.

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4. Overall Conclusions

This thesis explored the influence of various agricultural management practices on soil health within the Central Claypan Area of the United States. The studies aimed to highlight potential soil health differences in different long-term management sites. The first study evaluated engineered fields with plots of unique depth to claypan (DTC) thicknesses. The second, three sites within the Long-Term Agroecosystem Research Network (LTAR) containing an aspirational (ASP) field conservatively managed without tillage and with the use of cover crops, a business-as-usual (BAU) field conventionally managed with tillage and without the use of cover crops, and Tucker Prairie (TP) serving as a reference site by which ASP and BAU were likely similar to prior to European settlement. These data have supported general conclusions outlined below:

Study 1: Soil Health Benefits of Perennial Biofuel Crops on Claypan Soils

- Overall, miscanthus and switchgrass exhibited significantly greater levels of soil organic carbon (24%), arylsulfatase activity (46 to 67%), β-glucosaminidase (58 to 80%), soil respiration (122 to 139%), total protein (23 to 37%), and potentially mineralizable nitrogen (33 to 51%) compared to the corn-soybean system.
- In the shallow (0-15 cm) DTC class, soil organic carbon was 36% greater, arylsulfatase activity was 77% to 98% greater, β-glucosaminidase activity was 73% to 111% greater, soil respiration was 181% to 193% greater, and

potentially mineralizable nitrogen was 76% to 89% greater under switchgrass and miscanthus compared to the corn-soybean system.

- Results showcase the potential for perennial switchgrass and miscanthus to improve or maintain the soil health of marginal, shallow land when compared to an annual corn-soybean rotation. This can be beneficial to farmers, because studies show that perennial biofuels could compete economically with corn-soybean farming on shallow soils.

Study 2: Sensitivity of Dynamic Soil Properties to Long-Term Agricultural Management on Claypan Soils

- Soil organic carbon and total nitrogen were not significantly different between ASP and BAU, but differences were shown in more dynamic soil health indicators.
- ASP had significantly greater soil health indicators than BAU for permanganate oxidizable carbon (7.4% higher), total protein (7.9% higher), AA-soil respiration (25.1% higher), and fresh soil respiration (12.2% higher).
- Results demonstrate that while soil organic carbon and total nitrogen are not different after 30 years of divergent management, differences do exist in more dynamic soil health indicators, showing that the ASP management practices do bring positive changes in soil health.

The first study of the thesis gives strong evidence to all growers in the region in supporting the implementation of perennial biofuels on marginal, potentially eroded soils. This could be in areas of greater slope, where chemical runoff is a concern, by serving as a buffer zone to nearby watersheds and water bodies. The results support that soil health can be maintained and even improved under perennial biofuel crops relative to annual corn-soybean rotations. The second study of the thesis shows that in a long-term diverse cropping system without tillage, dynamic soil health indicators may be greater when compared to a conventional annual corn-soybean rotation, highlighting a potential alternative cropping system to growers concerned about decreasing soil health on their land. Results of these studies show the effects of varying management practices on soil health indicators in the Central Claypan Area. These results can be applied to fit a wide range of scenarios for farmers in the region, applicable to aspiring perennial biofuel growers, and to corn-soybean growers concerned about the long-term sustainability of their soil health.