Assessing the impact of cover crops on soil health in a long-term no-till rotation: A case study from Northeast Kansas

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Abstract

Maintaining soil health is essential for sustaining agricultural productivity and ecosystem resilience. Conservation agriculture, known for minimal soil disturbance and permanent soil cover, offers strategies to address soil degradation associated with conventional management practices like conventional tillage and prolonged fallow periods. Despite its recognized benefits, there are concerns regarding potential drawbacks, such as nutrient stratification and soil acidification over time. Cover crops have emerged as a promising tool for enhancing soil health, however, long-term effects of cover crops on soil proprieties under no-till management and various intensification schemes remain to be fully understood. This study aimed to assess (i) how different preceding cover crop types and varying nitrogen (N) rates applied to corn influence pH, soil organic carbon (SOC), and nutrient concentrations and distribution in the soil profile, and (ii) the effects of different intensification strategies on soil biological indicators and their persistence throughout subsequent years of the rotation. A long-term experiment consisting of a no-till three-year wheat (*Triticum aestivum* L.), sorghum [*Sorghum bicolor* (L.) Moench], soybean [*Glycine max* (L.) Merr] rotation was established in 2007 near Manhattan, Kansas. Sorghum was replaced with corn (*Zea mays* L.) in 2020. The different fallow managements imposed between wheat and corn included chemical fallow (CF), double-crop soybean (DSB), and four cover crop treatments.

To meet the first objective of this study, soil profile samples were collected in 2021 and 2022 after corn harvest to determine how different fallow management preceding corn and different N rates applied to corn affect soil pH, SOC, Total N, and Melich-3 Phosphorus (P) concentration and their distribution in the soil profile. Nutrient concentrations were higher near the soil surface, and pH tended to decrease in the upper soil layers. Differences among the fallow

management options were most pronounced at the 0-5 cm depth for all measurements. Soil organic carbon was enhanced by adding cover crops and DSB compared to CF. Similarly, Total N was greater whenever any cover crop treatment or DSB replaced a portion of fallow. Soil pH increased with cover crops and DSB and decreased with higher N rates only at surface layers. Phosphorus concentrations decreased with DSB and remained similar to CF for most cover crops. Overall, cover crops and intensification alternatives like double-cropping showed potential to improve SOC and N levels in the soil surface and enhance its capacity to buffer changes. Further research should address managing the negative impacts of P stratification on soil health and water quality.

To meet the second objective, soil samples were collected in May 2022 and April 2023 in all crop phases, to capture the effect of fallow management on biochemical indicators at different time intervals after cover crop termination: immediately after cover crop termination (T0), in corn stubble one year after cover crop termination (T1), and in growing wheat two years after cover crop termination (T2). Samples were collected at the 0-5 cm depth and analyzed for the following biochemical indicators: POXC, soil protein, glucosidase and glucosaminidase activities, and soil respiration. Cover crops positively impacted all soil biochemical indicators immediately after cover crop termination (T0), increasing POXC by 30%, soil protein by 17%, glucosidase activity by 63%, glucosaminidase activity by 82%, and soil respiration by 116% compared to CF. In general, cover crops with larger biomass inputs provided the largest increases in biochemical indicators. Double-crop soybeans exhibited indicator levels similar to or slightly greater than CF, yet lower than most other cover crops. These responses were most evident immediately after cover crop termination and persisted to some extent for one year thereafter. However, the responses diminished over time and were not detectable two years after cover crop termination. This information could assist in determining how frequently cover crops should be integrated into cropping systems and identify the most effective mixtures and species for sustaining soil health benefits over time.

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Chapter 1 - Literature Review

The need for a sustainable agriculture system

Agricultural sustainability is defined as the ability of a crop production system to continuously produce food without environmental degradation (Tahat et al., 2020). Currently, food production systems face a significant challenge due to the exponential growth of the human population, which has risen from 3 billion in the 1960s to 8 billion in 2023 (World Bank, 2023), and is projected to surpass 9 billion by 2050 (Zarocostas, 2022). In this context, assuming a linear increase in consumption and no improvement in food waste reduction, food production will need to increase by 60% relative to current levels to meet human consumption needs.

Agriculture is also facing the challenge of climate change, which refers to long-term shifts in global or regional climate patterns, mainly caused by human activities, especially the emission of greenhouse gases (USGCRP, 2018). The concentrations of these gases, including CH_4, CO_2, and nitrous oxide (N_2O), has increased by 156%, 47%, and 23%, respectively, since 1750 (AR6 Synthesis Report, 2023). Although these gases are produced naturally in the environment, human activities such as industry, transportation, and agriculture can accelerate their production. Because they trap and redistribute heat, their accumulation contributes directly to global temperature increases and associated problems for agriculture, such as alterations in rainfall patterns and more frequent climate extremes like high temperatures or drought (Gowda et al., 2018). High temperatures can reduce yields by surpassing the maximum temperature threshold of certain crops, leading to a reduction in length of their growing season. Likewise, prolonged drought periods can also result in poor yields and depletion of water resources. Extreme precipitation events, on the other hand, threaten sustainable crop production by causing excessive runoff, leaching, and flooding, which can lead to soil loss, soil structure degradation, and nutrient depletion (Rashmi et al., 2022).

Yield sustainability in agriculture is threatened by soil degradation resulting from conventional farming practices. Heavy machinery and intensive tillage can cause degradation of soil structure, leading to decreased water infiltration, soil organic matter (SOM) content, and nutrient availability for crops (Pagliai et al., 2004). Furthermore, monoculture systems are more susceptible to pest and disease outbreaks, as the continuous planting of the same crop provides a favorable environment for pests and pathogens to thrive. To address the challenges of soil degradation caused by conventional farming practices, conservation agriculture emerges as a promising solution.

Conservation agriculture, which can be defined as minimal soil disturbance and permanent soil cover combined with crop rotations, can help mitigate the negative effects of continuous cropping systems and provide various benefits for soil fertility and the environment. Studies by Hobbs et al., (2008) and Büchi et al., (2018) have highlighted the potential of conservation agriculture as a sustainable farming option. For instance, Hobbs et al. (2008) examined the outcomes of a field trial where water infiltration under long-term conservation tillage was up to a 400 % greater compared to conventional tillage on a grey cracking clay and a sandy loam soil in south-eastern Australia. Similarly, Büchi et al. (2018) demonstrated that cover crops cultivated for a brief two-month period between two winter wheat (*Triticum aestivum* L.) crops can provide numerous benefits for cropping systems in clay and loam soils in Switzerland. Their findings revealed that high biomass cover crops efficiently suppressed weed biomass across different tillage treatments, demonstrating superior weed control compared to the control group where no cover crops were used.

No-tillage management is a key component of conservation agriculture that plays a crucial role in supporting sustainable crop production by ensuring permanent soil cover through the presence of either living plants or their residues, along with diversified cropping systems that may involve more than one harvest per year (Fuentes-Llanillo et al., 2021). The no-till approach not only contributes to the elevation of soil organic carbon (SOC), typically accompanied by an increase in soil microfauna biodiversity (Bartz, 2020; Kan et al., 2020) but can also result in improved yields over time (Page et al., 2019). Some of the reasons for these benefits may be reduced soil loss, improved water storage, and overall improvement of soil health. At the same time, rainfall impact on soil and water runoff can be minimized by maintaining the soil covered, mitigating erosion processes (Fuentes-Llanillo et al., 2021).

Cover cropping can also be an alternative for enhancing sustainability by increasing surface residue and reducing soil erosion. The introduction of cover crops in the rotation has the potential to provide multiple benefits in a cropping system, such as improving soil physical and biological properties, supplying nutrients, suppressing weeds, improving soil water storage, etc. (Blanco-Canqui et al., 2015a). By adding organic compounds to the soil through cover crop residues, soil structure is improved, leading to better water infiltration, reduced compaction, and increased aeration. Moreover, the root systems of cover crops enhance soil microbial activity and diversity, promoting nutrient cycling and soil health. Additionally, cover crops can serve as habitat and food sources for beneficial insects and other organisms, contributing to overall ecosystem resilience. These combined benefits make cover cropping a valuable practice for sustainable agriculture, offering a holistic approach to soil and environmental management.

Combining no-till management with cover cropping can result in synergistic effects that amplify the benefits of both practices (Mitchell et al., 2017). The combined presence of cover crop residues and no-till practices acts as a protective layer against erosion and runoff, enhancing soil structure and microbial activity . These practices also serve as a buffer against the effects of climate change and the negative impacts of conventional agriculture, while also helping to meet the growing demand for food needed to feed an expanding population.

Soil health indicators

Intensive agricultural practices aimed at maximizing crop productivity have prompted the emergence of the concept of soil health. The Soil Science Society of America Ad Hoc Committee on soil quality defines soil health as "the capacity of a specific type of soil to operate, within natural or managed ecosystem boundaries, in sustaining plant and animal productivity, preserving or improving water and air quality, and promoting human health and habitation" (Karlen et al., 1997). More recently, More recently, the Intergovernmental Technical Panel on Soils (ITPS) defined soil health as “the ability of the soil to sustain the productivity, diversity, and environmental services of terrestrial ecosystems (ITPS, 2020). Soil functions go beyond supporting crop growth but also serve as a foundational source of vital ecosystem services. These services include climate regulation, nutrient cycling, flood regulation, carbon sequestration, water purification, and the provision of habitats for microorganisms, as elucidated by Karlen et al. (1997). In this sense, a healthy soil would maintains its ability to function optimally over time.

Optimization of chemical, physical, and biological processes in soil is important to build soil health by sustaining crop productivity and environmental quality. Monitoring changes in chemical, physical, and biological indicators provide a means to evaluate soil health across various production systems (Doran et al., 1997). Assessment enables the evaluation of how effectively the soil performs its functions and the preservation of these functions for future use (Rinot et al., 2019). The essential factors for establishing effective soil health indicators includes their responsiveness to changes in soil management, correlation to other indicators capable of forecasting soil functioning, consistency in analyses, and consideration of cost and user-friendly application (Hurisso et al., 2018; Laishram et al., 2012a).

Physical indicators

An agricultural soil presenting good physical quality should be “strong” enough to maintain a good structure, hold crops upright, and resist erosion and compaction, and “weak” enough to allow unrestricted root growth and proliferation of soil flora and fauna (Reynolds et al., 2002). This soil should also present fluid transmission and storage characteristics that allow the correct proportions of water, nutrients, and air for both maximum crop performance and minimum environmental impact. Assessment of physical qualities such as soil infiltration and aeration, water holding capacity, drainage, aggregate stability, and compaction allow for a comprehensive understanding of soil physical properties and their impact in soil health.

Some of the most common soil physical indicators to assess the previously mentioned soil functions are bulk density, water infiltration, and aggregate stability. Bulk density is a routine assessment that characterizes soil compactness in response to land use and management and is related to soil functions like aeration and infiltration (Reynolds et al., 2009). Soil structure is physical characteristic that determines the resistance of soil aggregates to external energy such as high intensity rainfall and cultivation (Moebius et al., 2007). Aggregate stability is a key indicator for assessing soil structure and can provide information about the capacity of a soil to function as it is directly linked with soil aeration and water movement (Aksakal et al., 2020). Finally, soil water infiltration represents the rate at which water enters the soil surface and moves through the soil profile and its assessment its crucial because slow infiltration rates can lead to issues like runoff, waterlogging, nutrient loss, and flooding (Joel and Messing, 2001). Other relevant soil physical indicators may be porosity, soil available water, or rooting depth (Allen et al., 2011). All the previous variables can change significantly with soil management; thus, they are suitable soil health indicators.

Adding cover crops represents an input of above- and below-ground biomass, which can improve soil physical proprieties and protect the soil from water and wind erosion (Fronning et al., 2008). Several cover crops studies showed an increase in aggregate stability by promoting the formation of soil aggregates through their root systems (Hermawan and Bomke, 1997; Liu et al., 2005a). In a study conducted by Folorunso et al. (1992) cover crops had a positive impact on soil by reducing penetration resistance and increasing infiltration under conventional tillage on a sandy loam and loam. Similarly, Liu et al. (2005) found that cover crops increased mean weight diameter and percentages of water-stable 2- to 6-mm aggregates.

However, cover crops effect on soil physical indicators has not always been consistent (Fronning et al., 2008; Mubiru and Coyne, 2009; Villamil et al., 2006a). While Blanco-Canqui et al. (2011) and Haruna & Nkongolo (2015) found a decrease in bulk density of 3.5 to 4% in cover crop plots, a study conducted on a fine sandy loam in North Carolina did not report differences in bulk density when cover crops were included in the rotation (Wagger and Denton, 1989). The lack of significant cover crop effect in bulk density is difficult to explain and may be due to the highly variable nature of these measurements.

Chemical indicators

Chemical attributes of soil health are correlated with the capacity of providing nutrients for plant uptake, as well as retaining elements that are harmful for the environment (Cardoso et al., 2013; Kelly et al., 2009). The presence and amount of macro- and micronutrients in agricultural soils play a crucial role in determining the likelihood of deficiencies or toxicities. These factors, in turn, have a direct impact on crop productivity. Conventional soil testing methods for row crops primarily center on the analysis of soil inorganic chemistry, with a specific emphasis on the presence of essential plant nutrients such as nitrogen (N), phosphorus (P), potassium (K), and micronutrients and soil pH (O'Neill et al., 2021). Among chemical indicators, SOC, SOM, total N, mineral nutrients, cation exchange capacity (CEC), and soil pH, are the main properties used for soil health assessment, especially considering a soil's capacity to support high-yield crops (Kelly et al., 2009).

Soil organic matter represents a continuum of organic compounds undergoing progressive decomposition (Lehmann and Kleber, 2015). Derived from plant residues, microbial biomass, and other organic inputs, SOM comprises a diverse array of materials that undergo several transformations within the soil matrix. As a foundational element in assessing soil health, SOM encompasses carbon (C), N, sulfur, and various other elements. Soil total C is a vital constituent of SOM, encompassing both organic and inorganic C compounds. It plays a central role in several critical soil processes, including nutrient availability, water retention, aggregate stability, and microbial activity (Bennet et al., 2010). Nitrogen stands out as the most essential plant nutrient and is present in various chemical compositions within the soil (Cardoso et al., 2013). Total N is another important indicator that represents the sum of the organic N pool plus the inorganic N fraction (NO_3^- and NH_4^+) and together with SOM are the major determinants and indicators of soil fertility and quality and are closely related to soil productivity (Al-Kaisi et al., 2005; Huang et al., 2007; Reeves, 1997; Susanne and Michelle, 1998). Notably, changes in SOC and Total N are hard to detect in the short-term due to the slow rate of turnover and accumulation of these elements in the soil (Bunemann et al., 2018). Consequently, accurately assessing the impact of management practices and environmental factors on SOC and Total N requires long-term monitoring.

Because short-term changes in SOC and Total N are difficult to detect, measurements of specific fractions from these pools have been suggested. Permanganate oxidizable C (POXC, i.e., active C), a pool of labile soil C processed by microbes, frequently displays heightened sensitivity to alterations in management or environmental conditions compared to SOC. This relatively new method can quantify labile soil C rapidly and inexpensively by measuring the portion of SOM that can serve as a readily available food and energy source for the soil microbial community, thus helping to maintain a healthy soil food web (Culman et al., 2012; Six et al., 1998; Wander, 2004). Soil protein represents the largest pool of organic N in the soil, and it can reflect the size of the organic N pool being depolymerized and can serve as a reservoir of N that is subsequently released through mineralization (Roberts and Jones, 2008). The autoclaved-citrate extractable (ACE) method, previously thought to only measure glomalin produced by arbuscular mycorrhizal fungi (Hurisso et al., 2018) is now widely recognized as a procedure extracting large quantities of non-mycorrhizal proteins, and is now used as a soil health indicator that reflects the primary pools of organically-bound N, and thus as a measure of potential available organic N.

Phosphorus (P) is also a key nutrient for agricultural productivity and is the second most required nutrient for plant growth making it essential in assessments of soil quality (Cardoso et al., 2013). This nutrient plays a vital role in numerous plant physiological processes, such as photosynthesis, energy transfer, and cell division. However, despite its significance, P uptake and utilization by plants are often limited, with less than 1% of total soil P being readily available to plants (Hansen et al., 2001). Although plants absorb P primarily in orthophosphate forms from the soil solution, most P remains immobilized in soil due to absorption onto mineral surfaces and precipitation as secondary P minerals.

Soil pH is a key chemical indicator as it correlates directly with nutrient availability, influencing their uptake by plants (Costantini et al., 2016; Gatica-Saavedra et al., 2023). At low pHs (<5.5): availability of macronutrients (N, P, K, Ca, Mg, S) is reduced and availability of metal micronutrients (Fe, Mn, Zn) increases (Husnain et al., 2021). Conversely, at high pHs (>7): P, Zn, Fe, Mn, Cu are less available. These factors collectively contribute to understanding and managing soil fertility and productivity in agricultural systems.

Cover crops have the potential for improving soil chemical proprieties due to their large root systems that allow them to cycle nutrients from deep soil layers and release them near the soil surface during residue degradation (Cunha et al., 2011; Duda et al., 2003; Nascente et al., 2015; Pacheco et al., 2011; Torres and Pereira, 2008). Therefore, understanding the influence of cover crops on nutrient cycling may help improve overall nutrient use efficiency for subsequent cash crops (Fabian, 2009). Several studies have demonstrated that including cover crops in crop rotations significantly increases SOC compared to rotations without cover crops (Blanco-Canqui, 2022; Poeplau and Don, 2015), thus cover crops present a potential to increase organic C and therefore improve soil C sequestration. Other studies reported an increase in CEC and available P and K when including cover crops in rotations (Bernardi et al., 2005; Crusciol et al., 2010; Cunha et al., 2011; Santos and Tomm, 2003). Although some studies have not shown a significant effect of cover crops on soil pH (Adetunji et al., 2021), others indicate an increase in alkalinity in the top 0 to 5 cm soil layer (Sharma et al., 2018). Tang and Yu (1999) suggested that the direction and magnitude of pH changes with cover cropping depends on the concentration of organic anions and N in the added plant material and the initial soil pH, therefore pH may behave differently with different cover crop species.

Another important benefit of cover crops may be their ability to reduce agricultural nutrient and sediment loss (Duncan et al., 2019; González-Chávez et al., 2010; van Kessel et al., 2013; Kuhn et al., 2016; Snapp et al., 2005). Research suggests that cover crops can effectively reduce soil erosion and sediment loss by providing ground cover and enhancing soil structure, thereby preventing soil particles from being washed away by water runoff and reducing sedimentation in water bodies downstream (Blanco-Canqui, 2018). Regarding P, its accumulation on the soil surface has generally been identified as the principal source in both surface runoff and leachate (Kleinman et al., 2011; McDowell and Sharpley, 2001). While soil health practices are often effective at reducing sediment delivery, there is evidence of P stratification and accumulation when cover crops were included in a conservation tillage cropping system (Carver et al., 2022; Jarvie et al., 2017; Smith et al., 2016; Tiessen et al., 2010). Finally, nitrate losses represent another concern that cover crops may be able to mitigate. Winter cover crops can potentially reduce NO_3^- leaching in corn soybean crop rotations by taking up water and NO_3 during the months between corn (*Zea Mays* L.) and soybean [Glycine max (L.) Merr] maturity and planting (Dabney et al., 2001; Kaspar and Singer, 2011). Previous studies by (Jackson et al., 1993) also presented similar results, where NO_3−N losses from tiles draining fields with cover crops were 69–90% less than from tiles draining fields without cover crops during winter and spring.

Biological indicators

A healthy soil should deliver multiple ecosystem services and maintain active biological processes, such as C cycling and nutrient mineralization (Cardoso et al., 2013). Soil biological indicators provide information about how living organisms respond to changes in their environment, as they connect roots with soil, decompose SOM, and recycle nutrients (Jacoby et al., 2017a; Tahat et al., 2020a) Unlike chemical and physical indicators, they are highly susceptible to changes imposed on the environment such as land use and management, facilitating early predictions of disruptions in environmental sustainability (Wink et al., 2005a).

Microbial activity indicates a wide range of physiological activities mediated by soil micro-organisms that include soil respiration and soil enzyme activities, etc. Soil respiration represents CO_2 produced by microorganism respiration, and is measured as produced or consumed CO_2 under different in situ or laboratory conditions (Arias et al., 2005; Dalal and Moloney, 2000; Haynes, 2008). Similarly, enzyme activity characterizes microorganism activity in the plant soil system because of its close relation to nutrient cycling, easily measured, integrate microbial status and physicochemical soil conditions information, and respond rapidly to soil management (Aon et al., 2001; García-Ruiz et al., 2009). Activities of β-glucosidase and β-glucosaminidase are commonly assayed as indices of C, and C and N cycles, respectively (Acosta-Martinez et al., 2018). Glucosidase plays a crucial role by catalyzing the ultimate step in the breakdown of cellulose, a component of plant cell walls. This process serves as a significant energy source for the soil microbial community (Luo et al., 2017). Glucosaminidase is involved in the hydrolysis of chitin, a crucial structural element found in insects and fungal mycelia. The breakdown of chitin into amino sugars is of particular importance, given that amino sugars represent a primary source of mineralizable N in soil (Ekenler and Tabatabai, 2002).

There is extensive evidence that cover crops can improve biological proprieties and therefore, soil health (Koudahe et al., 2022a; Mullen et al., 1998). Several studies have reported a significant increase in SOC when implementing cover crops, mainly through the addition of plant residue in the cropping (Liu et al., 2005a; Poeplau and Don, 2015; Steenwerth and Belina, 2008; Wulanningtyas et al., 2021). Wood and Bowman (2021) presented data from a farmer-led trial of cover crops on 1,522 strip-years across 9 US states over 5 years, and found active C increased 2.2

ppm more per year on cover crop strips than no cover crop strips. Similarly, Liang et al. (2014) found cover crop treatments significantly increased β-glucosidase and β-glucosaminidase activity in two study sites in southeastern USA. A meta-analysis compiling results from 60 relevant studies showed CO_2 respiration and β-glucosaminidase rate suggested positive cover crops effects on soil microbial activity (Kim et al., 2020). Cover cropping has also been demonstrated to increase soil protein levels. Feng et al., (2021) found an increase in soil protein when winter rye or a cover crop mix was included in a corn-soybean rotation in Iowa and South Dakota with a similar soil texture to the present in this study (silty clay loam). On the other hand, no differences in soil protein were observed when cover crops were included in a no-till dryland soybean system in Mississippi (Pokhrel et al., 2021).

Role of cover crops in cropping systems and soil health

The maintenance of soil health in agriculture systems is essential for sustaining agricultural productivity. Through its extensive positive impacts on various soil physical, chemical, and biological processes, keeping soil covered can be a key component of sustainable agricultural production systems. Cover crops, defined as any living ground cover planted into or after a cash crop that is commonly killed before the next cash crop is planted (Hartwig and Ammon, 2002), can play a crucial role in maintaining soil cover and, ultimately, soil health. By effectively providing continuous ground cover between cash crop cycles, cover crops help to mitigate soil erosion, reduce nutrient leaching, enhance water retention, and suppress weed growth (Blanco-Canqui et al., 2015). Typically, cover crop species include legumes, brassicas, and grasses (Chapagain et al., 2020), and each of them play different roles when providing services in agriculture systems.

The main agricultural advantage of legume cover crops, such as crimson clover (*Trifolium incarnatum* L.), hairy vetch (*Vicia villosa* L.), and sunn hemp (*Crotalaria juncea* L.), lies in their ability to fix atmospheric N via symbiosis with root nodule bacteria, *Rhizobium* (Hatch et al., 2007; Ledgard and Steele, 1992). When used as cover crops or with the intention of providing sources of nutrients as living or dead plants, legumes may build soil N and supply it to the subsequent crop (Koudahe et al., 2022a), which is of great importance, especially in areas where synthetic fertilizers are scarce or costly (Dabney et al., 2001). All N fixed by the plant will be returned as a direct contribution to soil organic N (Maseko et al., 2020:4; Zalak and Parthsinh, 2021), and the final amount of N supplied will depend on cumulative biomass production. Inoculation of legume seeds with the corresponding bacteria is essential for maximum efficiency of N fixation (SARE, 2012).

Grass cover crops, such as cereal rye (*Secale cereale* L.), sorghum sudan (*Sorghum* × *drummondii*), and triticale (×Triticosecale Wittmack), commonly produce fine and fibrous root systems with the potential to reduce soil erosion and improve soil structure when used as a cover crop (Mcgourty and Reganold, 2005). This dense network of roots helps bind soil particles together, preventing erosion caused by wind and water. Moreover, grass cover crops are selected for their ability to establish quickly and provide rapid ground cover, thereby outcompeting weeds for light, water, and nutrients (Baraibar et al., 2018). This rapid growth also shades the soil surface, creating an unfavorable environment for weed seed germination and growth. Additionally, grass cover crops support a diverse community of beneficial soil macro- and micro-organisms (Vukicevich et al., 2016). The root exudates released by grasses provide a food source for soil microbes, fostering their growth and activity.

Finally, non-legume broadleaf species like brassicas are also popular as cover crops. Brassicas and legumes typically have a lower C:N ratio than grasses at maturity and therefore have the capacity for rapid residue decomposition, releasing nutrients and enhancing soil structure. In addition, brassicas such as radish (*Raphanus sativus* L.) and turnip (*Brassica rapa var. rapa* L.) are characterized by their ability to penetrate compacted soils with their taproots, which makes them a suitable option when dealing with soil compaction (Blanco-Canqui and Jasa, 2019; Chan and Heenan, 1999; Haynes and Beare, 1997). Other common brassica cover crops, like canola (*Brassica napus*, L.) are prized for their rapid growth, high biomass production, and dense canopy, all of which improve soil structure, increase organic matter content, and suppress weeds (Haramoto and Gallandt, 2004).

Cover crops can improve agricultural soil condition and sustainability and thus, soil health, by controlling soil erosion, maintaining soil moisture, suppressing weeds, enhancing C sequestration, improving soil and water quality, controlling insect and pest populations, and providing economic benefits (Sharma et al., 2018). A study conducted by García-González et al. (2018) across a large area of irrigated crops in Madrid, Spain, found that cover crops promoted C sequestration at a rate of 180 kg C ha^{-1} $year^{-1}$ and N retention at a rate of 13 kg N ha^{-1} $year^{-1}$. In the same experiment, a barley (*Hordeum vulgare* L.) cover crop had enhanced soil structure, water holding capacity, infiltration rate, and saturated hydraulic conductivity in contrast to those characteristics in the fallow treatment, with the vetch cover crop treatment having an intermediate effect. The barley cover crop also mitigated nitrate leaching risk by reducing inorganic N content in the top 4 m of the soil profile. In another study, Mitchell et al. (2017) analyzed the effect of cover crops on soil health in a long-term trial in California, where soil aggregation, water infiltration, C and N content, water extractable organic C and organic N, residue cover, and biological activity were all increased by both no-tillage and cover crops relative to standard tillage and no cover cropping, indicating an overall increase in soil health. Clearly, cover crops provide a wide range of benefits for soil health.

Corn response to cover crops

Although cover crops have the potential to offer both agronomic and environmental benefits, their effects on subsequent crop yields have been reported to vary considerably depending on the region, soil type, and management practices employed (Marcillo and Miguez, 2017). Despite farmers acknowledging the value of winter cover crops in safeguarding the soil and environment, studies have revealed that knowledge gaps related to their costs, management, and apprehensions about potential impacts on yields have curtailed more widespread adoption (Singer et al., 2007).

Several studies explain how legume cover crops fix atmospheric N_2 and have the potential to improve N availability through its residue mineralization, and consequently, potentially help improving corn yields (Mahama et al., 2015; Marcillo and Miguez, 2017). However, the magnitude of N supplied varies among studies because winter cover crops respond differently across regions, soils, climates, and management practices. Pott et al. (2021) estimated that a hairy vetch cover crop supplied biologically fixed N equivalent to approximately 45 to 151 kg ha^{-1} of fertilizer N to corn in a field experiment in Brazil. Similarly, cover crops such as hairy vetch crimson clover, and sunn-hemp, have been reported to supply 55 to 130 kg N ha^{-1} to maize (Balkcom and Reeves, 2005). One major limitation for planting cover crops before corn in Northeast Kansas may be the early planting dates compared to other grass crops like sorghum [Sorghum bicolor (L.) Moench] (Staggenborg et al., 1999). This, in turn, may determine the length of the growing season and limit the growth of some cover crop species (Baraibar et al., 2020).

Another area of interest relies on the potential of cover crops to suppress weeds. Cover crops contribute to weed suppression through various mechanisms, including competition (Creamer et al., 1996; Mennan et al., 2020), smothering (Islam and Sherman, 2021), or allelopathic activity (Kunz et al., 2017; Osipitan et al., 2018). For instance, weed biomass was reduced by 98% with winter triticale cover crop relative to weedy fallow in western Kansas (Petrosino et al., 2015). Cover crop mixtures are an attractive alternative to suppress weeds, because mixtures produce more above-ground biomass than constituent species grown alone (Antosh et al., 2020).

Although cover crops have demonstrated several agronomic benefits, their impact on subsequent crop yields can vary. While some studies show no or minimum yield reductions due to cover crops (Chim et al., 2022; Jacobs et al., 2022; Raimondi et al., 2023) others document significant yield losses with the implementation of this practice (Alvarez et al., 2017; Garba et al., 2022; Spencer et al., 2022). The introduction of cover crops in corn systems imposes several limitations that can directly affect corn yield. One such limitation is the concern over water availability, because cover crops use soil moisture that would be available for the subsequent corn, potentially inducing water stress during critical growth stages. A recent study evaluating maize and soybean yields in the US revealed that planting non-legume cover crops could lead to a 3.9 ± 3% reduction in maize yield primarily due to intense competition for resources, including water and N (Qin et al., 2021). Immobilization of N by cover crops, which depends on the amount of biomass produced as well as the C/N ratio, may result in reduced N availability for the following cash crop. Several studies have reported N immobilization by decaying cover crop grasses residue that negatively affected corn development and yield (Kessavalou and Walters, 1999; Marcos et al., 2023, Fontes et al., 2017).

Another significant factor to consider is the impact of cover crop termination dates on corn yield. Delaying the termination of a cover crop can significantly impact maize yield through various mechanisms. Firstly, late-terminated cover crops continue to deplete soil moisture, which is essential for optimal maize growth and development (Rosa et al., 2021). This excessive consumption of soil water limits the availability of moisture crucial for germination, root development, and overall crop growth during critical stages of maize growth. Moreover, late-terminated cover crops prolong the period of competition with the emerging maize crop for essential resources such as sunlight, nutrients, and space. Lastly, delayed termination of cover crops can result in delayed planting of maize, further exacerbating yield losses (Qin et al., 2021). Further research is crucial to understand the specific responses of corn to different cover crop species, soil types, weather conditions, and management strategies.

Project objectives and hypotheses

Although the literature suggests the potential of cover crops to enhance soil health, long-term studies are essential for comprehensively understanding their regional benefits and potential drawbacks. The objective of this study was to evaluate the impact of various intensification strategies on soil health indicators within a three-year no-till winter wheat – corn – soybean cropping system in northeast Kansas. More specifically, the study aimed to assess (i) how different preceding cover crop types and varying N rates applied to corn influence pH, SOC and nutrient concentration and distribution in the soil profile, and (ii) the effects of different intensification strategies on soil biological indicators and their persistence throughout subsequent years of the rotation.

The research hypotheses were as follows: (i) Cover crops will contribute to heightened stratification of SOC, total N, and immobile nutrients as time progresses, while pH stratification will decrease. Furthermore, we anticipate that higher N rates will correspondingly decrease pH and available P near the soil surface and increase SOC and Total N concentrations; (ii) cover crops will enhance soil biological indicators immediately following cover crop termination and throughout the subsequent years of the crop rotations, with the greatest benefits anticipated immediately after termination.

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Chapter 2 - Assessment of Soil Organic Carbon, pH, and Nutrient Dynamics After Fifteen Years of No-Till and Cover Crops in Northeast Kansas.

Abstract

Conservation agriculture, known for minimal soil disturbance and permanent soil cover, offers strategies to address soil degradation associated with conventional management practices like conventional tillage and prolonged fallow periods. Although conservation agriculture practices have demonstrated benefits such as increased soil organic carbon (SOC) and reduced erosion, they can also result in nutrient stratification within the soil profile and surface soil acidification over time. A long-term experiment consisting of a no-till, three-year wheat (*Triticum aestivum*), sorghum [*Sorghum bicolor* (L.) Moench], soybean [*Glycine max* (L.) Merr] rotation was established in 2007 near Manhattan, Kansas. All phases of the three-year rotation were present every year. Sorghum was replaced with corn (*Zea mays* L.) in 2020. After five cycles of this rotation, soil samples were collected from plots that were between the corn and soybean phases in 2022 and 2023 to determine how different cover crop types preceding corn and different Nitrogen (N) rates applied to corn affected soil pH, SOC, and nutrient concentration and their distribution in the soil profile. The different fallow management imposed between wheat and corn included chemical fallow (CF), double-crop soybean (DSB), and four cover crop treatments. The N fertilizer was subsurface banded at five different rates soon after planting the corn. Profile soil samples were divided into five depth increments (0-5, 5-10, 10-15, 15-30, and 30-60 cm) and analyzed for soil organic carbon (SOC), total N, pH, and available phosphorous (P). Fallow management improved SOC stratification in the cover crops and DSB treatments compared to CF, with an average

increase of 0.3 Mg C cm^{-1} ha^{-1} at the surface layer. Similarly, Total N stratification was enhanced by all treatments compared to CF, with a 12% increase at the 0-5 cm layer. Soil pH stratification slightly decreased with cover crops and DSB with an average increase in 0.2 units at the 0-5 cm layer. Increasing N rates greatly impacted pH with a decrease of 0.5 units of pH with the highest N rate at the surface layer. Phosphorus removal by DSB decreased Melich-3 P in all soil layers except for the 30-60 cm and remained similar to CF for most cover crops. Overall, cover crops and intensification alternatives like double-cropping showed potential to improve carbon and N levels in surface soil layers and enhance its capacity to buffer changes.

Introduction

Productive agricultural soils are essential for sustaining the food needs of a growing global population (Thaler et al., 2021). However, erosion-induced degradation of soil quality diminishes crop yields, potentially exacerbating food insecurity. This phenomenon is partly attributed to conventional management practices such as intensive tillage and prolonged fallow periods. Conservation agriculture (CA), which usually includes minimal soil disturbance and permanent soil cover combined with diverse crop rotations offers alternatives for dealing with soil degradation problems (Hobbs et al., 2007).

Evidence exists that implementation of CA can reduce erosion and nutrient runoff (Van Pelt et al., 2017), and increase soil organic carbon (SOC) (Blanco-Canqui and Lal, 2008; Lal, 2004; Nicoloso and Rice, 2021). Continuous residue additions associated with minimum soil disturbance gradually promote the stratification of SOC in the soil profile (de Oliveira Ferreira et al., 2013). Although this approach is a promising alternative for addressing soil degradation, it is important to consider and accurately quantify potentially negative effects over time. Indeed, other studies documented that CA practices could result in vertical stratification of nutrients in the soil profile (Fernández and Schaefer, 2012; Mallarino and Borges, 2006). Several studies reported a decrease in soil pH, and an increase in immobile nutrients such as phosphorus (P) near the soil surface resulting from CA practices (Blanco-Canqui et al., 2011; Crozier et al., 1999). This suggests a potential environmental hazard for water quality, particularly if the P near the surface leaves the field through runoff and enters water bodies, thus contributing to eutrophication. Mitigation strategies to address these concerns may include an adequate nutrient management plan that considers 4R nutrient stewardship (Johnston and Bruulsema, 2014), as well as fallow management such as planting cover crops to improve SOC, reduce soil erosion, and minimize nutrient loss (Daryanto et al., 2018). Long-term studies are necessary to capture the gradual changes and long-term trends in soil chemical properties that may not be evident in short-term assessments.

Heavy machinery utilized for tillage and excessive chemical inputs (e.g., fertilizers, pesticides, herbicides), as well as monoculture crop cultivation and, in some cases, groundwater exploitation for supplemental irrigation are some common conventional agricultural practices that have led to a progressive decline in soil quality (Daryanto et al., 2018; Lal et al., 2015). Although soil loss is the most visible consequence of those practices (Thaler et al., 2021), other well-known consequences of tillage-based conventional agriculture include SOC decline, water runoff, and soil erosion (Derpsch, 1991).

Moreover, most nitrogen (N) fertilizer sources eventually convert to the nitrate-N form, which is not held tightly by soil particles and can be lost by leaching (Grant et al., 2006), with N losses increasing substantially with increasing fertilization (Bergström and Brink, 1986). The addition of synthetic N (particularly from ammonium-based fertilizers) is an important factor in enhancing soil acidification (Tian and Niu, 2015) as a result of the nitrification process where NH_4^+ is transformed to NO_3^- releasing protons in the process (Shi et al., 2019). As pH decreases, crops can experience toxicities of aluminum and manganese, as well as deficiencies of P, base cations (calcium, potassium, and magnesium), and molybdenum (Dai et al., 2017). The fact that nutrients are unavailable for plant uptake, along with the increasing availability of toxic forms, can decrease soil quality and reduce crop yields.

With all these challenges, CA practices known for increasing soil health and providing ecosystem services such as climate moderation, food security, nutrient cycling, and erosion control have gained importance in recent decades (Jayaraman et al., 2021). No-till, crop rotation, and cropping system intensification with double-cropping and cover crops are sustainable agricultural practices that can potentially alleviate soil degradation by increasing soil organic matter (SOM) via the input of crop residues (Caviglia et al., 2011; Duval et al., 2018; Villamil et al., 2006). Crop intensification refers to the practice of increasing the productivity and efficiency of crop production within a given area of land. As an example, double cropping is a farming practice in which two different crops are grown sequentially on the same piece of land within a single growing season and is commonly adopted in parts of Kansas as winter wheat-soybean double-cropping systems (Kelley, 2003). Double-crop soybeans have the potential to enhance soil health by facilitating N_2 fixation and improving SOM with the residue they leave behind (Amuri et al., 2008).

Another alternative intensification practice is the use of cover crops, defined by the Soil Science Society of America as "close-growing crops that provide soil protection and soil improvement between periods of normal crop production, or between trees in orchards and vines in vineyards" (SSSA, 1997). Introducing cover crops in crop rotations can provide several agronomic benefits such as improved soil structure and aggregation (Blanco-Canqui and Ruis, 2020; Liu, et al., 2005b), water infiltration (Folorunso et al., 1992b; Haruna et al., 2020), nutrient cycling (DuPont et al., 2009), SOC and biological activity (Adetunji et al., 2020; Jian et al., 2020), and decreased nutrient loss through leaching or runoff (Abdalla et al., 2019; Blanco-Canqui, 2018). Moreover, legume species can fix atmospheric N_2 through a symbiotic relationship with bacteria, providing available N for the subsequent cash crop (Kuo and Sainju, 1998; Quemada and Cabrera, 1995). Most of these benefits are highly dependent on cover crop management, including planting and termination dates, as well as the amount of biomass produced, and species selected for the cover crop (Adetunji et al., 2020).

Despite cover crop's potential to provide several benefits to cropping systems, other studies suggest that as nutrients move from deeper soil layers to cover crop above-ground plant tissue, the decaying tissue on the soil surface enhances nutrient stratification (Chen and Weil, 2010; Smith et al, 2017). This is particularly true under no-till systems, where the lack of soil disturbance can cause nutrient stratification because most of the changes in soil properties occur near the surface (Blanco-Canqui and Ruis, 2018). In some ways, the degree of carbon (C) stratification in the soil profile can be considered a good indicator of soil quality, because surface SOM is essential for erosion control, water infiltration, and conservation of nutrients (Franzluebbers, 2002). However, a potential negative consequence of cover crop no-till systems could be increased P concentration near the soil surface, resulting in greater dissolved P (both organic and inorganic forms) in runoff water (Carver et al., 2022; Smith et al., 2017). However, other studies highlight that cover crops can potentially reduce the environmental impacts of P by minimizing erosion, runoff, and leaching rates via greater soil P retention (Horst et al., 2001; Maltais-Landry et al., 2015). Cover crops can also convert low-availability P forms retained in soils into labile P by altering rhizosphere properties such as pH and enzyme activity.

In the context of potentially problematic unintended consequences from CA practices, a better understanding of how different cover crops and N rates may affect pH, SOC, and nutrient concentrations and stratification in the soil profile is needed. The objective of this study was to determine how different cover crop types preceding corn and different N-rates applied to corn affect pH, SOC, and nutrient concentration and distribution in the soil profile after five cycles of a no-till wheat-corn or sorghum-soybean rotation. Producers looking to include cover crops in their crop rotations can benefit from gaining a comprehensive understanding of the advantages and disadvantages associated with intensification in the central Great Plains region. We hypothesize that the incorporation of cover crops into agricultural systems will lead to significant improvements of SOC, total N and pH at the surface through increased organic matter. Moreover, this increase in surface layers will heighten stratification of SOC, total N, and immobile nutrients as time progresses, while pH stratification will decrease due to an enhanced buffering capacity in the surface. Furthermore, we anticipate that higher N rates will correspondingly decrease pH and available P near the soil surface and increase SOC and Total N concentrations.

Material and methods

Site Description and Experimental Design

The study consisted of a three-year wheat (Triticum aestivum), corn (*Zea mays* L.), soybean [Glycine max (L.) Merr] rotation, where all phases were present every year and were randomized within each replication. The long-term experiment was established in 2007 at the Kansas State University Research Farm (39º 07’ N lat, 96º 38’ W long) located approximately 8 km south of Manhattan, KS. The altitude is approximately 311 m, and the region is characterized by a hot humid continental climate (Köppen Climate Classification System: Dfa). The mean annual temperature is 12.9 ºC and the average annual precipitation is 889 mm. Precipitation data from 2008 to 2016 is reported in Fontes et al., (2017). The soil is a moderately well-drained Wymore silty clay loam (fine, smectite, mesic Aquertic Argiudoll), with 0 to 1% slopes. Since the initiation of this study, no lime application has been conducted, with an initial soil pH averaging 6.1 at the 0-15 cm soil depth (Arnet, 2010).

The experimental design was a randomized complete block design with a split plot arrangement, with four replications of each phase of the rotation. Fallow management treatments imposed between wheat harvest and corn planting were set as whole plots, and five N-rates (0, 45, 90, 180, and 270 kg ha^{-1}) during the corn phase were subplots. Every whole plot was 6 by 70 m, and each subplot was 6 by 14 m. Fallow management consisted of chemical fallow (CF), double crop soybean (DSB), plus four cover crop treatments: crimson clover (CC), cereal rye (CR), cereal rye and crimson clover mix (CR/CC), and a diverse seven species cover-crop mix (DM). The N fertilizer treatments during the corn phase were applied as 28% urea ammonium nitrate (UAN) in subsurface bands roughly 19 cm on either side of each corn row at corn developmental stage V4-6 (Ritchie, Hanway, and Benson 1989)

Cropping system management

Winter wheat and cover crop phase

Winter wheat was planted immediately after soybean harvest on October 24, 2019, and October 15, 2020, with a target seeding rate of 130 kg ha^{-1} drilled on 19-cm rows using a John Deere 1590 no-till drill (Deere & Co., Moline, IL). Diammonium phosphate was applied at planting at a rate of 130 kg ha^{-1} to supply 60 kg P ha^{-1} and 23 kg of N ha $^{-1}$. In February 27 of 2020 and March 22 of 2021, wheat was top-dressed with 67 kg N ha^{-1} soon after Feekes stage 4-5 (Miller, 1992) using 28% UAN, applied in streams spaced every 10 cm. Weeds were controlled by applying 2-methyl-4-chlorophenoxyacetic acid and 10.5 g a.i. ha^{-1} of tribenuron-methyl (MCPA Ester and Harmony® Extra SG) at Feekes stage 4-5 in both years. Winter wheat was harvested on June 26, 2020, and June 22, 2021.

Chemical fallow and DSB fallow management treatments remained constant throughout the years, whereas cover crop treatments varied since the initiation of the study. See details about the first three cycles in Preza et al. (2017) and fourth cycle in Nielsen (2020). In 2019, DSB was planted in all plots except for the CF plots. The current set of winter cover crop treatments was planted on September 2, 2020, and September 10, 2021. The target seeding rate was 67 kg ha^{-1}

for CR, 28 kg ha^{-1} for CC, 34 kg ha^{-1} for CR and 14 kg ha^{-1} for the CC in the CR/CC treatment, and 75 kg ha^{-1} for DM. Non-hardy winter cover crop species were killed by freezing temperatures, which usually occurred during November. On April 14 of 2021 and April 26 of 2022, cover crop plots were terminated by spraying with a mix of glyphosate, 2,4-D, and dicamba in 2021, and a mix of glyphosate, 2,4-D in 2022 to terminate surviving cover crops, volunteer wheat, and other winter annual weeds.

Double-crop soybean was planted on July 2 of 2020 and July 9, 2021, with a target seeding rate of 445,000 seeds ha^{-1}. At DSB planting, CF and DSB plots were sprayed with mix of paraquat, glyphosate, and flumioxazin and pyroxasulfone in 2020 and flumioxazin and pyroxasulfone in 2021 at label rates to control volunteer wheat and other weeds. In addition, 44 kg P ha^{-1} was applied to all plots at DSB planting in both years. Chemical fallow and DSB plots were sprayed again with glufosinate on August 15 of 2020 and with glufosinate and clethodim on August 5 of 2021 to maintain plots free of weeds and volunteer wheat. A third application was done in the CF treatment with a mix of paraquat and glyphosate on September 9 of 2021. The DSB plots were harvested with a combine on November 2 of 2020 and hand-harvested on November 5 of 2021. All CF and DSB plots were sprayed with the same herbicides applied to terminate cover crop treatments to control volunteer wheat, and other annual winter weeds.

Corn phase

Initially, the rotation consisted of wheat, grain sorghum, and soybean (Fontes et al. 2017). However, grain sorghum was replaced by corn beginning in 2020. Corn was planted on April 30 of 2021 and May 11 of 2022. The target seeding rate was 69000 seeds ha^{-1} of Pioneer P0977AM hybrid in 2021 and 67000 seeds ha^{-1} of Pioneer P0995AM hybrid in 2022. The N fertilizer rates were applied as 28% UAN with 45 kg ha^{-1} applied at planting and the balance applied at V4-6

(June 3 of 2021 and June 15 of 2022). The UAN was applied with a flat-colter liquid fertilizer applicator to inject N fertilizer below the residue layer. No P or K fertilizer was applied in the corn phase. Weeds were controlled with a pre-emergence herbicide application applied within 2 days of planting and a post-emergence herbicide application approximately 20 days after planting (Mezotrione, S-Metalochlor. Atrazine, Glyphosate, and AMS in May 2021 and AMS, Acuron, MSO, 2,4-D and Glyphosate in May 2022). After corn reached physiological maturity and had dried sufficiently for machine harvest, yield was determined by harvesting plants from the center two rows of each subplot with a combine on September 17, 2021, and October 4, 2022.

Soil sampling and laboratory analysis

Plots that had been planted to corn in the previous growing season were sampled in March 2022 and 2023 by taking two cores (4-cm diameter) from each plot. The cores were collected to a 60-cm depth using a tractor-mounted hydraulic probe (Model GSRTS, Giddings Machine Company, Inc., Windsor, CO), dividing each core into five depth increments (0-5, 5-10, 10-15, 15-30, and 30-60 cm), and compositing by depth for a total of five samples per plot. Samples were dried in a forced-air oven at 50 °C until constant weight, ground, and passed through a 2-mm sieve using a soil grinder equipped with a ceramic mortar, steel screw type grinding head, and stainless-steel screen (Nasco-Asplin, Fort Atkinson, WI). After grinding, samples were submitted at the Kansas State University Soil Testing Laboratory to be analyzed for SOC, Total N, pH and P.

Soil pH was measured potentiometrically from 1:1 soil-water slurries using a dual electrode robotic pH analyzer (Skalar SP50, Bufurd, GA & Breda, Netherlands) equipped with glass pH electrodes. Ten-gram aliquots of soil and 10 mL of deionized water were dispensed into cups, stirred for 5 seconds, and allowed to stand for 10 minutes (Peters et al., 2012). Subsequently, the sample was stirred again, and the pH was recorded by the instrument. Phosphorus concentrations were determined using the Mehlich-3 method (Frank et al., 1998). Two grams of soil were reacted with 20 mL of Mehlich-3 extracting solution in flasks and shaken for 5 minutes. The suspensions were then filtered, and the concentration of orthophosphate in the filtrates was quantified via molybdate-blue colorimetry at 660 nm using a flow injection analyzer (Lachat QuikChem 8500 Series 2, Hach Company, Loveland, CO). Total C and N were determined by direct combustion using a C/N analyzer (Model LECO TruSpec, LECO Corporation, St. Joseph, MI). Total C was assumed to be equivalent to SOC due to the absence of carbonate in the soil (Pribyl, 2010).

During 2023, bulk density sampling was carried out using two methodologies. In spring 2023, a 4-cm diameter core for the depths of 15-30 cm and 30-60 cm was obtained for each fallow management, using a tractor-mounted hydraulic probe. The collected samples were dried in a forced-air oven at 105°C until a constant weight was achieved. In the summer of 2023, soil samples were gathered from depths of 0-5 cm, 5-10 cm, and 10-15 cm, employing the method described by (Blake and Hartge, 2018). The rings were pushed into the soil surface using a slide hammer and wooden block and then placed into an autoclavable plastic bag to be dried at 105°C until constant weight was reached. For both methodologies, the resulting dry weight was used to calculate the bulk density (Equation 1). We assumed that bulk density remained constant over time due to the lack of soil mixing and low machinery traffic overall and that the N rate did not significantly affect the bulk density. Bulk density values were used to transform nutrient concentrations into nutrient per volume of soil (Equation 2). Later, profile nutrient stocks were calculated as the sum of the nutrient per volume of each layer of soil multiplied by each layer depth (Equation 3).

$$Bulk\ density\ \left(\frac{g}{cm^3}\right) = \frac{Mass\ of\ dry\ soil\ (g)}{Soil\ core\ volume (cm^3)} \qquad \text{Eq. [1]}$$

$$\text{Nutrient per volume} \left(\frac{mg}{cm^3}\right) = Nutrient\ concentration \left(\frac{mg}{kg}\right) \times Bulk\ density \left(\frac{kg}{cm^3}\right) \qquad \text{Eq. [2]}$$

$$Stocks\ \left(\frac{Mg}{ha\ cm}\right) = \frac{\sum \text{Nutrient per volume}\left(\frac{mg}{cm^3}\right)}{10\ \frac{ha\ mg}{cm^2\ Mg}} \qquad \text{Eq. [3]}$$

Statistical analysis

Data were analyzed assuming a split-plot randomized complete block design, where fallow management was the whole-plot factor, and N rate applied to the corn crop was the subplot factor. Data were tested for normal distribution and equal variances using the Shapiro-Wilk Test and Levene's Test to fulfill assumptions for ANOVA. The effects of Fallow management, N rate, and their interaction at each Depth on nutrient stocks and pH were evaluated using ANOVA, where the Year and block were included as random factors, and Fallow management and N rates were fixed effects. We considered Year as a random factor in the ANOVA to account for potential variability between study years, considering the stability of soil chemical properties over time. The Fallow management by block interaction was used as the error term to test Fallow management effects, and the residual error was used to test N-rate and Fallow management by N-rate effects to account for the split-plot treatment structure. Main effects and all interactions were considered significant at $P \leq 0.1$. If Fallow management x Depth, N x Depth or Depth main effects were significant, an ANOVA was conducted by depth. Pairwise differences were performed to assess the difference between the least squares means of the treatments. Both ANOVA and mean separation differences were carried out using PROC GLIMMIX in the SAS® software (SAS Institute Inc, Cary, NC).

Results and discussion

Temperatures and precipitation

Manhattan, Kansas, typically has a temperate climate with distinct seasons. The 30-year normal values of precipitation and temperature for Riley County are presented in Figure 2.1.

Over the last 30-years, the average winter temperatures averaged 0°C, while the summer average temperatures were 25°C (Figure 2.1; Kansas Mesonet, 2024). Rainfall was unevenly distributed throughout the year, with May and June typically being the wettest months.

Weather conditions from wheat harvest until corn planting were evaluated due to the direct relation between water uptake by cover crops during their growing period and the benefits they can provide to a cropping system (Blanco-Canqui et al., 2015). Cumulative precipitation preceding the 2021 corn growing season exceeded the 30-year normal values for Riley County by 11 % (Table 2.1, Figure 2.1, Kansas Mesonet, 2024). On the other hand, cumulative precipitation preceding 2022 corn was 8% less than normal. During the corn growing season, cumulative precipitation was 10% less than normal in 2021 and 18% more than normal in 2022. Temperatures during the period preceding 2021 corn planting were close to normal, but maximum temperatures during the period preceding the 2022 corn planting were 2.1 °C higher than normal. During the corn growing season, temperatures were slightly higher than normal in both years. Particularly, maximum temperatures were 1°C and 1.6°C above normal in 2021 and 2022, respectively.

Soil organic C

The Fallow management × Depth interaction was significant, indicating that fallow management treatments influenced SOC stratification differently (Table 2.2). Both Fallow management and Depth main effects were significant. Average across the entire 0-60 cm sampling depth any treatment other than CF increased SOC stocks cm^{-1} by 4%. Given the significant Fallow management × Depth interaction, a two-way ANOVA evaluating Fallow management, N rate, and their interaction effects on SOC was conducted for each sampling Depth. Neither the Fallow management × N interaction nor the N main effect was significant at any depth (Table 2.3). Fallow management had a significant effect in the surface layers (0-5 and 5-10 cm) (Figure 2.3). On average, all treatments increased SOC by 13 % at the 0-5 cm layer and by 5% at the 5-10 cm layer when compared to CF. Averaged across Fallow management treatments, SOC decreased from 2.6 Mg C stocks per cm^{-1} in the 0-5 cm soil layer to 1.2 Mg C stocks per cm^{-1} in the 30-60 cm soil layer (Figure 2.2).

Soil organic C is the most often reported attribute in long term studies because of its impact on other chemical, physical and biological indicators of soil health (Reeves, 1997). In the present study, SOC stocks were higher in the topsoil and decreased with depth (Figure 2.). When associated with no-till, continuous residue input gradually promotes the stratification of SOC in the profile (de Oliveira Ferreira et al., 2013). For instance, Blanco-Canqui & Lal (2007) found that soils managed under no-tillage for 10 years, receiving 8 and 16 Mg ha^{-1} yr^{-1} of wheat straw mulch, developed a distinct dark soil layer <5 cm near the surface, with increases in SOC limited to the 0- to 10-cm depth. Contrary to other nutrients, stratification of SOC is considered an indicator of soil quality because surface SOM is essential to erosion control, water infiltration, and conservation of nutrients (Franzluebbers, 2002). Fallow management × N rate was not significant for any of the Depth increments (Table 2.3). Moreover, N rate did not affect SOC at any depths (Figure 2.2 B). This was unexpected given the fact that previous studies showed that high N rates can lead to increasing crop biomass and C input, which could be translated into more SOC (Presley et al., 2012). However, it's important to note that in this study, N rates were applied once every three years, unlike the annual application in the study by Presley et al. (2012).

Fallow management's main effect was significant for the surface layers (0-5 and 5-10 cm) (Figure 2.3). This aligns with research suggesting that this layer is most sensitive to agricultural management practices and root activity (Castellano-Hinojosa et al., 2023). In the 0-5 cm layer, all alternatives had significantly more SOC than CF (Figure 2.3 A), which may be explained by C inputs through elevated plant biomass production and reduced C loss by protection provided by soil cover (Mazzoncini et al., 2011; Sainju et al., 2002). The DSB, CR/CC, CC and DM treatments had 11% more SOC than CF, and CR had the highest SOC, with 24% more SOC than control. Soil organic C was slightly less with DSB compared to the cover crops, which have living roots in the ground for a longer period, while DSB is grown from July to November when it is commonly harvested in Kansas. Cereal rye had the largest SOC mass cm^{-1} for the 0-5 cm soil layer, which could be explained by its greater biomass production and therefore C inputs (Grünberg et al., 2024). Similar results were observed when comparing C inputs from grass species to those from legume cover crops in a silt loam in Santa Fe, Argentina (Duval et al., 2016). While the CR/CC treatment presented even higher cover crop biomass and thus C input, this set of winter cover crops was imposed since 2020, and the resulting SOC may be potentially affected by the variability in cover crop species used in previous years (Fontes et al., 2017; Nielsen, 2020). Although Finney et al. (2016) reported that cover crop mixes led to enhanced SOC accumulation compared to single species cover crops in a silt loam in Pennsylvania, this was not the case for our study where DM and CR/CC had similar SOC than CR alone. The amount of biomass from cover crops dictates C inputs and can consequently influence SOC levels (Huang et al., 2021). Although biomass production from CC was less than the rest of the treatments, SOC did not differ significantly within cover crops. This can be explained by the preference for low C:N residues to be incorporated into microbial biomass. As soil microbial biomass turns over and interacts with the soil matrix, it contributes to the formation of stable soil C (Cotrufo et al., 2013; Liang et al., 2007).

The cumulative SOC stocks across the 0-60 cm soil layer did not have significant Treatment × N interaction, Fallow management, or N rates main effects were significant (Table 2.3). However, SOC stocks in the 0-60 cm soil layer tended to be greater in DSB and cover crops compared to CF (Figure 2.4).

Total N

Neither the Fallow management × N interaction nor the Depth × N nor the N main effect were significant, but the Fallow management × Depth interaction and the Fallow management and Depth main effects were significant for Total N (Table 2.2, Figure 2.5). Across the entire 0-60 cm sampling depth any treatment other than CF increased total N by 4%. Given the significant Fallow management × Depth interaction, a two-way ANOVA evaluating Fallow management, N rate, and their interaction effects on Total N was conducted for each sampling depth. Neither the Fallow management × N interaction nor the N main effect was significant at any depth (Table 2.3). Fallow management's main effect was significant only in the 0-5 cm soil layer, with an average increase in Total N of 13% for all treatments as compared to CF (Figure 2.6). Averaged across Fallow management treatments, Total N decreased from 0.2 Mg N stocks per cm^{-1} at the 0-5 cm soil layer to 0.1 Mg N stocks per cm^{-1} at the 30-60 cm soil layer (Figure 2.5).

Total N represents the sum of all N forms in the soil and influences plant productivity and nutrient cycling (Bauer and Black, 1994). In this study, Total N was stratified near the soil surface and declined with depth (Figures 2.5 A and B). This pattern is because as OM breaks down, it releases C and N into the soil. Therefore, areas with SOC also tend to have more Total N (Gál et al., 2007). Fallow management significantly affected Total N only in the 0-5 cm soil Depth (Table 2.3, Figure 2.5 A). In this layer, CF had 12% less Total N than the remaining treatments which did not differ statistically from each other (Figure 2.6). Small increases in total N by the addition of cover crops or DSB in a crop rotation were previously documented by Sharma et al. (2018b) in a silt loam in Nebraska. Cover crop biomass and DSB residue can contribute to an increase in total N through both N uptake from the soil and N_2 fixation, particularly in legume species (Sainju et al., 2002). This N is then incorporated into the crop biomass and becomes part of the total N pool as it decomposes.

Previous long-term studies documented an increase in Total N from the addition of grass (Adetunji et al., 2021; Wulanningtyas et al., 2021) as well as legume cover crops (Mazzoncini et al., 2011). In a study conducted by Sainju et al. (2003) in a sandy loam in Georgia, crimson clover had greater Total N than cereal rye, suggesting that legumes may be more effective than non-legume cover crops in increasing soil N pools, probably due to increased N inputs from N fixation. However, no differences between legume (CC and DSB) and grass treatments were observed in the current study. Although Mazzoncini et al., (2011) also documented more Total N in the soil after legume cover crops compared to grasses, legumes had greater biomass production compared to the grasses in that study conducted in a loam soil in Italy. The significantly less biomass production of CC and compared to the other cover crops in our study may have limited their potential for increasing the Total N pool. Compared to cover crops, the DSB is combined-harvested, and the residue has some level of processing that can limit increases in C input and therefore Total N.

In the 5-10 cm layer, the Fallow management × N rate interaction was significant (Table 2.3). When single ANOVA was conducted for each Fallow management separately, N rate significantly affected Total N in the CC treatment and was not significant for the rest of the treatments. In this treatment, the lowest N rate presented the highest Total N, and contrary, the highest N rate (240-N) led to the lowest amount of Total N (Table 2.4). This inhibitory effect of N fertilization on nodulation and N_2 fixation of legumes, resulting in lower Total N has been documented previously (Li et al., 2009). Fallow management × N rate, and Fallow management and N rate main effects were not significant for any of the remaining Depths. Lastly, no Fallow management × N rate and Fallow management and N rate main effects were observed in Total N mass cm^{-1} (Table 2.2).

pH

In the present study, only the N rate × Depth, Depth, and Fallow management effects were significant for pH (Table 2.2). Across the entire 0-60 cm sampling depth any treatment other than CF increased pH from 5.9 to 6.0 (Figure 2.7). A two-way ANOVA evaluating Fallow management, N rate and their interaction effects on pH was conducted for each sampling Depth. While the Fallow management x N interaction was only significant at the 15-30 cm depth, Fallow management and N rate's main effects resulted significant in the surface layers (0-5 cm and 5-10 cm). Averaged across Fallow management treatments, pH increased from 5.8 in the 0-5 cm soil layer to 6.5 in the 30-60 cm soil layer.

Fallow management had a significant impact on pH in the 0-5 cm and 5-10 cm topsoil layers only (Table 2.3, Figure 2.7 A). At the 0-5 cm layer, CF had a lower pH than any other treatment (Figure 2.8 A). Cover crops have the potential to increase levels of SOM in the soil, which might stabilize the soil pH and help in plant nutrient uptake (Campbell et al., 1996). Although some studies found that cover crops did not affect soil pH (Sharma et al., 2018b), an increase in soil pH due to the addition of cover crops has been previously documented in four different sites on silt loam soils in Virginia (Strickland et al., 2019). The cover crop effect in this layer may be related to greater additions of organic residues and greater activity of micro and macro fauna and roots in the topsoil as compared to bare soil (Dozier et al., 2017). Slightly higher pH values were observed for the DSB, CC, and CR/CC treatments, with an average unit increase of 0.13 compared to CF. Greater values were observed for DM and CR, with the highest value for CR, which had 0.32 units greater than CF. This cereal cover crop treatment also had the highest SOC in this layer and thus organic matter (Figure 2.3), which can lead to increased buffering capacity in the soil (Campbell et al., 1996). As C input decreased among treatments, so did the buffering capacity (i.e. pH decreased). Compared to cover crops, the DSB is combined-harvested, and the residue left has some level of processing that can limit C input and therefore and consequently diminish buffering capacity. Moreover, lower soil pH in legumes like CC and DSB compared to grasses has been suggested to be a result of the effect of soil N_2 fixation, where legumes tend to take up more positively charged ions relative to negatively charged ions, further contributing to soil acidity in the rhizosphere (Maltais-Landry, 2015; Nuruzzaman et al., 2006). In the 5-10 cm layer, only the CR treatment presented a higher soil pH than any other treatment, which could be related to its high C input and consequently increased buffering capacity (Figure 2.8 B).

The initial pH at the start of the study in 2007 averaged 6.1 across N rates. However, in this recent sampling, the average pH in the 0-5 cm layer is 5.8 (Arnet, 2010). In the top 0-5 cm and 5-10 cm layers of the soil profile, increasing N rates resulted in an average pH unit decrease of 0.4 in pH observed from the 270-N compared to the 0-N, and the remaining soil layers were not affected by N rate main effect (Figure 2.7 B). Notably, with each additional 40 kg of N applied, there was a pronounced decline in pH. The application of N fertilizers to the soil surface under no-till has been shown to lead to the stratification of pH and other soil acidity parameters near the soil surface (Souza et al., 2023). In the 0-5 cm layer, pH decreased by 0.5 units when the highest N rate was applied, compared to the 0-N rate (Figure 2.9 A). In the following layer (5-10 cm), the decrease in pH when the highest N rate was applied was 0.2 units, compared to the 0-N rate (Figure 2.9 B). The decrease in soil pH with greater N application rates in the upper soil layers (0-5 cm and 5-10 cm) is supported by previous research showing that long-term N fertilization with increasing N rates can significantly reduce soil pH near the soil surface (Schroder et al., 2011), especially in fertilizers containing ammonium, since the transformation of NH_4^+ to NO_3^- by nitrification produces 2 H^+. The fertilizer utilized in this study was UAN, which contains urea and ammonium nitrate. For instance, when urea undergoes the hydrolysis process, it slightly increases the pH, however, the NH_4^+ released as a product of this reaction does through the nitrification process leading to a net acidification of 2 H^+ per 2 moles of urea-N (Bouman et al., 1995). Therefore, both urea and ammonium nitrate have the potential to decrease soil pH, and that may explain the decrease in soil pH in this study with increasing N rates.

In the 15-30 cm layer, a significant Fallow management x N interaction was observed for pH. When looking at each treatment individually, the only treatment affected by N rate main effect was CF, however when looking at differences between N rates means, they didn’t follow any specific pattern (Table 2.5). No significant Fallow management or N rate effects were observed for the 30-60 cm layer. The lack of Fallow management effect with depth could be attributed to the significant buffering capacity of the soil below a Depth of 5 cm (Sharma et al., 2018a).

Mehlich-3 phosphorous

All interactions and main effects of Fallow management, N rate, and Depth were significant for P (Table 2.2). Given this interaction, a two-way ANOVA evaluating Fallow management, N rate and their interaction effects on Melich-3 P was conducted for each sampling

Depth. The Fallow management by N interaction was not significant for any soil depth, and the Fallow. Fallow management and N rate main effects were significant for Melich-3 P at multiple depths (Table 2.3). Results from the current study revealed that P stocks were notably higher in the soil surface and gradually decreased with increasing soil depth, with an average of 5.7 Mg of P stocks per cm^{-1} at the 0-5 cm layer and 0.1 Mg of P stocks per cm^{-1} at the deepest soil layer (Figure 2.10). Phosphorus stratification is a typical outcome of no-till practices, where this immobile nutrient is found in higher concentrations near the soil surface compared to deeper layers (Chakraborty and Prasad, 2021; Kleinman et al., 2011; McDowell and Sharpley, 2001).

Fallow management significantly influenced P at all Depths, except the 30-60 cm soil layer (Figure 2.10 A). Starting in the top 5 cm of the soil profile (0-5 cm) lower stocks per cm^{-1} of P were observed in the DSB treatment compared to the CF, CR, and DM treatments, and the rest of the treatments did not differ from CF (Figure 2.11 A). Although previous studies indicate a potential for cover crops to increase P concentration near the soil surface (Carver et al., 2022; Smith et al., 2017), CF had either higher or similar P to most cover crop treatments in the current study. In the following layer (5-10 cm), CF had greater P stocks per cm^{-1} compared to R/C, DM, and DSB treatments, and did not differ from the rest of the treatments (Figure 2.11 B). Notably, DSB had the least P and was significantly less than DM, CR, CC, and CF, but did not differ from the CR/CC treatment. In the next layer (10-15 cm), the CF and CR treatments had greater P than DSB and CC but were not significantly different from DM and R/C (Figure 2.11 C). At the 15-30 cm soil layer, the DSB treatment had less P than CF, CR, and CR/CC treatments, and did not differ from the rest of the treatments (Figure 2.11 D).

The lower values for DSB could be explained by the fact that DSB removes an average of 6.3 kg of P per Mg of grain (Salvagiotti et al., 2021). In this study, the DSB grain yield averaged

1600 kg ha^{-1} over the years, therefore after five cycles of this crop rotation, that would be translated to an average P removal of 50 kg ha^{-1} by this treatment. The CR/CC treatment produced more biomass than other cover crops (Grünberg et al., 2024), which could have led to a greater P uptake at the 5-10 cm and 10-15 cm depths (Dube et al., 2014). Legume cover crops like CC, as well as radishes that are included in the DM treatment, have a greater soil P mobilization capacity, due to the exudation of malic and citric acids that enhance P solubility (Maltais-Landry et al., 2015; Soltangheisi et al., 2018). Therefore, more P could have been mobilized by the R/C, DM, and CC treatments from deeper soil layers to the above-ground tissue.

Nitrogen rate significantly affected Melich-3 P stocks per cm^{-1} in the 0-5 cm and 5-10 cm soil layers (Figure 2.10 B). At 0-5 cm, the highest N rates (180-N and 270-N) had higher P stocks per cm^{-1} than the 45-N and 90-N rates, and did not differ from 0-N (Figure 2.12 A). Lower pH at the higher N rates could have affected P availability and plant uptake (Figure 2.8). Acidic conditions could lead to the formation of insoluble forms of P, thereby impeding the uptake of P by corn roots (Penn and Camberato, 2019). At the 5-10 cm depth, lower P stocks per cm^{-1} in the 45-N and 90-N rates compared to 0-N may be related to higher plant productivity by reduced N limitation that could improve overall P uptake (Kamprath, 1987), however, no differences were observed compared to the higher N rates at this soil depth (Figure 2.12 B).

Conclusion

The integration of cover crops into crop rotations resulted in significant benefits for soil health, including increased SOC and total N in the upper soil profile. Furthermore, cover crops played a crucial role in buffering pH levels in the upper soil layers, maintaining a more neutral state around pH 7. Among cover crop treatments, those with greater C inputs resulted in more

SOC accumulation, while the DSB treatment contributed moderately to building SOC in the 0-10 cm surface layer. It is plausible that the observed increase in SOC would facilitate the storage and mineralization of more N, leading to the 12% rise in Total N observed in the 0-5 cm layer when the fallow period was reduced by planting either DSB or cover crops. Fallow management had a notable impact on soil pH, particularly at the surface layer, with grass cover crops exhibiting higher pH levels compared to legumes and CF, suggesting their potential to enhance soil buffering capacity. These findings support our hypothesis regarding the positive influence of integrating cover crops into agricultural systems on pH, SOC, and Total N concentrations. Although Mehlich-3 P was reduced by certain cover crop treatments in specific subsoil layers, the most significant impact on P stocks per cm^{-1} was observed with the DSB treatment, indicating minimal effects of cover crops on P stratification.

The influence of N rate on soil pH confirms our initial hypothesis, indicating a long-term negative impact of N fertilization on soil acidification, particularly in shallow soil layers. However, the effect of N rates on P stocks per cm^{-1} was less clear and no significant effects of N rates on SOC and Total N amounts and distribution in the soil profile were observed. Cover crops and intensification alternatives like double-cropping show the potential to improve C and N levels in the surface soil and enhance its buffering capacity. Further research should address managing the negative impacts of P stratification on soil health and water quality.

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Table 2.1 Cumulative precipitation and mean, maximum, and minimum air temperatures during the fallow period preceding corn planting and during corn growing season in 2021 and 2022 and respective departures from normals for Riley County, KS (Kansas Mesonet, 2024).

Year	**Period**	**Cumulative precipitation**	**Departure from Normal†**	**Mean temperature**	**Departure from Normal†**	**Max. temperature**	**Departure from Normal†**	**Min. temperature**	**Departure from Normal†**
		— mm —		— °C —					
2021	Wheat harvest 2020 - corn planting 2021	680	76	11	0	17.8	0.2	4.9	0
2022	Wheat harvest 2021 - corn planting 2022	560	-44	12	1	19.7	2.1	5.5	0.6
2021	Corn growing season	458	-46	23.4	1	29.6	1	17.1	0.7
2022	Corn growing season	541	83	23.6	1.2	30.2	1.6	17	0.6

Table 2.2 Summary of the ANOVA significance levels for pH, Soil Organic Carbon (SOC), Total Nitrogen (N) and Melich -3 Phosphorus (P) stocks per cm^{-1} and SOC and Total N profile stocks. Bolded values represent statistical significance at p <0.1 (Mgmt: management).

Source of variation	**SOC**	**Total N**	**pH**	**Mehlich-3 P**
Fallow mgmt.	**0.0016**	**0.0098**	**0.0019**	**0.0092**
N rate	0.8421	0.4500	**<.0001**	**0.0010**
Fallow mgmt. x N rate	0.1227	0.9427	0.1612	**0.0175**
Depth	**<0.0001**	**<0.0001**	**<.0001**	**<0.0001**
Fallow mgmt. x Depth	**<0.0001**	**<0.0001**	**0.0741**	**<0.0001**
N rate x Depth	0.2294	0.7599	**<.0001**	**0.0009**
Fallow mgmt. x N rate x Depth	0.9967	0.9992	0.9905	**0.0469**

Table 2.3 Summary of the ANOVA significance levels within soil depth for Soil Organic Carbon (SOC), Total Nitrogen (N), pH and Melich-3 Phosphorus (P). Bolded values represent statistical significance at p <0.1 (Mgmt: management).

	Depth					
Source of variation	**0-5 cm**	**5-10 cm**	**10-15 cm**	**15-30 cm**	**30-60 cm**	**0-60 cm**
			----------------------- SOC ---------------------			
Fallow mgmt.	**<.0001**	**0.0082**	0.1494	0.8996	0.7146	0.1279
N rate	0.2819	0.1988	0.2772	0.1434	0.7200	0.7282
Fallow mgmt. x N rate	0.8291	0.4899	0.3080	0.4030	0.2080	0.3696
			------------------------ Total N ----------------------			
Fallow mgmt.	**<.0001**	0.6202	0.9514	0.5992	0.817	0.210
N rate	0.1358	0.1116	0.7026	0.4808	0.516	0.832
Fallow mgmt. x N rate	0.9940	**0.0091**	0.2203	0.4110	0.758	0.648
			----------------------- pH -----------------------			
Fallow mgmt.	**<.0001**	**0.0602**	0.4498	0.4866	0.4706	-
N rate	**<.0001**	**<.0001**	0.5000	0.3452	0.3440	-
Fallow mgmt. x N rate	0.2915	0.6222	0.3178	**0.0308**	0.4898	-
			----------------------- Mehlich-3 P -----------------------			
Fallow mgmt.	**0.0839**	**0.0083**	**0.0202**	**0.0540**	0.3275	-
N rate	**0.0761**	**0.0776**	0.2908	0.2501	0.3292	-
Fallow mgmt. x N rate	0.3283	0.6391	0.3795	0.1021	0.3855	-

Table 2.4 Least Squares (LS) means for Total Nitrogen (N) in the 5-10 cm soil layer for each Fallow management. Letters and bolded values indicate significant difference at $p < 0.1$ (0-N, 0 kg N ha^{-1}; 45-N, 45 kg N ha^{-1}; 90-N, 90 kg N ha^{-1}; 180-N, 180 kg N ha^{-1}; 270-N, 270 kg N ha^{-1}, CF: chemical fallow, DSB: double-crop soybean, CR: cereal rye, CC: crimson clover, CR/CC: cereal rye and crimson clover mix, DM: diverse mix).

N rate	**CF**	**DSB**	**CC**	**CR**	**CR/CC**	**DM**
	---------------------------- Mg N ha^{-1} ----------------------------					
0-N	0.2068	0.2122	**0.2220 a**	0.1955	0.2031	0.2048
45-N	0.1994	0.2067	**0.1980 bc**	0.2047	0.2011	0.2031
90-N	0.1993	0.1974	**0.2030 bc**	0.2085	0.1954	0.1995
180-N	0.2084	0.2067	**0.2100 ab**	0.2050	0.2008	0.1974
270-N	0.1939	0.2047	**0.1970 c**	0.2030	0.2084	0.2143

Table 2.5 Least Squares (LS) means for pH in the 15-30 cm soil layer for each Fallow management. Letters and bolded values indicate significant difference at $p < 0.1$ (0-N, 0 kg N ha^{-1}; 45-N, 45 kg N ha^{-1}; 90-N, 90 kg N ha^{-1}; 180-N, 180 kg N ha^{-1}; 270-N, 270 kg N ha^{-1}, CF: chemical fallow, DSB: double-crop soybean, CR: cereal rye, CC: crimson clover, CR/CC: cereal rye and crimson clover mix, DM: diverse mix).

N rate	CF	DSB	CC	CR	CR/CC	DM
0-N	**6.1 ab**	6.1	6.0	6.0	6.0	6.1
45-N	**6.2 a**	6.0	6.0	6.3	6.0	6.0
90-N	**6.0 ab**	6.0	6.0	6.0	6.1	6.0
180-N	**5.9 b**	6.1	6.1	6.1	6.2	6.2
270-N	**6.1 a**	6.0	5.9	6.1	6.1	6.0

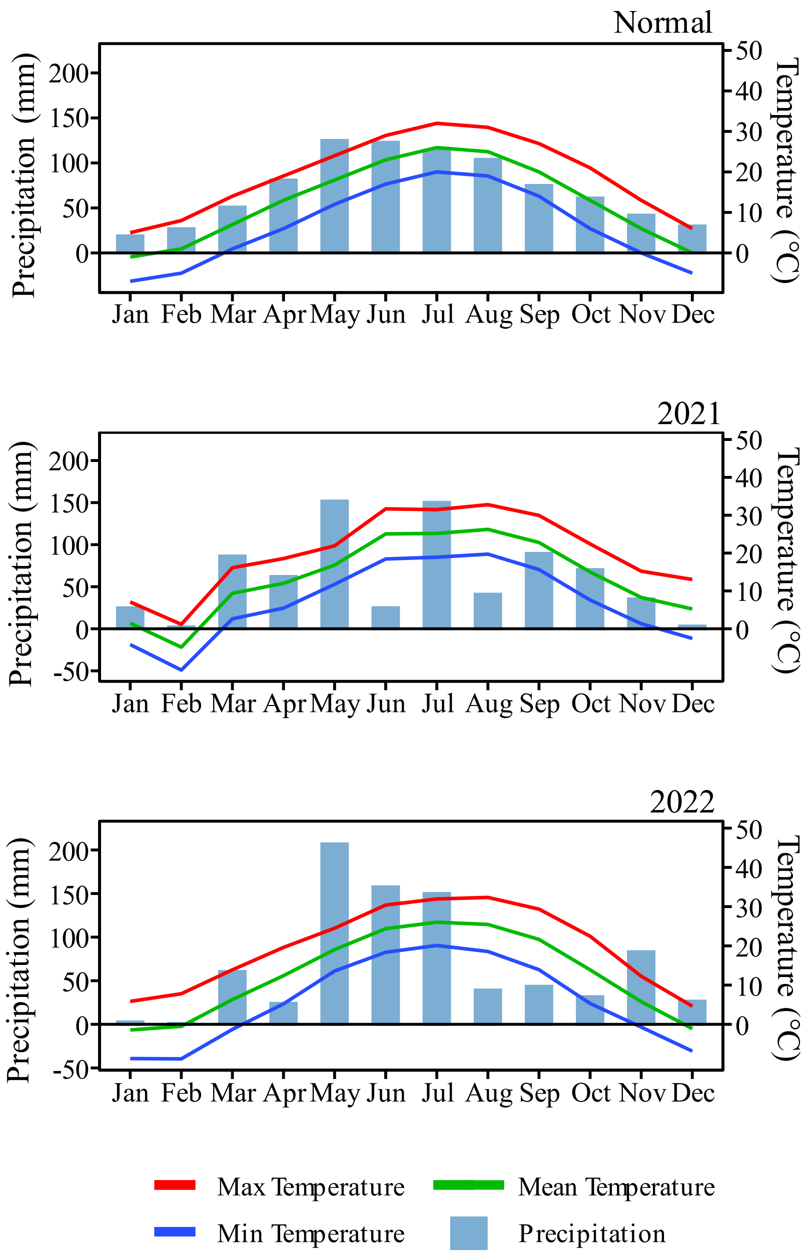

Figure 2.1 Normal, 2021, and 2022 monthly maximum, minimum, and mean air temperatures and monthly precipitation. Values for 2021 and 2022 recorded by Kansas Mesonet station located adjacent to experiment site. Normal represent 30-year averages for Riley County, KS (Kansas Mesonet, 2024).

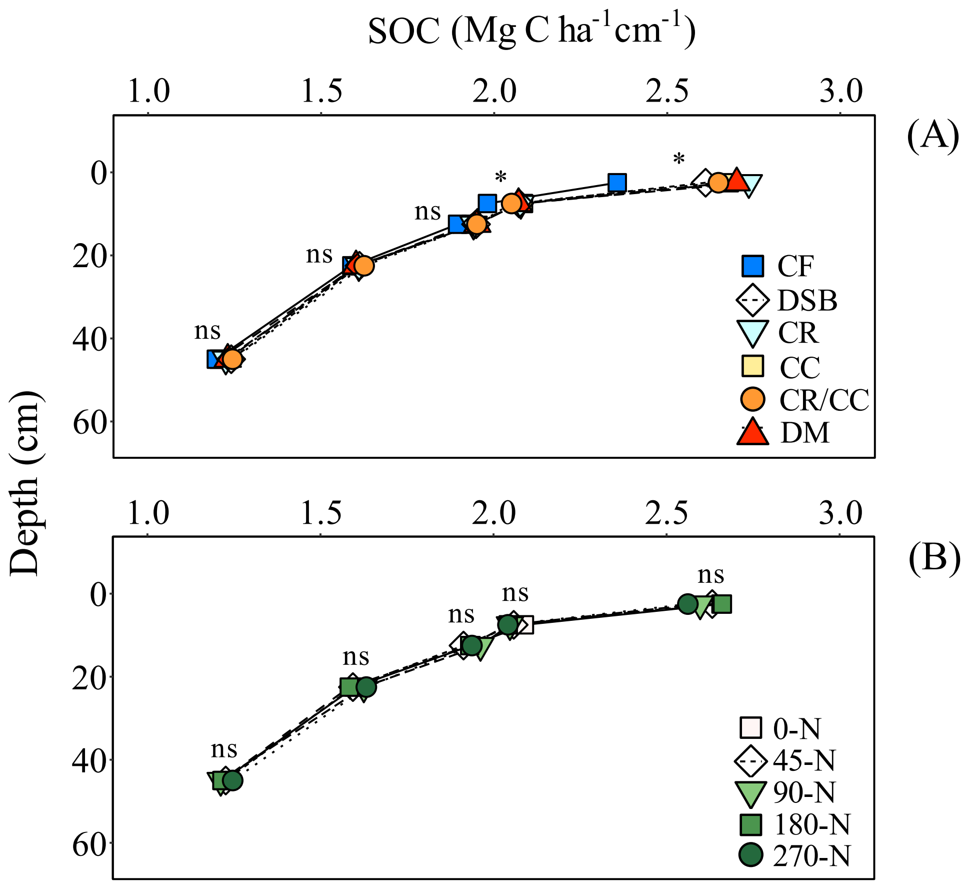

Figure 2.2 Vertical distribution of soil organic carbon (SOC) as affected by Fallow management (A) and Nitrogen (N) rate (B). (CF: chemical fallow, DSB: double-crop soybean, CR: cereal rye, CC: crimson clover, CR/CC: cereal rye and crimson clover mix, DM: diverse mix; 0-N, 0 kg N ha^{-1}; 45-N, 45 kg N ha^{-1}; 90-N, 90 kg N ha^{-1}; 180-N, 180 kg N ha^{-1}; 270-N, 270 kg N ha^{-1}; *, p-value <0.1; ns, not significant.).

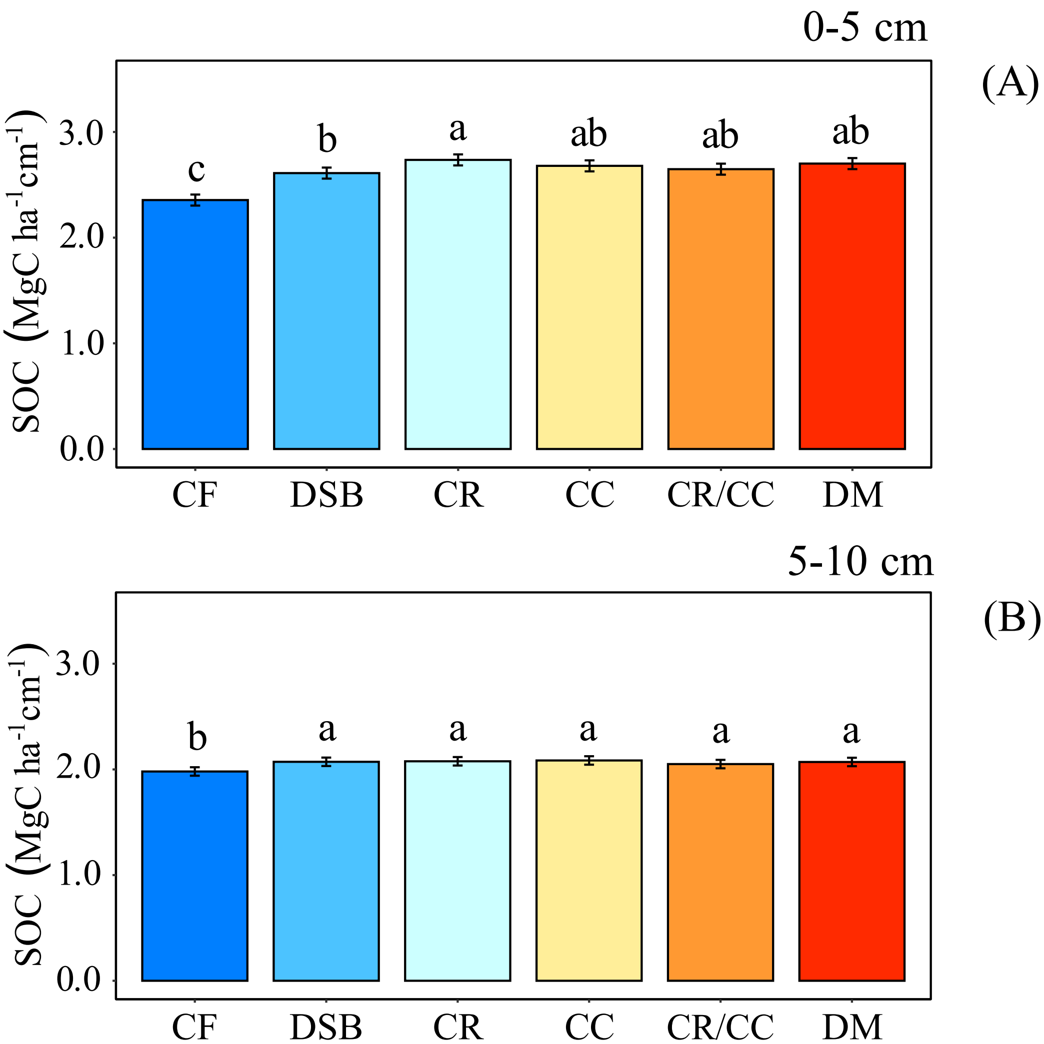

Figure 2.3. Fallow management effect in soil organic carbon (SOC) at 0-5 cm (A) and 5-10 cm (B) soil layers. Letters indicate significant difference at $p < 0.1$ and error bars represent standard error of the mean (CF: chemical fallow, DSB: double-crop soybean, CR: cereal rye, CC: crimson clover, CR/CC: cereal rye and crimson clover mix, DM: diverse mix).

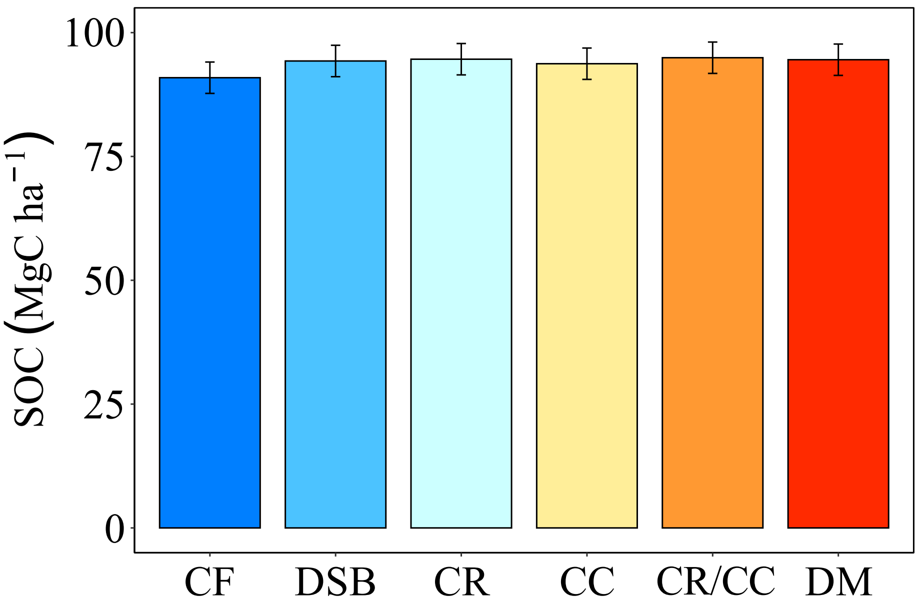

Figure 2.4 Fallow management effect on soil organic carbon (SOC) in the 0-60 cm soil layer. Letters indicate significant difference at $p < 0.1$ and error bars represent standard error of the mean (CF: chemical fallow, DSB: double-crop soybean, CR: cereal rye, CC: crimson clover, CR/CC: cereal rye and crimson clover mix, DM: diverse mix).

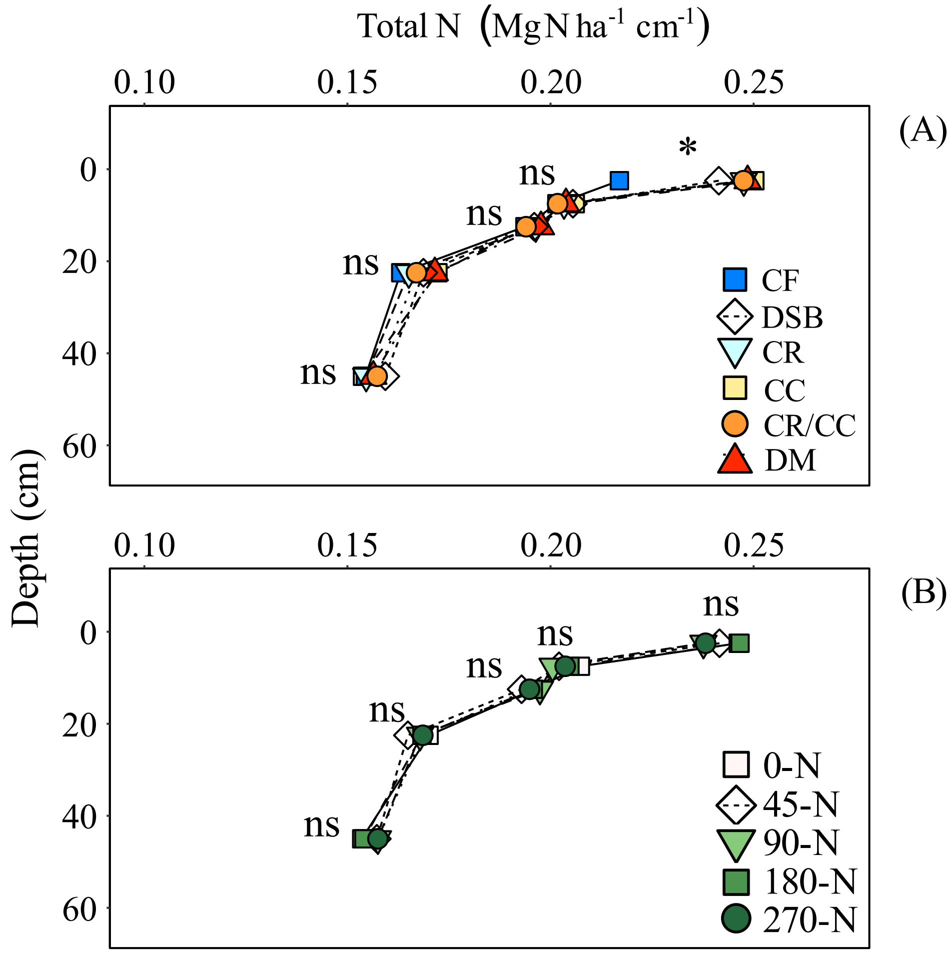

Figure 2.5 Total nitrogen (N) stocks per cm^{-1} with soil depth as affected by Fallow management (A) and Nitrogen (N) rate (B). (CF: chemical fallow, DSB: double-crop soybean, CR: cereal rye, CC: crimson clover, CR/CC: cereal rye and crimson clover mix, DM: diverse mix; 0-N, 0 kg N ha^{-1}; 45-N, 45 kg N ha^{-1}; 90-N, 90 kg N ha^{-1}; 180-N, 180 kg N ha^{-1}; 270-N, 270 kg N ha^{-1}; *, p-value <0.1; ns, not significant.).

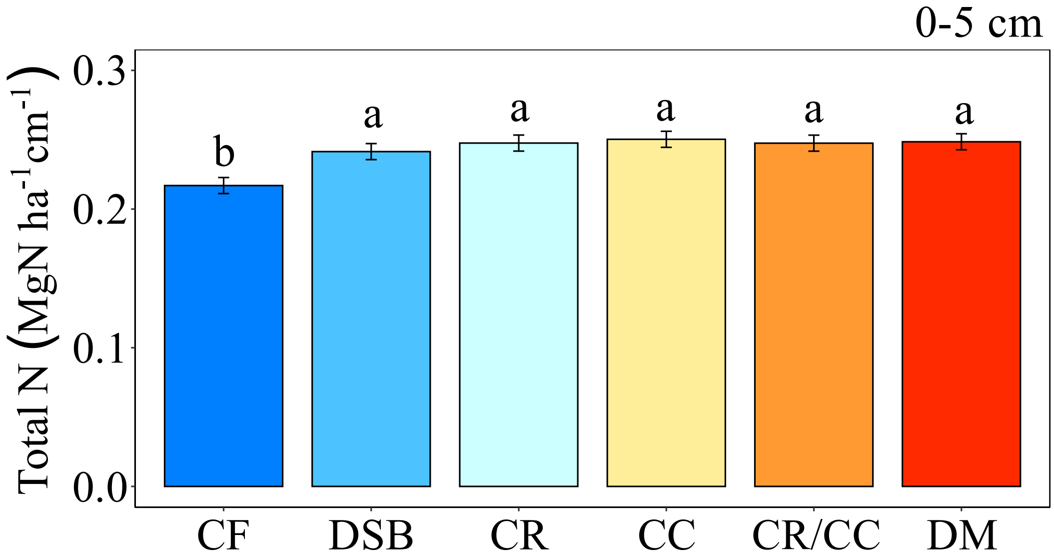

Figure 2.6 Fallow management effect in Total nitrogen (N) at the 0-5 cm soil layer. Letters indicate significant difference at $p < 0.1$ and error bars represent standard error of the mean (CF: chemical fallow, DSB: double-crop soybean, CR: cereal rye, CC: crimson clover, CR/CC: cereal rye and crimson clover mix, DM: diverse mix).

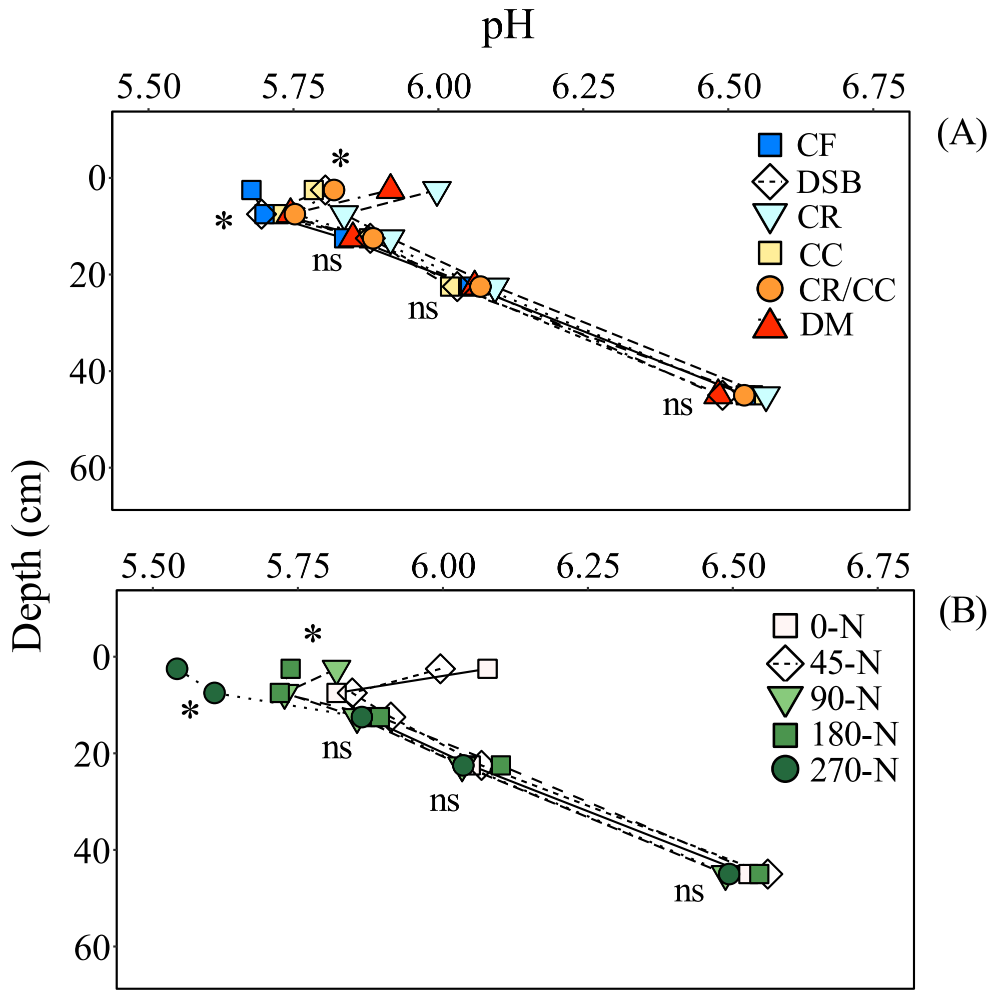

Figure 2.7 Vertical distribution of pH in the soil profile as affected by Fallow management (A) and nitrogen (N) rate (B). (CF: chemical fallow, DSB: double-crop soybean, CR: cereal rye, CC: crimson clover, CR/CC: cereal rye and crimson clover mix, DM: diverse mix; 0-N, 0 kg N ha^{-1}; 45-N, 45 kg N ha^{-1}; 90-N, 90 kg N ha^{-1}; 180-N, 180 kg N ha^{-1}; 270-N, 270 kg N ha^{-1}; *, p-value <0.1; ns, not significant.).

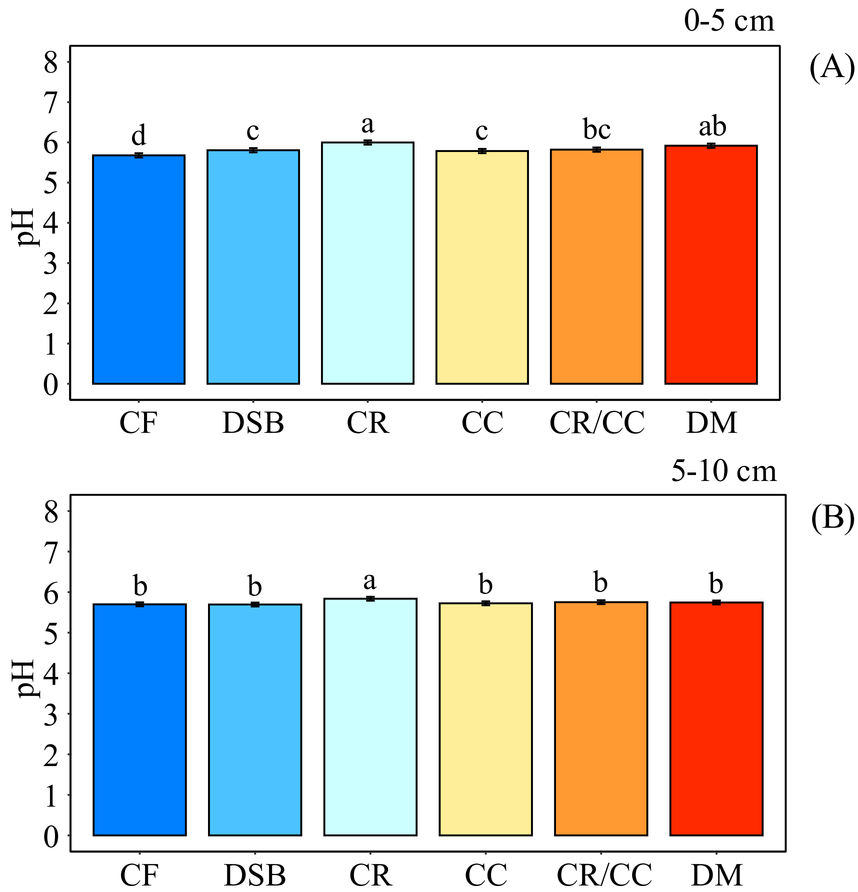

Figure 2.8 Fallow management effect in pH at the 0-5 cm (A) and 5-10 cm (B) soil layer. Letters indicate significant difference at $p < 0.1$ and error bars represent the standard error of the mean (CF: chemical fallow, DSB: double-crop soybean, CR: cereal rye, CC: crimson clover, CR/CC: cereal rye and crimson clover mix, DM: diverse mix).

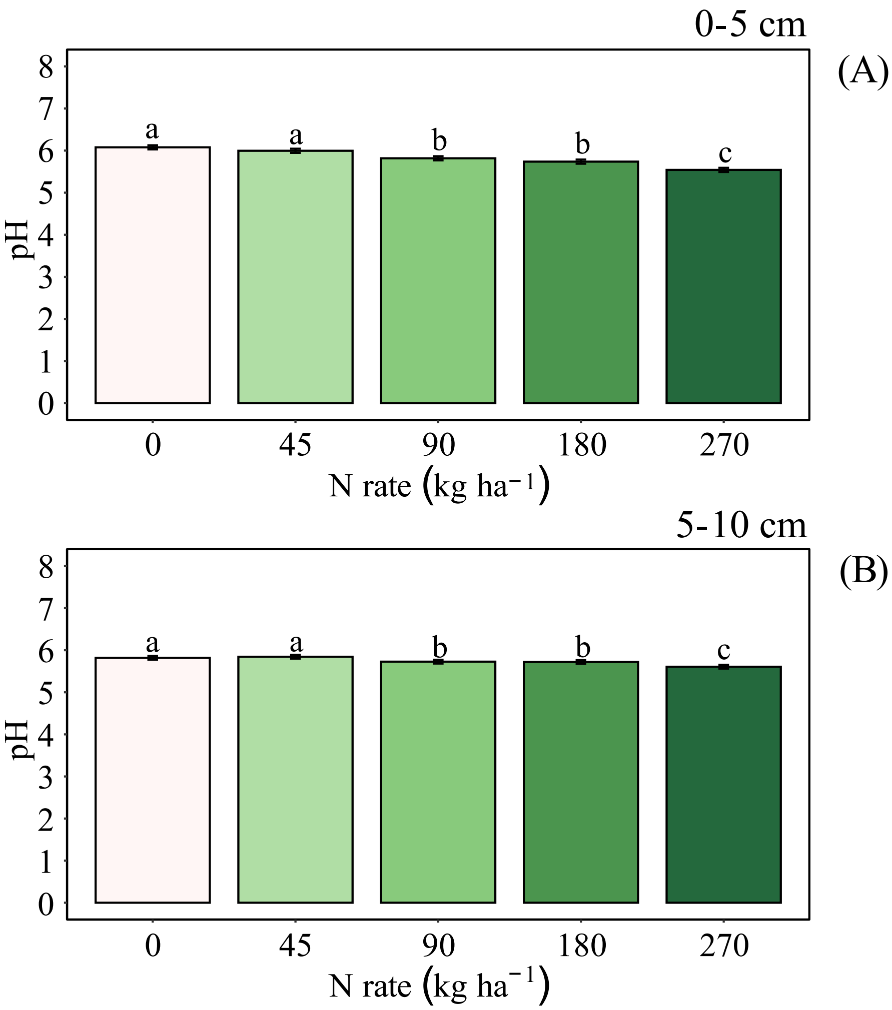

Figure 2.9 Nitrogen (N) rate effect in pH at the 0-5 cm (A) and 5-10 cm (B) soil layers. Letters indicate significant difference at $p < 0.1$ and error bars represent standard error of the mean (0-N, 0 kg N ha^{-1}; 45-N, 45 kg N ha^{-1}; 90-N, 90 kg N ha^{-1}; 180-N, 180 kg N ha^{-1}; 270-N, 270 kg N ha^{-1}).

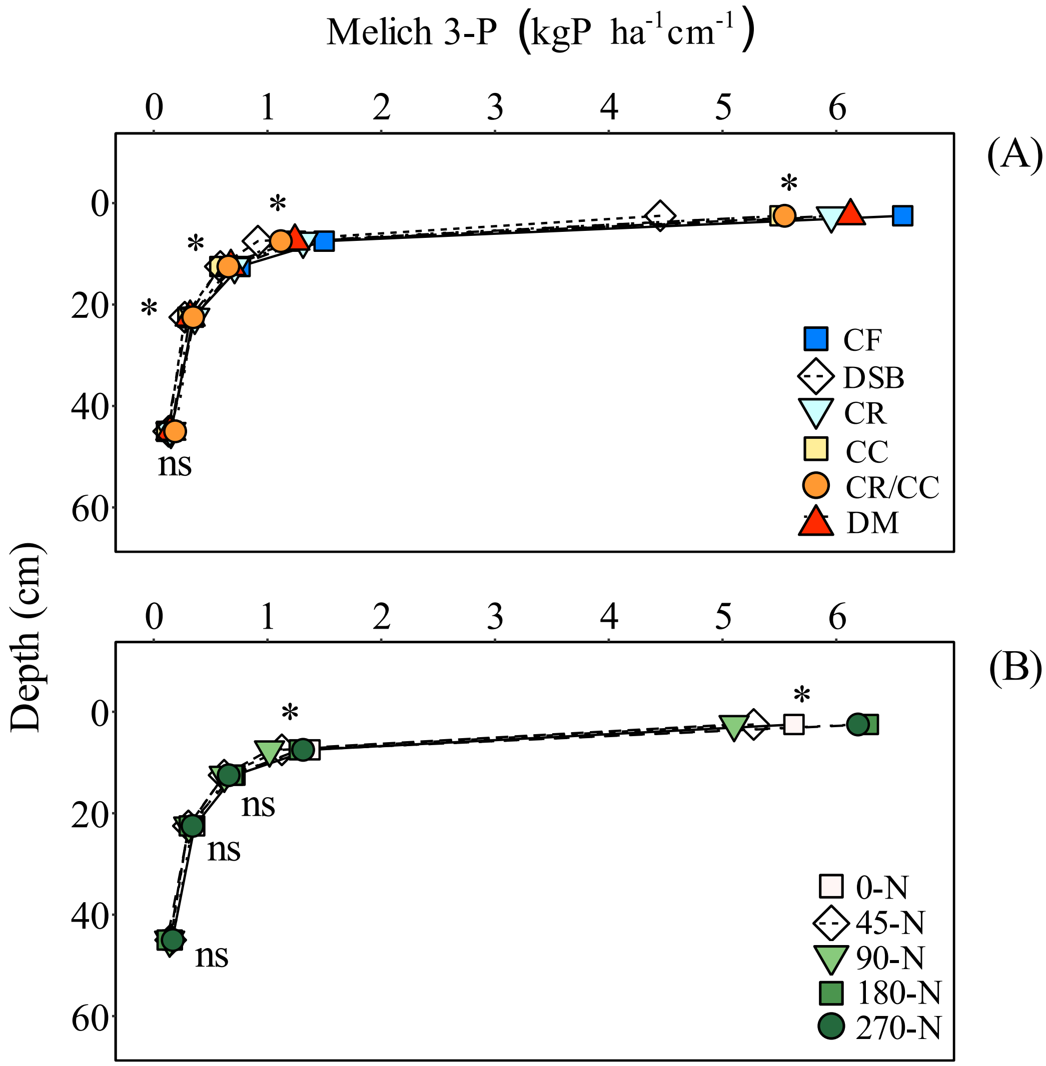

Figure 2.10 Effects of Fallow management (A) and nitrogen (N) rate (B) on Mehlich-3 phosphorus (P) stocks per cm^{-1} in the soil profile (CF: chemical fallow, DSB: double-crop soybean, CR: cereal rye, CC: crimson clover, CR/CC: cereal rye and crimson clover mix, DM: diverse mix; 0-N, 0 kg N ha^{-1}; 45-N, 45 kg N ha^{-1}; 90-N, 90 kg N ha^{-1}; 180-N, 180 kg N ha^{-1}; 270-N, 270 kg N ha^{-1}; *, p-value <0.1; ns, not significant).

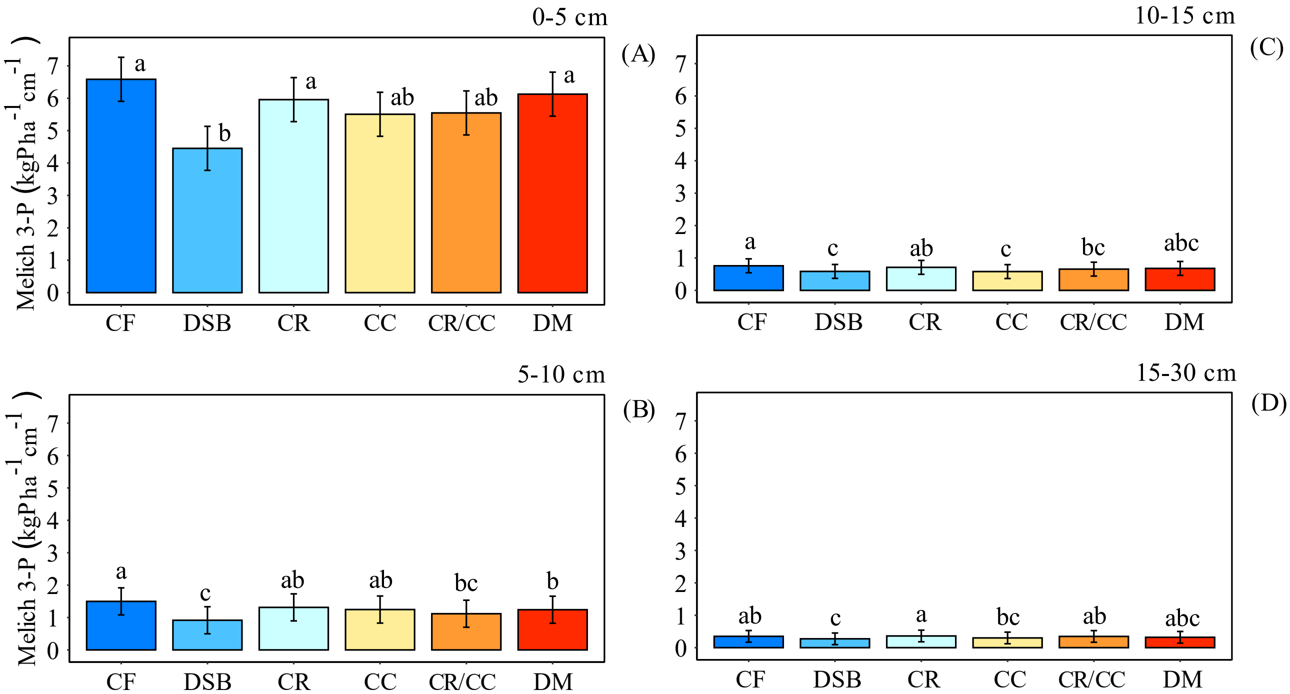

Figure 2.11 Fallow management effect on Mehlich-3 phosphorus (P) at the 0-5 cm (A), 5-10 cm (B), 10-15 cm (C) and 15-30 cm (D) soil layers. Letters indicate significant difference at $p < 0.1$ and error bars represent standard error of the mean (CF: chemical fallow, DSB: double-crop soybean, CR: cereal rye, CC: crimson clover, CR/CC: cereal rye and crimson clover mix, DM: diverse mix).

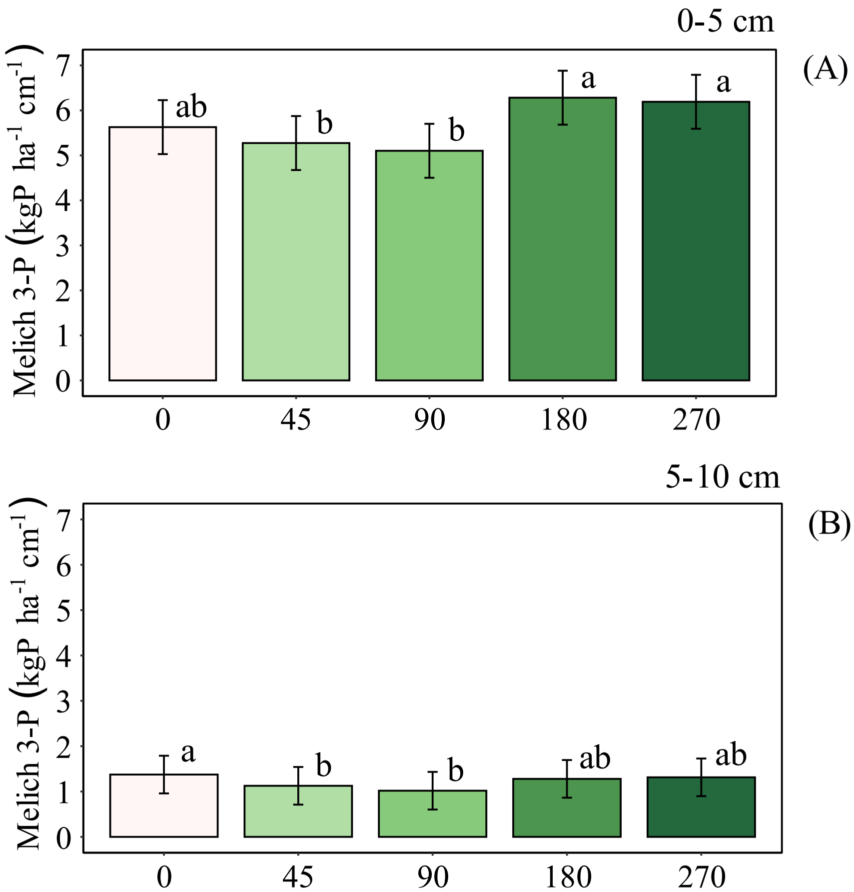

Figure 2.12 Nitrogen (N) rate effect on Mehlich-3 phosphorus (P) at the 0-5 cm (A) and 5-10 cm (B), soil layers. Letters indicate significant difference at $p < 0.1$ and error bars represent standard error of the mean (0-N, 0 kg N ha^{-1}; 45-N, 45 kg N ha^{-1}; 90-N, 90 kg N ha^{-1}; 180-N, 180 kg N ha^{-1}; 270-N, 270 kg N ha^{-1}).

Chapter 3 - Exploring the Legacy Effects of Cover Crops on Soil Biochemical Properties in a No-Till Rotation in Northeast Kansas

Abstract

A healthy soil should deliver multiple ecosystem services and maintain active biological processes, such as carbon (C) cycling and nutrient mineralization. Cover crops present the potential to improve soil biological properties, although the response and persistence of that response for these indicators to long-term no-till management and different intensification schemes still needs clarification. A study conducted since 2007 in a silty clay loam near Manhattan, Kansas, examined effects of fallow management options in a no-till wheat (*Triticum aestivum*) -corn (*Zea mays L.*) -soybean [*Glycine max* (L.) Merr] rotation. The fallow management imposed between wheat and corn included chemical fallow (CF), double-crop soybean (DSB), and four cover crop treatments. Soil samples were collected in May 2022 and April 2023 in all crop phases, to capture different time intervals after cover crop termination: immediately after cover crop termination (T0), in corn stubble one year after cover crop termination (T1), and in growing wheat two years after cover crop termination (T2). Samples were collected at the 0-5 cm depth and analyzed for the following biological and biochemical indicators: active C, soil protein, glucosidase and glucosaminidase activity, and soil respiration. Cover crops positively impacted all soil indicators immediately after cover crop termination (T0), increasing active C by 30%, soil protein by 17%, glucosidase activity by 63%, glucosaminidase activity by 82%, and soil respiration by 116% compared to CF. In general, cover crops with larger biomass inputs provided the largest increase in biochemical indicators. Double-crop soybean exhibited indicator levels similar to or slightly greater than CF, yet lower than most cover crops. These responses were most evident immediately after cover crop

termination and persisted to some extent one year later. However, the responses diminished over time, and most were not detectable two years after cover crop termination. This information could assist in determining how frequently cover crops should be integrated into cropping systems and identify the most effective mixtures and species for enhancing soil health benefits and sustaining them over time.

Introduction

Soil degradation is one of the main results of repeated tillage operations, long fallow periods, and lack of crop rotation (Ghimire et al., 2018). These practices create a favorable environment for wind or water erosion, which decreases soil fertility and organic matter and, therefore, reduces the land's potential for crop production. These issues are especially evident in the US Great Plains, where nutrient cycling and crop productivity are constrained by water limitation and soil fertility loss (Nielsen et al., 2011). As climate change intensifies, the challenges outlined above are expected to be exacerbated. Between 1895 and 2015, Kansas experienced a notable increase in average temperatures, with an observed rise of 0.06 ± 0.03 °C per decade and annual mean precipitation for the western third, the central third, and the eastern third of Kansas being 531 mm, 660 mm, and 945 mm, respectively (Lin et al., 2017; Rawat et al., 2023). These uneven changes in temperature from north to south, and in precipitation from east to west, have been demonstrated to affect crop yields (Maitah et al., 2021). In the present context of climate change, soil capacity must be improved to face current and anticipated changes in the environment.

Soil health is understood as the continued capacity of the soil to function as a living ecosystem that sustains plants, animals, and humans (Lehmann et al., 2020). In cropping systems, it refers to the capacity of the soil to respond to an agronomic intervention, so that it can continue supporting plant growth and maintaining crop production as well as other ecosystem services, including water retention and supply, nutrient cycling, resistance to degradation, etc. (Kibblewhite et al., 2007; Laishram et al., 2012b). Changes in chemical, physical, and biological properties can be monitored to assess soil health in different production systems through their corresponding soil indicators (Cardoso et al., 2013). Previously, indicators of soil function primarily encompassed chemical parameters such as pH, cations exchange capacity (CEC), and soil organic matter (SOM) as well as physical attributes like infiltration, aggregation, and bulk density (Karlen and Stott, 1994). However, a shift is underway towards incorporating biological and biochemical indicators, due to certain advantages they have compared to physicochemical methods (Alkorta et al., 2003)

Soil biological indicators provide information about how living soil organisms respond to changes in their environment as they function to connect roots with soil, decompose SOM, and recycle nutrients (Jacoby et al., 2017b; Tahat et al., 2020b; Pandolfini et al., 1997). Unlike chemical and physical indicators, biological indicators are highly sensitive to changes imposed on their environment such as land use and management, facilitating early predictions of changes in environment (Cardoso et al., 2013; Wink et al., 2005b). According to the Natural Resource Conservation Service, a good soil health indicator should be management-sensitive, easy to use, cost-effective, repeatable, and interpretable for agricultural management decisions (Stott, 2019). Typical biochemical indicators used in commercial soil health assessments include soil organic carbon (SOC), SOM, active carbon (C), soil respiration, soil enzyme activities, and extractable soil protein (Nunes et al., 2020). Together they reflect the amount and quality of SOM, abundance, and activity of soil microorganisms, and are tied to several soil functions and processes.

Although the impact of different soil management practices on SOC measurements may require several years to manifest, active C, a pool of labile soil C processed by microbes, frequently displays heightened sensitivity to alterations in management or environmental conditions and therefore is widely used as a soil health indicator (Weil et al., 2003). Soil enzymes are excreted by microorganisms and plants to catalyze the decomposition of SOM.

Because of their sensitivity to management practices, activities of enzymes are considered good soil health indicators, because they provide information about the quantity and quality of soil SOM and related biochemical cycles (Ferraz-Almeida et al., 2015). Glucosidase plays a crucial role in the C cycle by catalyzing the ultimate step in the breakdown of cellulose, a component present in plant cell walls that serves as a significant energy source for the soil microbial community (Luo et al., 2017). Glucosaminidase is involved in the hydrolysis of chitin, a crucial structural element found in insects and fungal mycelia. The breakdown of chitin into amino sugars is of particular importance, given that amino sugars represent a primary source of mineralizable nitrogen (N) in soil (Ekenler and Tabatabai, 2002). Assessing soil protein levels provides insight into the size of the organic N pool being depolymerized, which serves as a source of N subsequently released through mineralization (Hurisso et al., 2018; Roberts and Jones, 2008). Lastly, soil respiration is an overall measure of microbial community activity, representing CO_2 produced by microorganism respiration (Arias et al., 2005; Haynes, 2008). Increased soil respiration is a good soil health indicator because it indicates the level of organism activity to perform functions such as residue breakdown and nutrient cycling.

No-till, crop rotation, and cropping system intensification through the use of double-cropping and cover crops are sustainable agricultural practices that can potentially alleviate soil degradation by increasing SOM via the input of crop residues (Caviglia et al., 2011; Duval et al. 2018; Villamil et al., 2006b). Mechanical disturbance, as shown in past studies (Alhameid et al., 2017, 2017; Kumar et al., 2017; Martínez et al., 2016), disrupts soil biological properties by accelerating the decomposition of plant biomass, reducing SOM content in the topsoil layer and reducing a soil's ability to retain nutrients and maintain physical conditions. Conversely, no-till combined with other conservation practices may increase SOM content, microbial biomass, and enzyme activity (Kinoshita et al., 2017). Implementing diverse crop rotations that include a variety of crops with different nutrient requirements and growth patterns can help mitigate soil degradation by promoting soil biodiversity, nutrient cycling, and overall soil fertility (Agomoh et al., 2021). Cropping systems can be intensified with double cropping, which is a farming practice in which two different crops are grown sequentially on the same piece of land within a single growing season. Double cropping is commonly adopted in Kansas as winter wheat (*Triticum aestivum* L.)– soybean [*Glycine max* (L.) Merr] cropping systems (Kelley, 2003). Double-crop soybeans have the potential to enhance soil health by decreasing fallow periods, facilitating N fixation, and improving SOM with the residue they leave behind (Amuri et al., 2008).

Cover cropping offers another option for intensifying cropping systems and has demonstrated the ability to significantly enhance various soil properties, including soil structure, SOC, microbial abundance, and diversity (Koudahe et al., 2022b). Several studies have suggested the benefits of cover crops for soil biological indicators. For instance, Strickland et al. (2019) found that a variety of cover crops such as triticale (×*Triticosecale Wittmack*), hairy vetch (*Vicia villosa* Roth), crimson clover (*Trifolium incarnatum* L.), and radish (*Raphanus sativus* L.), increased bioavailable C by 37% in four silt loam fields in Virginia. Similarly, Nunes et al. (2018) found that a mix of cover crop species increased autoclaved citrate extractable (ACE) protein content by 8% in a silt loam in New York. Moreover, Brennan and Acosta-Martinez (2019) found that different cover crop treatments including mustard (*Brassica juncea* L.), legume-rye (*Secale cereale* L.) and rye alone increased glucosidase and glucosaminidase activity by at least 66% and 79%, respectively, in a loamy sand in California. Although it may take several years for noticeable increases in SOM, the biological indicators mentioned above are often correlated with this measure and offer earlier insights into the chemical-biological processes driving SOM enhancement (Weil et al., 2003).

The benefits obtained from planting cover crops for soil proprieties are directly dependent on successful germination and growth of the cover crops (Obrycki et al., 2018). Cover crops with longer growing periods and higher biomass values may accrue greater soil benefits. Cover crop species selection may also have implications for how biological indicators respond. Grass cover crops tend to have dense rooting systems, high above-ground biomass production, and high C/N ratio at maturity, providing a rich C source for diverse groups of soil organisms and maintaining below-ground food chains (Carrera et al., 2007; Dahal et al., 2020). On the other hand, legume cover crops have a lower C/N ratio and decompose faster (Murungu et al., 2011), leading to an increase in microbial proliferation and nutrient cycling (Thapa et al., 2021). Brassicas decompose more rapidly than grasses and have a tap root system that can help alleviate soil compaction (Blanco-Canqui and Jasa, 2019). Several studies have reported that mixtures of cover crops can boost soil microbial activity by providing a variety of substrates and altering habitat conditions over time (Carrera et al., 2007; Njeru et al., 2014). Lastly, cover crop termination date needs to be considered in management planning. Delayed termination increases biomass production of cover crops but also results in higher residue C:N ratios, increasing the risk of potential N immobilized in soil, and increased soil water extraction, potentially compromising soil water available for the subsequent cash crop. (Blanco-Canqui et al., 2015b).

Although cover crops have the potential to improve soil biological properties, the response of these indicators to long-term no-till management and different intensification schemes in Kansas still needs clarification. Furthermore, understanding the persistence of these effects over subsequent years of crop rotation is essential. The objective of this study was to determine the effect of different intensification strategies on soil biological indicators in a three-year, no-till winter wheat– corn (*Zea mays* L.) – soybean rotation in northeast Kansas. In addition, this study aims to evaluate the persistence of these effects in subsequent years of the rotation. We hypothesize that cover crops will enhance soil biological indicators immediately following cover crop termination and in subsequent years of the crop rotations, with the greatest benefits anticipated immediately after termination. This information could assist in determining how frequently cover crops should be integrated into cropping systems and identify the most effective mixtures and species for sustaining soil health benefits over time.

Materials and Methods

Site Description and Experimental Design

The study consisted of a three-year, no-till winter wheat– sorghum [Sorghum bicolor (L.) Moench] or corn–soybean rotation, where all phases of the rotation were present every year. The long-term experiment was established in 2007 at the Kansas State University Department of Agronomy Research Farm (39º 07’ N lat, 96º 38’ W long) located approximately 8 km south of Manhattan, KS. The altitude is approximately 311 m, and the region is characterized by a hot humid continental climate (Köppen Climate Classification System: Dfa). The mean annual temperature is 12.9 ºC and the average annual precipitation is 889 mm (Kansas Mesonet, 2024) . The soil is a moderately well-drained Wymore silty clay loam (fine, smectitic, mesic Aquertic Argiudoll), with 0 to 1% slopes.

The experimental design was a randomized complete block, with a split-plot treatments structure and four replications. Crop phases were whole plots, and six Fallow management treatments during the wheat-fallow phase were subplots. Every whole plot was 36 by 70 m, and each subplot was 6 by 70 m. Further details about the experimental design and site can be found in Fontes et al. (2017).

Fallow management

Cover crop treatments have varied since initiation of the study by including both winter and summer cover crops during the first three cycles (Preza et al., 2017) and different degrees of intensification with cover crops in the fourth cycle (Nielsen, 2020). Despite variations in treatments over the years, each treatment consistently exhibited a specific cover crop characteristic, such as crop type and/or biomass production potential. The CF and DSB treatments were applied consistently since the study was initiated. In 2019, the Fallow management treatments consisted of only DSB planted in all plots other than CF. The current set of winter cover crop treatments were planted in September 10 of 2021 and August 24 of 2022 and included a winter legume cover crop (crimson clover, CC), winter non-legume cover crop (CR), a mix of the rye and the clover (CR/CC), and a diverse mix (DM) including, winter peas (*Pisum sativum* L.), sorghum-sudangrass (*Sorghum bicolor* L.), turnips (*Brassica rapa* L.), radishes and rape (*Brassica napus* L.).

All treatment plots were sprayed at DSB planting with flumioxazin (N-(prop-2-yn-1-yl)-2H-1,4-benzoxazin-3(4H)-one) and pyroxasulfone (3-{[5-(Difluoromethoxy)-1-methyl-3-(trifluoromethyl)-1H-pyrazol-4-yl]methanesulfonyl}-5,5-dimethyl-4,5-dihydro-1,2-oxazole) in 2021 and mix of paraquat (1,1'-Dimethyl-4,4'-bipyridinium dichloride), glyphosate (N-(phosphonomethyl)glycine), and 2,4-D (2,4-Dichlorophenoxyacetic acid) in 2022 at label rates to control volunteer wheat and other weeds and to provide residual suppression of weed emergence. In addition, 44 kg P ha^{-1} applied to all plots at DSB planting in both years. The target seeding rate was 67 kg ha^{-1} for CR, 28 kg ha^{-1} for CC, 34 kg ha^{-1} for CR and 14 kg ha^{-1} for the CC in the

CR/CC treatment, and 75 kg ha^{-1} for DM. Non-hardy winter cover crop species were killed by freezing temperatures, which usually occurred during November of each year. On April 14 of 2021 and April 26 of 2022, all plots were sprayed with a mix of glyphosate, 2,4-D, and dicamba (3,6-dichloro-2-methoxybenzoic acid) in 2021, and a mix of glyphosate and 2,4-D in 2022 to terminate surviving cover crops, volunteer wheat, and other winter annual weeds.

Double-crop soybean was planted at a target seeding rate of 445,000 seeds ha^{-1} on July 9 of 2021 and July 9, 2022, which was 17 and 9 days after wheat harvest in each year, respectively. Chemical fallow and DSB plots were sprayed again with glufosinate (2-Amino-4 [hydroxy (methylphosphonoyl)]butanoic acid) and clethodim (2-{(E)-1-{3-chloroallyloxyimino] propyl]-5-{2-(ethylthio)propyl}-hydroxycyclohexen-2-one(1)) on August 5 of 2021 and a mix of glufosinate and glyphosate on August 24 of 2022 to maintain plots clean of weeds and volunteer wheat. A third application in the CF treatment consisted of a mix of paraquat and glyphosate on September 9 of 2021 and a mix of clethodim and glufosinate on October 15 of 2022. The DSB plots were hand-harvested on November 5 of 2021 and combine harvested in October 26 of 2022.

Corn phase

Initially, the rotation consisted of wheat, grain sorghum, and soybean (Fontes et al., 2017). However, grain sorghum was replaced by corn in 2020. Corn was planted soon after cover crop termination on April 30 of 2021 and May 11 of 2022. The target seeding rate was 69000 seeds ha^{-1} of Pioneer P0977AM hybrid in 2021 and 67000 seeds ha^{-1} of Pioneer P0995AM hybrid in 2022. The N fertilizer rate of 90 kg ha^{-1} was applied as 28% UAN, split with 45 kg ha^{-1} at planting and balanced at V4-6 (June 3 of 2021 and June 15 of 2022). The UAN was applied with a flat-colter liquid fertilizer applicator to inject N fertilizer below the residue layer. No P or

K fertilizer was applied in the corn phase. Weeds were controlled with a pre-emergence herbicide application applied within 2 days of planting and a post-emergence herbicide application approximately 20 days after planting. The application consisted of Mezotrione (2-[4-(methylsulfonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione), S-Metalochlor (2-chloro-N-(2-ethyl-6-methylphenyl)-N-[(2S)-1-methoxypropan-2-yl]acetamide), Atrazine (1-Chloro-3-ethylamino-5-isopropylamino-2,4,6-triazine), Glyphosate, and AMS in May 2021 and AMS (Ammonium sulfamate), Acuron (mix of atrazine, mesotrione and S-meolachlor), 2,4-D and Glyphosate in May 2022. After corn reached physiological maturity and had dried sufficiently for machine harvest, yield was determined by harvesting plants from the center two rows of each subplot with a plot combine equipped with an on-board weigh system in September 17 of 2021 and October 4 in 2022.

Soybean phase

A Pioneer 38A49L (LL) soybean variety was planted on April 30 of 2021 and May 12 of 2022 at a seeding rate of 432,000 seeds ha^{-1}, with 76-cm row spacing. Ammonium polyphosphate was applied as a starter fertilizer with planter at a rate of 130 kg ha^{-1}, resulting in 10 kg P ha^{-1} and 13 kg N ha^{-1} applied. Before soybean planting, all plots were sprayed with 1.46 kg a.i. ha^{-1} of glyphosate 0.53 kg a.i. ha^{-1} of 2,4-D to kill winter annual weeds. In both years, weeds were controlled after planting by applying 1.46 kg a.i. ha^{-1} of glyphosate, and 1.56 kg a.i. ha^{-1} of alachor,2-chloro-2,6-diethyl-N (methoxymethyl) acetanilide (N-phenylacetamide) as a pre-emergence herbicide. Soybeans were harvested on November 5 of 2021 and October 10 of 2022.

Wheat phase

Winter wheat was planted immediately after soybean harvest in October 2021 and November 2022 with a target seeding rate of 130 kg ha^{-1} on 19-cm rows using a John Deere 1590 no-till drill (Deere & Co., Moline, IL). Diammonium phosphate was applied at planting at a rate of 130 kg ha-1 to supply 60 kg P ha^{-1} and 23 kg of N ha^{-1}. On March 22 of 2021 and March 1 of 2022, wheat was top-dressed with 67 kg N ha^{-1} soon after Feekes stage 4-5 (Miller 1992) using 28% UAN, applied in streams spaced every 10 cm. Weeds were controlled by applying 2-methyl-4-chlorophenoxyacetic acid and 10.5 g a.i. ha^{-1} of tribenuron-methyl (MCPA Ester and Harmony® Extra SG) after Feekes stage 4-5 in both years. Winter wheat was harvested on June 22, 2021, and June 30, 2022.

Soil sampling and laboratory analysis

Soil sampling was conducted on May 9 of 2022 and April 25 of 2023 in all crop phases, which captured different time intervals after cover crop termination: immediately after cover crop termination (T0: at corn planting), one year after cover crop termination (T1: at soybean planting), and two years after cover crop termination (T2: wheat at F6). Given that the plots from 2022 reflected a particular phase of rotation, which transitioned to the next phase year by year, the plots sampled in 2022 immediately following cover crop termination (T0) transitioned to become the plots one year after cover crop termination (T1) in 2023, and this pattern continued subsequently. The only set of plots sampled that were not preceded by the current set of cover crop treatments are the T2, two years after cover crop, sampled in 2022. This set of plots with wheat at the F6 stage at the sampling time, were preceded by double crop soybeans during the 2019 fallow after wheat harvest in place of previous cover crop and other intensification treatments in addition to the consistent CF and DSB treatments.

A total of ten cores per plot were collected with a manual soil probe and then composited into one sample using nitrile gloves and disinfecting between samples with ethanol to prevent cross contamination. Soil samples were collected from the upper 5 cm where microbial populations and activity are highest making it the most indicative layer for biological/biochemical soil health properties (Omer et al. 2023). Samples were kept in a cooler during in-field sampling and transport to the lab, air-dried, ground, and passed through a 2-mm sieve. To address the objective of this study, the following soil biological/biochemical indicators were analyzed: POXC (permanganate oxidizable C, indicator of labile fraction of SOC), soil protein (indicator of bioavailable N), β-glucosidase and N-acetyl-β-glucosaminidase activities (C and N cycling enzymes), and soil respiration (indicating microbial activity).

Permanganate oxidizable Carbon

Permanganate oxidizable C (POXC) was determined by following the methodology described in Weil et al. (2003). Briefly, 2.5 g of air-dried soil were weighed into 50-ml Falcon tubes in duplicate, where 18 ml of deionized (DI) water and 2 ml of 0.2M K_2MnO_4 were added. Tubes were capped and shaken at 120 rpm for 2 min and left to settle on a lab bench for 8 min to continue the oxidation reaction. Subsequently, an aliquot of 0.2 ml of the solution was taken and dispensed into tubes with 20 mL of DI water to stop the reaction. The absorbance of each sample was analyzed colorimetrically on a spectrophotometer at 550 nm (Shimadzu Scientific Instruments, Columbia, MD, USA), and POXC concentration was finally obtained with the following equation (Equation 1):

$$POXC\left(\frac{\text{mg}}{\text{kg}}\right) = \left[0.02\ \frac{mol}{L} - (m \times \text{absorbance (x)} + b)\right] \times \left(9000\ \frac{mg\ C}{mol}\right) \times \left(\frac{0.02\ L}{0.0025\ kg}\right) \quad \text{Eq. [1]}$$

Where 0.02 mol L^{-1} is the initial concentration of the K_2MnO_4 solution, b is the intercept, x is the absorbance and m is the slope of the standard calibration curve where 9000 mg C is assumed to be oxidized when 1 mol MnO_4 changes from Mn^{7+} Mn^{2+}, 0.02 L is the volume of the K_2MnO_4 reacting with the samples, and 0.0025 kg is the mass of soil used for the reaction.

Soil protein

Soil protein concentration was determined using the Autoclaved Citrate Extractable (ACE) protein method following the methodology described by Hurisso et al. (2018), and Wright and Upadhyaya (1996, 1998). A total of 3 g of air-dried and sieved soil was weighed in two 50-mL centrifuge tubes, and 24 ml of 20 mM neutral sodium citrate buffer solution (pH 7) was added to each tube labeled as A and B replicates. Tubes were shaken for 5 min at 180 rpm and later subjected to a high temperature and pressure in an autoclave (121°C and 15 psi, 30 min). The soil extractant mixture was allowed to cool to room temperature and then shaken again for 1 minute (180 rpm). An aliquot of 1.75 ml was transferred to a 2-ml microcentrifuge tube and then clarified by centrifugation (10,000 x g, 3 min). Subsequently, another 1-ml aliquot was transferred to a 1.5-ml microcentrifuge tube, and the centrifugation step was repeated. For quantification, a dry heat block (VWR heat block, 97043-610, USA) was heated to 61.5˚C, and Pierce bicinchoninic acid (BCA) working solution was prepared. Ten µL of the standard were added to the reaction plate using a multichannel pipettor, followed by duplicate columns of each strip of eight sample tubes into plate wells. Each well of the reaction plate received 200 µL of working solutions, then sealed with sealing tape and placed on the heat block for one hour. The plate was allowed to cool for 10 min and then read with a plate reader (BioTek Synergy H1, USA) at 562 nm.

Soil Enzyme Activities

The activities of glucosidase and glucosaminidase were measured as given by Eivazi and Tabatabai (1988) and Parham and Deng (2000), respectively. Each enzyme methodology differed in substrate, start, and stop buffer (Table 3.1). Briefly, three subsamples of 0.5 g of air-dried soil were weighed into 20-mL scintillation vials, labeled A and B for the two replicates, and C for the control. Start buffer (2 mL) was added to all flasks, while substrate (0.5 mL) was added only to A and B replicates Vials were capped and swirled before incubating at 37°C for 1 hour. To stop the reaction, 0.5 M $CaCl_2$ (0.5 mL) was added to all flasks, followed by a stop buffer (2 mL), and swirled gently after each addition. Substrate (0.5 mL) was then added only to C vials. Each solution was poured into a filter-lined funnel and captured in test tubes for ~30 minutes until fully filtered (Ahlstrom 642, 11 pore size filter paper). The filter was removed, and the test tubes were placed into a rack. If necessary, each sample was diluted to get an absorbance ≤1.3 (using the same dilution for all A and B samples), and the samples were read in a spectrophotometer. A calibration curve was prepared with p-nitrophenol (PNP) for each run. To determine the dilute concentration of PNP in the sample from the input of the spectrophotometer reading, a linear standard curve equation was utilized where: y represented the concentration of PNP in each sample, m was the slope of the standard curve, x was the spectrophotometer reading from each sample, and b was the y-intercept of the standard curve. Lastly, to account for the dilution and soil weight, the calculated dilute concentration was multiplied by the dilution factor and divided by the equivalent dry weight of the sample.

Soil respiration

The rate of carbon dioxide (CO_2) respired from soil during a 96-hr incubation was determined following the alkali trap method described by Haney and Haney (2010). Briefly, 20 g of air-dried and sieved soil was weighed into an aluminum weigh boat (diameter 51 mm) that was perforated nine times (three by three array) and placed onto two filter papers (qualitative 413-VWR North America). The alkali trap was then placed on two staggered filter papers in a standard mason jar, with a trap assembly (a 10 ml glass beaker secured to a plastic tripod 'pizza stool') containing 9 ml of 0.5 M KOH placed in the jar. Next, 7.5 ml of distilled DI water was pipetted to the inside edge of the jar to wet the filter papers and soil, and the jar was sealed tightly and incubated for 4 days at room temperature. During incubation, the CO_2 was absorbed by the KOH in the trap, causing a decline in electrical conductivity. After incubation, the conductivity of the trap solution was measured, and the amount of CO_2 respired was calculated by comparing the conductivities of the original trap solution with a solution representing the trap if saturated with CO_2 (0.25 M K_2CO_3).

Statistical Analyses

Data were tested for normal distribution and equal variances using the Shapiro-Wilk Test and Levene's Test to fulfill assumptions for ANOVA. Data were analyzed using a statistical model to account for the split-plot randomized complete block design, where time since cover crop termination was the whole-plot factor, and Fallow management preceding the corn was the subplot factor. The effects of Time since cover crop, Fallow management, Year and their interaction for soil biochemical indicators were determined using analysis of variance (ANOVA), where each of them was considered fixed effects and block was considered a random effect. Given the natural variability of these data main effects and all interactions for soil biochemical indicators were considered significant at $\alpha = 0.1$. Both ANOVA and mean separations were carried out using PROC GLIMMIX in the SAS® software (SAS Institute Inc, Cary, NC).

Results and discussion

Temperatures and precipitation

Over the last 30 years, the average winter temperature has been 0°C, while the summer average temperature was 25°C at the experiment site (Figure 3.1). Rainfall is unevenly distributed throughout the year, with May and June typically being the wettest months. Weather conditions preceding each sampling time were summarized to capture the potential impact of weather from those periods on treatment effects. Cumulative precipitation varied significantly among years but only slightly among phases of the rotation within each year (Table 3.2). The weather conditions for T1 and T2 were identical because they corresponded to the same months. Cumulative precipitation for all three times since termination for the 2022 sampling were significantly less than the 30-year normal for Riley County, with 7% less cumulative precipitation in T0, and 18% less in T1 and T2 compared to normal. In 2023, T0 was also drier than normal with 19% less precipitation, and T1 and T2 received more cumulative precipitation than normal (~5%). The period preceding T0 averaged 36% less cumulative precipitation over the two years compared to the period preceding T1 and T2. Regarding temperature, all times since termination in both 2022 and 2023 were within 0 to 1 °C of normal.

Soil biochemical indicators

The three-way interaction of Fallow management, Time since termination, and Year effects were not significant for any of the indicators (Table 3.3). The two-way interaction of Time since termination and Year was significant only for POXC, soil protein, glucosidase, and glucosaminidase activity. The interaction among Fallow management treatments and Time since termination was significant for all soil biochemical indicators, conversely the interaction between Fallow management and Year was not significant for any indicator. Year significantly affected all biochemical indicators except POXC. Time since termination affected soil protein, glucosidase activity, and soil respiration. Lastly, Fallow management significantly affected all soil biochemical indicators. Given these results, particularly, the significant Fallow management × Time since termination and Fallow management × Year interactions for nearly all indicators, one-way ANOVA analyses were conducted separately for each combination of Year and time since termination for all indicators.

Permanganate oxidizable carbon

Fallow management significantly affected POXC at all times since termination (T0, T1, and T2) in the 2022 sampling year but only T0 and T1 in 2023 (Table 3.4). The most substantial increases in POXC by the different Fallow management compared to CF were observed at T0, soon after cover crop termination (Figure 3.2). Previous research examining the impact of cover crops on the soil labil C pool has yielded varied results. Some studies report an increase in POXC attributed to the inclusion of cover crops in crop rotations (Ghimire et al., 2019; Jokela et al., 2009; Sainju et al., 2007; Wang et al., 2017), while others indicated that cover crops did not influence POXC (Pokhrel et al., 2021; Steele et al., 2012). The lack of effect is often attributed to differences in environmental conditions; For instance, Steele et al., (2012) did not find a consistent effect of winter cover crops in POXC in two sites in Maryland, where mean annual precipitation is 1033 mm yr^{-1}. However, in our study, winter cover crops significantly increased the POXC. While this site in Maryland had more mean precipitation than Manhattan KS, a dry spring season during cover crop growth could explain the lack of effect in the Steele et al., (2012) study.

A similar POXC response was observed at T0 in both 2022 and 2023 samplings years, where CF had the lowest POXC, DSB did not differ from CF, and the cover crop treatments further increased POXC by 30% on average compared to CF, with the highest POXC corresponding to the CR/CC treatment. However, overall higher POXC was observed in 2022 than in the 2023 sampling year, probably related to the higher cumulative precipitation during that year (Table 3.4). One year after cover crop termination (T1), treatment differences became less pronounced (Figure 3.2). In 2022 at T1, CF had the lowest POXC, while the rest of the treatments except CR increased POXC compared to CF by 28 % compared to CF. In 2023 at T1, POXC was 18% greater for the DM than CF, but the rest of the treatments did not differ from CF (Figure 3.2). Although plots that had long-term DSB or some version of cover crop or double-crop soybean improved POXC compared to CF two years after cover crop termination (T2) in the 2022 sampling year, no treatment differences were observed two years after cover crop termination in 2023.

Fallow management treatments displaying larger increases in POXC in our experiment also had more biomass production compared to treatments with small increases in POXC (Grünberg et al., 2024). Cover crop and crop residue inputs serve as C sources for microbes. Consequently, the absence of crop C inputs in the CF treatment likely led to the lowest POXC values (Spaeth et al., 1984, Burroughs et al., 2022). Furthermore, although DSB may leave a similar amount of residue as a cover crop biomass at termination, this residue undergoes processing during the combine-harvest and may not remain as closely attached to the soil as cover crop residue does after termination (Spaeth et al., 1984, Burroughs et al., 2022). Moreover, it's worth considering that DSB is harvested in the fall while cover crops are terminated in the spring, which could help explain the differences observed in POXC between most cover crops and DSB.

These findings align with conclusions drawn by various researchers (Ghimire et al., 2019; Lou et al., 2007; Wu et al., 1993). Unlike grass-based cover crops, most legumes do not produce a large amount of biomass, which may explain the lack of effect from the CC treatment.

Cover crop mixtures are widely used due to the potential multi-benefits this diversity may bring to the system (Chu et al., 2017; Lavergne et al., 2021). The DM treatment included grasses like Sorghum/Sudangrass, as well as a legume like pea, and brassicas like and turnip, which behave differently as they mineralize due to their differences in C/N ratio and root exploration. Among our treatments, the DM was the only one with more POXC compared to CF one year after termination in both sampling years. Other researchers have reported symbiotic effects on microbial activity when a higher C/N residue (like grasses) was mixed with a low C/N residue (like legumes) (Lavergne et al., 2021; Snapp et al., 2005). These previous studies suggested that the observed benefit could rely on an increase in microbial activity by the addition of high amounts of C inputs together with more N available for microorganisms, which is crucial for them to function. In another study, Pokhrel et al. (2021) did not find an effect of either single or mixed species cover crops on labil C concentrations compared to a non-cover control. While that study was conducted in a coarse soil, our study had a silty clay loam texture. Fine et al. (2017) reported that coarse-soils had significantly less active C compared to fine-textured soils.

Although a Fallow management effect was observed for all sampling times in the 2022 sampling year, only T0 and T1 were significantly affected by Fallow management in the 2023 samples. Immediately after cover crop termination, cover crop residues serve as a readily available C source, stimulating microbial growth (Lehman et al., 2012). As cover crop biomass undergoes decomposition, the reduced C input availability may potentially limit overall microbial activity (Aime et al., 2023). While plots at T2 in the 2022 sampling year were affected by Fallow management, the CCs (average cover crop treatments) and DSB treatments had a high grain yield that could imply a high residue input (Spaeth et al., 1984). On the other hand, plots from T2 in the 2023 sampling year were preceded by lower biomass and Fallow management did not affect POXC. Additionally, the residue input from corn, soybean, and wheat crops greatly exceeded that from Fallow management treatments, and it also influenced microbial activity differently based on residue quality (Hsiao et al., 2019). For example, the average corn residue exceeded the average cover crop biomass by up to 8 times (Liu et al., 2020, Grünberg et al., 2024). Hence, this could potentially contribute to obscuring the effects of Fallow management.

Soil protein

Fallow management response across time since cover crop termination varied with sampling year (Table 3.4). Fallow management affected soil protein only at termination (T0) in the 2022 sampling year and only at T0 and two years after termination (T2) in the 2023 sampling year (Figure 3.3). Inconsistent effects of cover crops on soil protein have been presented in the literature. Although certain studies have reported significant positive effects of cover crops on soil protein concentrations (Balota et al., 2014; Cordeiro et al., 2021; Feng et al., 2021), others have found minimal impact resulting from the incorporation of cover crops (Wood & Bowman, 2021; Wu et al., 2024). Feng et al. (2021) found an increase in soil protein when winter rye or a cover crop mix was included in a corn-soybean rotation in Iowa and South Dakota with a similar soil texture to this study (silty clay loam). On the other hand, no differences in soil protein were observed when cover crops were included in a no-till dryland soybean system in Mississippi (Pokhrel et al., 2021). Elevated soil moisture levels in this Mississippi site, in contrast to Manhattan, KS, may have restricted the production of protein-rich fungal hyphae potentially reducing soil protein content.

At cover crop termination (T0) in the 2022 sampling year, soil protein was similar in the CF, DSB, and CC treatments and significantly increased in the CR, CR/CC, and DM treatments compared to CF (Figure 3.3). At T0 in the 2023 sampling year, only CR and CR/CC treatments had significantly greater soil protein than CF, and the rest did not differ significantly from CF. At T1, Fallow management did not affect soil protein content in either sampling year. This lack of Fallow management effect could be explained by a simultaneous effect of N fertilization in microbial communities during the corn phase which could affect soil protein response among treatments (Sainju et al., 2021). Finally, two years after cover crop termination (T2) in the 2022 sampling year Fallow management didn’t affect soil protein. However, in the 2023 sampling year at T2, the CR/CC and DM treatments had significantly more soil protein than CF, and the remaining treatments were not significantly different from CF. As the cover crop biomass undergoes decomposition, the reduced substrate availability for microorganisms may potentially limit overall microbial growth and protein production. Furthermore, over time within the rotation, the proportion of cover crop residue gradually decreases relative to the total residue contributed by subsequent cash crops grown following termination of the cover crop. This gradual decline in cover crop residue as a fraction of total residues could obscure Fallow management effects at T1 and T2.

For this study, cover crop treatments exhibiting more biomass accumulation (CR/CC, DM (Grünberg et al., 2024), tended to have greater values for POXC and soil protein ($r = 0.5$, p-value: 0.003). Others have reported a correlation between soil protein and measures of C (Fine et al., 2017; Marshall et al., 2022), therefore the greater values for soil protein observed right after cover crop termination in the DM, CR and CR/CC treatments might be caused by stimulation of microbial activity by increased substrate availability (Feng et al., 2021). On the other hand, the lack of effect in the CC and DSB treatment is not surprising because the literature indicates that systems with legumes and high nitrate content in the soil tend to have low mycorrhizal fungi populations and low levels of glomalin-related protein soil content (Detheridge et al., 2016). The increased soil protein observed by the mixture treatment (DM) in most sampling times may be explained by the increased C input together with a synergic effect of multiple species that may stimulate microbial activity (Feng et al., 2021).

Enzyme activities

Similar trends were observed in glucosidase activity in both sampling years (Table 3.4). In both years, most alternatives to CF significantly increased both enzyme activities at T0 and T1 (Figures 3.4 and 3.5). Previous studies reported increases in enzyme activity due to the inclusion of a cover crop (Chavarría et al., 2016; Eivazi et al., 2024; Feng et al., 2021). For instance, Feng et al. (2021) observed an increase in glucosidase activity when cover crops were included in a rotation with similar soil texture to this study in Iowa and South Dakota. Cover cropping and no-till may can increase substrate availability, which can promote the induction of extracellular enzymes for breaking down residues and enhancing nutrient cycling (Balota et al., 2014).

At T0 in both sampling years, all cover crop treatments increased the glucosidase activity compared to CF, and DSB was not different than CF (Figure 3.4). At T1 in the 2022 sampling year, a greater glucosidase activity was observed in all treatments except CR compared to CF. At T1 in the 2023 sampling year, CF and DSB had the least glucosidase activity, CC, CR/CC, and

DM did not differ from CF, and the only treatment with higher glucosidase activity than CF was CR. The higher C content in the CR and high biomass in this treatment could be responsible for the tendency of higher glucosidase activity in most sampling times because this enzyme is positively correlated with C inputs in the soil (Tian and Shi, 2014). Although CC slightly increased glucosidase activity at certain times after cover crop termination, this treatment tended to have less activity than the rest of the cover crops, possibly due to reduced C inputs, which can limit the production of this enzyme (Chavarría et al., 2016). Although DSB may leave a similar amount of residue as a cover crop biomass at termination (Spaeth et al., 1984, Burroughs et al., 2022), glucosidase activity in this treatment was less than the rest of the cover crops. The DSB residue undergoes processing during the combine-harvest and this processing likely accelerated its breakdown, potentially leading to increased mineralization and reduced amount of C available to stimulate microbial glucosidase activity at the sampling time (Tian and Shi, 2014). Treatment differences tended to diminish from T0 to T1, and no Fallow management effect on glucosidase and glucosaminidase activity was observed two years after cover crops were terminated (T2) in either year. As cover crop biomass undergoes decomposition, the absence of available substrates can inhibit enzyme production (Chavarría et al., 2016). Furthermore, as the rotation advances, the proportion of cover crop residue gradually decreases relative to the total residue with each successive cash crop grown after the cover crop has been terminated. This gradual decrease in residue could mask the effects of at T1 and T2.

In 2022 and 2023, all cover crop treatments had more glucosaminidase activity than CF right after cover crop termination (T0) and one year after termination (T1) (Figure 3.5). Similarly, the DSB treatment had a higher glucosaminidase activity than CF for most sampling times. Overall, the highest glucosaminidase activity was observed for CR and CR/CC treatments, while DSB, DM, and CC had intermediate values. The most significant increases were observed in treatments with higher biomass production (i.e. CR/CC, CR, and DM) which included at least one grass species. Although cover crops don't add chitin directly as the substrate, the mechanism behind this may be that litter addition could fuel microbial growth and turnover, thereby increasing the concentration of fungal chitin in soil and thus enhancing microbial production of glucosaminidase (Tian and Shi, 2014). The lower glucosaminidase activity in the CC treatment compared to other cover crop treatments is unexpected given that the increased N-availability expected from a legume cover crop could potentially favor glucosaminidase activity (Ekenler and Tabatabai, 2002; Tian and Shi, 2014), however, similar results were obtained in a field study in Mississippi by Tyler (2020) when the legume cover crop biomass production was notably less than the other cover crops, as it was in this study (Grünberg et al., 2024). The slight increase in glucosaminidase activity in the DSB treatment compared to CF may also be explained by the induction of this enzyme with low C/N ratio residue from soybean (Tian and Shi, 2014). However, the magnitude of this increase likely was limited by the amount and quality of residue left by the DSB after harvest.

Higher glucosaminidase activities were observed at T0 in the 2022 sampling year compared to 2023, probably due to a lower cumulative precipitation during the preceding period in 2023 (Table 3.2). As with glucosidase, no Fallow management effect was observed in glucosaminidase activity two years after cover crop termination in either sampling year. This lack of a Fallow management effect at T2 for the activity of both enzymes may be attributed to the reduced substrate available for enzymes following prolonged decomposition (Chavarría et al., 2016). Moreover, as time progresses within the rotation, the residue of the cover crop gradually diminishes as a proportion of the total residue with each subsequent cash crop grown after the cover crop has been terminated. This gradual decrease in residue could mask the effects of at T1 and T2.

Soil respiration

As with the enzyme activities, soil respiration had similar trends across sampling times in both sampling years, where Fallow management significantly affected soil respiration at T0 and T1, but no effect was observed at T2 (Table 3.4). Several studies suggested the increase in microbial respiration with the addition of cover crops is primarily attributable to increased C substrate availability, as well as root and associated heterotrophic respiration (Kim et al. 2020; Nunes et al., 2018; Wood and Bowman, 2021). For instance, Nunes et al. (2018) observed that a cover crop mix of annual ryegrass (*Lolium multiflorum),* red clover (*Trifolium pratense*), crimson clover, and hairy vetch (*Vicia villosa*) improved soil heterotrophic respiration in a silt loam in New York right after termination. Similarly, Starr et al. (2019) observed an increase of 38% in soil respiration after termination of a cereal cover crop included in a continuous no-till corn-soybean rotation in Northeast Kansas, compared to fallow.

At T0 in the 2022 sampling year, all treatments exhibited, on average, an 8 mg CO_2 per gram of soil greater soil respiration rate than CF (Figure 3.6). Intermediate soil respiration was observed in DSB and CC treatments, while CR, CR/CC, and DM had the most soil respiration. At T0 in the 2023 sampling year, all cover crop treatments had an 8 mg CO_2 per gram higher soil respiration than CF, and DSB did not differ from CF, CC, and the DM treatment. At T1 in 2022, all cover crop treatments had a 4 mg CO_2 per gram higher soil respiration than CF, with slightly less activity observed for DSB. At T1 in the 2023 sampling year, the only treatment that had higher soil respiration than CF one year after cover crop termination (T1) was DM, with an additional 4 mg CO_2 per gram respired in this treatment. In general, differences in soil respiration rates between treatments diminished from T0 to T1.

Overall, the treatments exhibiting greater biomass, such as CR/CC, CR, and DM (Grünberg et al., 2024), had the most notable enhancements in soil respiration compared to CF. It is possible that an increase in C substrate availability in treatments with greater biomass compared to others contributed to enhanced microorganism activity and soil respiration rates (St Aime et al., 2023). This processing likely accelerated its breakdown, potentially leading to increased mineralization, resulting in a reduced amount of C available to stimulate soil respiration at the sampling time (Tian and Shi, 2014). Crimson clover and DSB had either slightly higher or similar soil respiration compared to CF. Although DSB may leave a similar amount of residue as a cover crop after harvest, this residue undergoes processing during the combine harvest and may not remain as closely attached to the soil as cover crop residue does after termination (Spaeth et al., 1984, Burroughs et al., 2022). In contrast, the average cover crop biomass was 1.2 Mg ha^{-1}, therefore the DSB contributed less C input for microbial activity compared to most cover crops (Grünberg et al., 2024). Although higher soil respiration rates could be expected in legumes due to the low C/N ratio that favors mineralization and promotes microbial growth and activity (Freidenreich et al., 2021), the low biomass production of the CC treatment in this study may be limiting the increase in soil microbial respiration. While cover cropping appeared to stimulate microbial activity compared to leaving land fallow, the impact did not last for longer than a year after termination. The absence of Fallow management effects at T2 could be explained by the amount of time for decomposition of cover crop biomass, leaving less C available for microorganisms to respire (Chavarría et al., 2016). Additionally, the residue input from corn, soybean, and wheat crops greatly exceeds that from cover crop treatments, and it also influences microbial activity and soil respiration differently based on residue quality (Hsiao et al. 2019). Hence, this could potentially contribute to obscuring the effects of Fallow management.

Conclusions

Cover crops positively impacted all soil biochemical indicators by increasing POXC, soil protein, enzymes activities and soil respiration immediately after cover crop termination. This supports our hypothesis that cover crops will improve all biochemical indicators evaluated in this study. While most indicators were also influenced by fallow management one year after cover crop termination, soil protein was the only indicator that was not affected by Fallow management at this sampling time. In general, cover crops with larger C inputs had the largest increases in biochemical indicators. Double-crop soybean exhibited levels similar to or slightly higher than CF for most of the indicators, yet less than most of the cover crops. This trend is likely attributed to its lower C input compared to the cover crops, along with the growing season difference—double-crop soybeans are harvested in the previous fall, whereas cover crops are terminated in spring. Furthermore, it is important to note that, although these benefits were evident immediately after cover crop termination and persisted for one year thereafter for most indicators, they diminished over time. Contrary to our initial expectations, the positive effects on biochemical indicators did not endure beyond the first year after cover crop termination. Among the management options evaluated in this study, the DM treatment impacted the indicators for a longer time after termination, mainly explained by a diversity of organic materials compared with the single species cover crops and, thus, stimulating microbial activity. This leads us to partially reject our initial hypothesis that cover crop-driven benefits in biochemical indicators will persist in the subsequent years of the crop rotation. Although cover crops have the potential to increase biological proprieties in the soil, continuous cover is needed to have a lasting impact on soil biological proprieties.

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Table 3.1 P-nitrophenol substrates and start and stop buffer for glucosidase and glucosaminidase enzyme assays.

Enzyme	**Start buffer**	**Stop buffer**	**Substrate**
Glucosidase	MUB pH 6	0.1 M THAM pH 12	0.05 M p-Nitrophenyl-β-D-glucopyranoside
Glucosaminidase	0.1 M Acetate Buffer	0.5 M NaOH	0.01 M ρ-Nitrophenyl-N-acetyl-β-D-glucosaminide

Table 3.2 Weather conditions since cover crop planting for T0 and since planting of the previous crop for T1 and T2 time for 2022 and 2023 samplings.

		Year					
		2022			2023		
Weather parameter		**T0[a]**	**T1**	**T2**	**T0**	**T1**	**T2**
Cumulative precipitation	(mm)	560	713	713	501	809	809
Departure from Normal[b]	(mm)	-44	-139	-139	-103	-63	-63
Mean temperature	(°C)	12	13	13	11	13	13
Departure from Normal	(°C)	1	0	0	0	0	0

[a] Time since cover crop termination: T0, immediately after; T1, one year after; T2, two years after.
[b] 30-year average for Riley County, 1993-2023 (Kansas Mesonet, 2024).

Table 3.3 Analysis of variance (ANOVA) table for POXC, soil protein, glucosidase (GLU), glucosaminidase (NAG) and soil respiration. Bolded values represent statistical significance at $p < 0.1$.

Source of variation	POXC	Soil protein	GLU	NAG	Soil respiration
Fallow management (mgmt.)	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Time since termination (TST)	0.5457	**0.0222**	0.9868	**0.0012**	**<0.0001**
Year	0.5614	**<0.0001**	**0.0002**	**<0.0001**	**<0.0001**
Fallow mgmt. x Year	0.1861	0.8301	0.413	0.1998	0.2523
Fallow mgmt. x TST	**0.0047**	**0.0199**	**0.0002**	**<0.0001**	**<0.0001**
TST x Year	**0.0005**	**0.0896**	**<0.0001**	**<0.0001**	0.1542
Fallow mgmt. x TST x Year	0.4359	0.4402	0.1828	0.8703	0.4117

Table 3.4 One way-ANOVA results for Fallow management effect by year and time since cover crop termination for POXC, soil protein, glucosidase (GLU), glucosaminidase (NAG), and soil respiration. Bold values represent statistical significance at $p < 0.1$.

Year	Time since termination	POXC	Soil protein	GLU	NAG	Soil respiration
2022	T0	**0.0004**	**0.0003**	**0.0007**	**<0.0001**	**<0.0001**
	T1	**0.0045**	0.1125	**<0.0001**	**0.0003**	**0.0042**
	T2	**0.0152**	0.6283	0.3031	0.1596	0.4769
2023	T0	**0.0007**	**0.0008**	**0.0003**	**<0.0001**	**<0.0001**
	T1	**0.0768**	0.4776	**0.0773**	**0.0249**	**0.0288**
	T2	0.3469	**0.0409**	0.2662	0.3119	0.5516

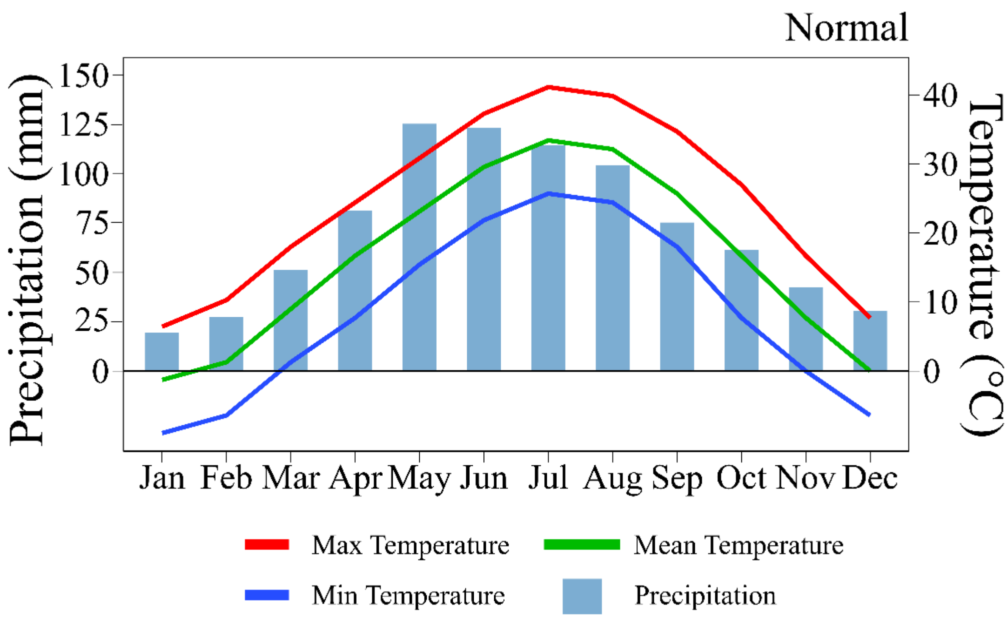

Figure 3.1 Monthly maximum, minimum and mean air temperatures, and monthly precipitation for 30-year Normals for Riley County, KS (Kansas Mesonet, 2024).

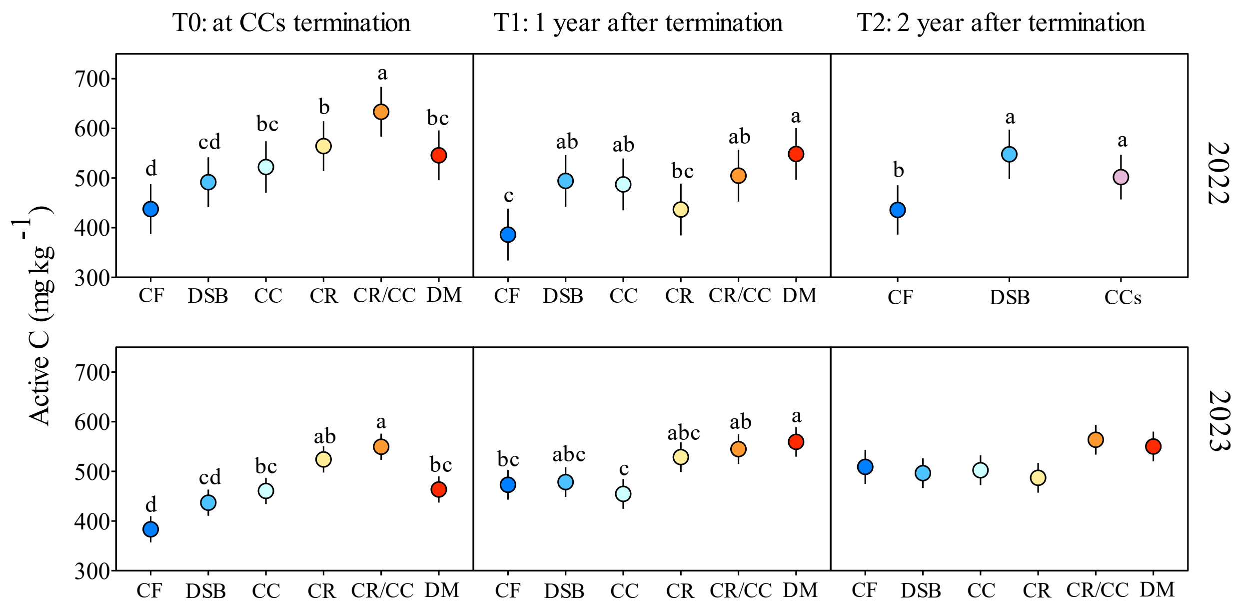

Figure 3.2 Permanganate oxidizable carbon (POXC) response to Fallow management for 0, 1, and 2 years after cover crop termination for 2022 and 2023 samplings. Letters indicate significant difference at α = 0.1 within each year and time since termination. Error bars represent standard error of the mean (CF: chemical fallow, DSB: double-crop soybean, CR: cereal rye, CC: crimson clover, CR/CC: cereal rye and crimson clover mix, DM: diverse mix, CCs: average of all cover crop plots).

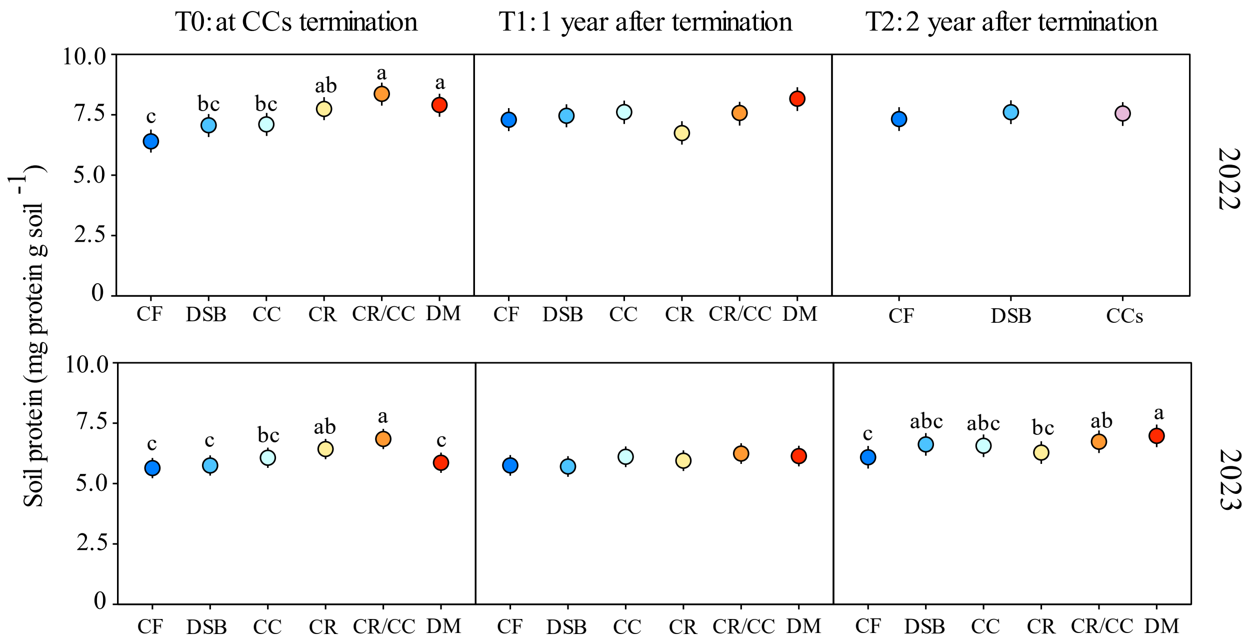

Figure 3.3 Soil protein response to Fallow management for 0, 1, and 2 years after cover crop termination for 2022 and 2023 samplings. Letters indicate significant difference at α = 0.1 within each year and time since termination. Error bars represent standard error of the mean (CF: chemical fallow, DSB: double-crop soybean, CR: cereal rye, CC: crimson clover, CR/CC: cereal rye and crimson clover mix, DM: diverse mix, CCs: average of all cover crop plots).

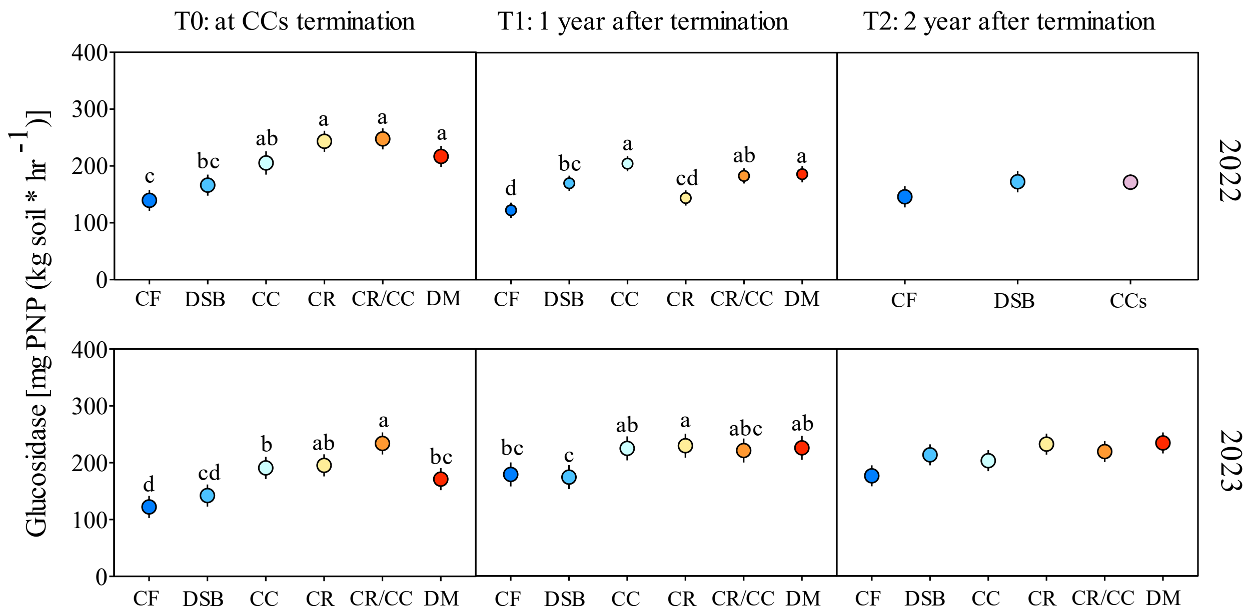

Figure 3.4 Glucosidase response to Fallow management for 0, 1, and 2 years after cover crop termination for 2022 and 2023 samplings. Letters indicate significant difference at $\alpha = 0.1$ within each year and time since termination. Error bars represent standard error of the mean (CF: chemical fallow, DSB: double-crop soybean, CR: cereal rye, CC: crimson clover, CR/CC: cereal rye and crimson clover mix, DM: diverse mix, CCs: average of all cover crop plots).

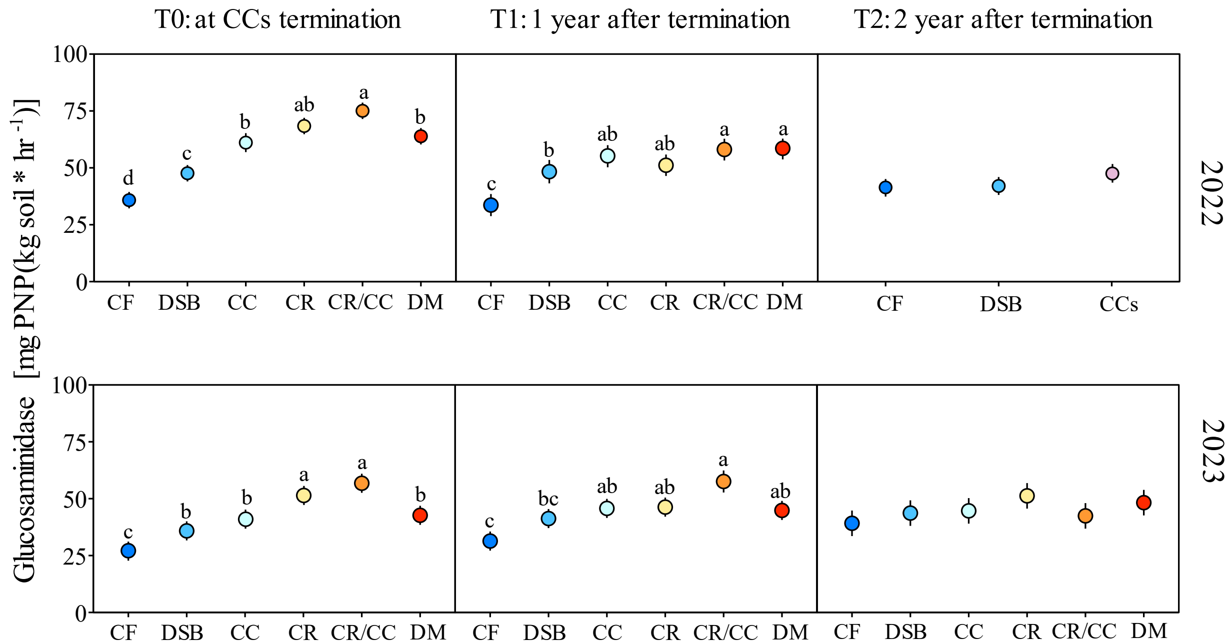

Figure 3.5 Glucosaminidase response to Fallow management for 0, 1, and 2 years after cover crop termination for 2022 and 2023 samplings. Letters indicate significant difference at α = 0.1 within each year and time since termination. Error bars represent standard error of the mean (CF: chemical fallow, DSB: double-crop soybean, CR: cereal rye, CC: crimson clover, CR/CC: cereal rye and crimson clover mix, DM: diverse mix, CCs: average of all cover crop plots).

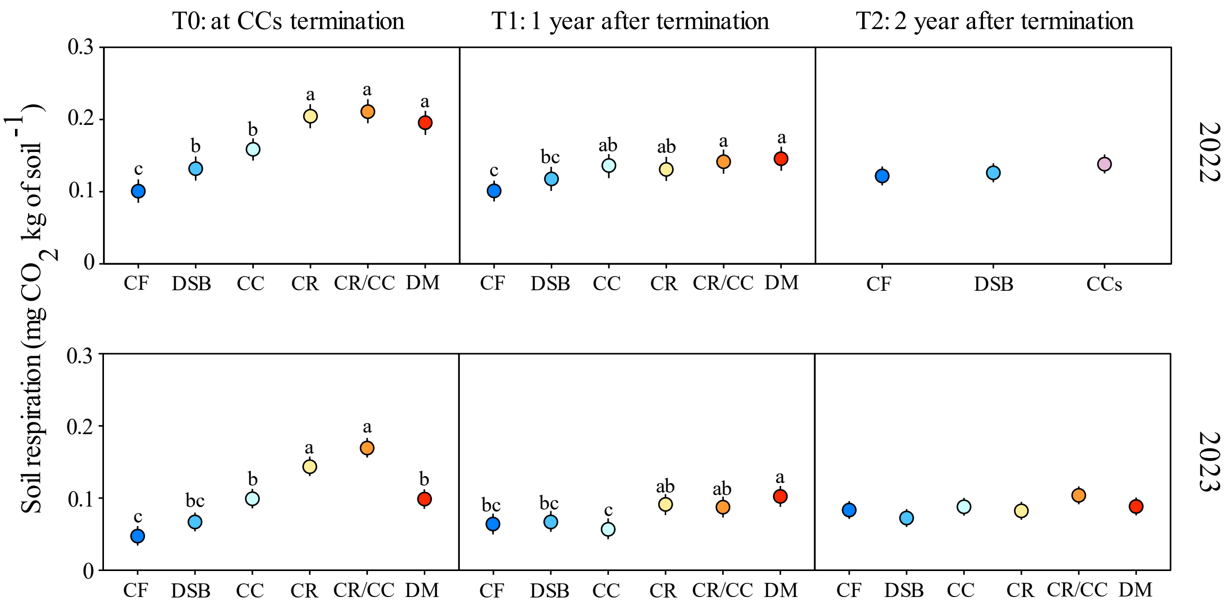

Figure 3.6 Soil respiration response to Fallow management for 0, 1, and 2 years after cover crop termination for 2022 and 2023 samplings. Letters indicate significant difference at α = 0.1 within each year and time since termination. Error bars represent standard error of the mean (CF: chemical fallow, DSB: double-crop soybean, CR: cereal rye, CC: crimson clover, CR/CC: cereal rye and crimson clover mix, DM: diverse mix, CCs: average of all cover crop plots).

Chapter 4 - General conclusions

In this study, the integration of cover crops into crop rotations showed significant benefits for soil health, particularly in terms of soil organic carbon (SOC), Total nitrogen (N), pH, and various soil biochemical indicators. Cover crops were instrumental in increasing SOC and Total N concentrations on the soil surface, with treatments characterized by higher carbon (C) inputs leading to greater SOC accumulation. Furthermore, the double-crop soybean treatment exhibited notable increases in these soil properties, although not surpassing those observed with cover crops. Moreover, cover crops, especially cereal rye, demonstrated an ability to buffer pH levels towards neutrality at the soil surface. These findings support the hypothesis that integrating cover crops into agricultural systems can positively influence soil health indicators. While cover crops have shown clear benefits, their impact on phosphorus (P) stocks per cm^{-1} is less pronounced. Certain cover crop treatments showed minimal effects on P stratification, and greater decreases in P were observed at the double-crop soybean treatment. These findings suggest a need for further research to manage the potential negative impacts of P stratification on P loss and water quality. The long-term negative impact of N fertilization on soil acidification, particularly in shallow soil layers, reinforces the need for sustainable N management practices in agricultural systems.

Cover crops also positively impacted soil biochemical indicators, such as permanganate oxidizable C, soil protein, enzyme activities, and soil respiration, immediately after termination. High biomass cover crops showed the greatest increase in biological indicators right after termination. However, these benefits tended to diminish over time, highlighting the importance of continuous cover cropping to sustain improvements in soil biological properties. Despite these limitations, cover crops, particularly those with diverse residue characteristics like the diverse mix treatment, can stimulate microbial activity and have a lasting impact on soil biochemical properties.

In conclusion, our results demonstrated that cover crops have significant potential to improve soil health indicators, especially when integrated into crop rotations with diverse species and management practices. However, to maximize these benefits, continuous cover cropping and sustainable management practices are essential. It is evident that cover crops can be a valuable tool for improving soil health, but their effectiveness depends on various factors, including local weather conditions, soil type, cover crop species selection and management. Further research is needed to complete the soil health assessment, including the evaluation of soil physical properties and main crop productivity. Overall, the findings suggest that cover crops are a beneficial practice for enhancing soil health and sustainability in agricultural systems.

Appendix A - Assessment of infiltration and bulk density after 15 years of no-till and cover crops

Introduction

Understanding soil physical properties is crucial for maintaining soil health and ensuring sustainable agricultural practices. An agricultural soil presenting good physical quality should be "strong" enough to maintain a good structure, hold crops upright, and resist erosion and compaction and "weak" enough to allow unrestricted root growth and proliferation of soil flora and fauna. This soil should also present fluid transmission and storage characteristics that allow the correct proportions of water, nutrients, and air for both maximum crop performance and minimum environmental impact (Reynolds et al., 2002). Key indicators for assessing soil physical health include soil infiltration, aeration, water holding capacity, drainage, aggregate stability, and compaction.

Bulk density, water infiltration, and aggregate stability are among the most common indicators used to evaluate soil physical functions (Reynolds et al., 2009; Moebius et al., 2007; Aksakal, 2020). Bulk density provides insights into soil compactness, which directly impacts soil aeration and infiltration rates. Soil structure, influencing aggregate stability, determines the soil's ability to withstand external forces such as intense rainfall or mechanical disturbances. Meanwhile, soil water infiltration reflects the rate at which water enters and moves through the soil profile, crucial for sustaining plant growth (Joel and Messing, 2001).

All the previous variables may change significantly with soil management, thus, they are suitable soil health indicators. Porosity, soil available water, and rooting depth are also essential factors to consider (Allen et al., 2011).

Introducing cover crops into agricultural systems can significantly impact soil physical properties. Cover crops represent an input of above and below-ground biomass, which can improve soil physical proprieties and protect the soil from water and wind erosion (Fronning et al., 2008). Studies have demonstrated that cover crops can increase aggregate stability, often measured by mean weight diameter (MWD) and the presence of wet or dry aggregates (Hermawan and Bomke, 1997; Liu et al., 2005). Folorunso et al. (1992) observed reduced soil penetration resistance and increased infiltration when multiple cover crop species were incorporated into conventional tillage systems.

However, the effects of cover crops on soil physical indicators can vary. While some studies report improvements in bulk density and other physical properties (Blanco-Canqui et al., 2011; Haruna & Nkongolo, 2015), inconsistencies have been noted in different soil types and management regimes (Fronning et al., 2008; Mubiru and Coyne, 2009; Villamil et al., 2006; Wagger and Denton, 1989; Sharma et al., 2018). Understanding these variations is essential for optimizing cover crop strategies to enhance soil health effectively. The objective of this study was to determine the effect of cover crops on soil physical properties in a long-term no-till rotation in Northeast Kansas.

Materials and Methods

Site Description and Experimental Design

The study consisted of a three-year winter wheat–corn–soybean rotation, where all phases of the rotation were present every year. The long-term experiment was established in 2007 at the Kansas State University Department of Agronomy Research Farm (39º 07’ N lat, 96º 38’ W long) located approximately 8 km south of Manhattan, KS. The altitude is approximately 311 m, and the region is characterized by a hot humid continental climate (Köppen Climate

Classification System: Dfa). The mean annual temperature is 12.9 ºC and the average annual precipitation is 889 mm (Kansas Mesonet, 2024). The soil is a moderately well-drained Wymore silty clay loam (fine, smectitic, mesic Aquertic Argiudoll), with 0 to 1% slopes.

The experimental design was a split-plot randomized complete block design, with four replications of each phase of the rotation. Crop phases were whole plots, and the six different Fallow management treatments during the wheat-fallow phase were subplots. Every whole plot was 36 by 70 m, and each subplot was 6 by 70 m. Further details about the experimental design and site can be found in Fontes et al. (2017). Fallow management treatments imposed during the wheat-fallow phase of the rotation in 2021 and 2022 were chemical fallow (CF) (control), double-crop soybean (DSB) (an alternative intensification option), winter legume cover crop (crimson clover, *Trifolium incarnatum* L. CC), winter non-legume cover crop (cereal rye, *Secale cereale* L., CR), a mix of the rye and the clover (CR/CC), and a diverse mix (DM) including triticale (×*Triticosecale Wittmack*), winter peas (*Pisum sativum* L.), sorghum-sudangrass (*Sorghum bicolor* L.), turnips (*Brassica rapa* L.), radishes (*Raphanus sativus* L.) and rape (*Brassica napus* L.).

Wheat-fallow phase

Winter wheat was planted immediately after soybean harvest in October 2021 and November 2022 using a John Deere 1590 no-till drill (Deere & Co., Moline, IL). Weeds were chemically controlled at Feekes stage 4-5 in both years. Winter wheat was harvested on June 22, 2021, and June 30, 2022.

Cover crop treatments have varied since initiation of the study (Fontes et al., 2017; Nielsen, 2020). The CF and DSB treatments were applied consistently since the study was initiated. The current set of winter cover crops treatments were planted in September 10 of 2021 and August 24 of 2022. Non-hardy winter cover crop species were killed by freezing temperatures, which usually occurred during November. On April 14 of 2021 and April 26 of 2022 cover crop plots were sprayed with a mix of herbicides in 2022 to terminate surviving cover crops, volunteer wheat, and other winter annual weeds.

Double-crop soybean was planted immediately after the wheat harvest on July 9 of 2021 and July 9, 2022. Weeds were chemically controlled throughout the DSB growing season.. The DSB plots were hand-harvested on November 5 of 2021 and combine harvested on October 26 of 2022.

Corn phase

Initially, the rotation consisted of wheat, grain sorghum, and soybean (Fontes et al., 2017). However, grain sorghum was replaced by corn in 2021. Corn was planted soon after cover crop termination on April 30 of 2021 and May 11 of 2022. Weeds were controlled with herbicides prior to and after corn planting. After corn reached physiological maturity and had dried sufficiently for machine harvest, yield was determined by harvesting plants from the center two rows of each subplot with a combine in September 17 of 2021 and October 4 in 2022.

Soybean phase

Soybeans were planted on April 30 of 2021 and May 12 of. In both years, weeds were controlled prior and after soybean planting with herbicides applications. Soybeans were harvested on November 5 of 2021 and October 10 of 2022.

Sampling

During 2023, bulk density soil sampling was carried out using two methodologies. In spring 2023, a 4-cm diameter core for the depths of 15-30 cm and 30-60 cm was obtained for each treatment, using a tractor-mounted hydraulic probe. The collected samples were subjected to drying in a forced-air oven at 105 °C until a constant weight was achieved. Furthermore, in the summer of 2023, soil samples were gathered from depths of 0-5 cm, 5-10 cm, and 10-15 cm, employing the core method described by (Blake & Hartge, 2018) . The rings were pushed into the soil surface with the help of a sledgehammer and wooden block and then placed into an autoclavable plastic bag to be dried at 105°C until constant weight was reached. For both methodologies, the resulting dry weight was then utilized to calculate the bulk density (g/cm³, Equation 1).

$$Bulk\ density\ (\frac{gr}{cm3}) = \frac{Mass\ of\ dry\ soil\ (gr)}{Core\ volume\ (cm3)} \quad \text{Eq. [1]}$$

Soil infiltration measurements were conducted in November 2022 and November 2023 in those plots where corn was harvested by using the Mini Disk infiltrometer (Model M1, Mini-Disk Infiltrometer, DecagonDevices, Inc.). This device consists of a single tube divided into two chambers: the lower one serves as a water reservoir (95 cm^3), closed at the bottom with the porous sintered stainless-steel disk (0.3 cm thick with a diameter of 4.5 cm); and the upper one serves for setting of the pressure head under which the infiltration takes place (from −7 to −0.5 cm). More detailed descriptions of the devices can be found in the User´s manuals (Decagon, 2005).

A total of three samples per plot were collected. Manual recordings of water level decline occurred at regular intervals of 30 seconds. The pressure head was applied -2 cm, as recommended for silty clay loams. Infiltration endured for at least 5 minutes, concluding when all water from the reservoir had infiltrated. The measured infiltration data were analyzed based on the steady-state data analysis method recommended in User´s manuals, fitting the cumulative infiltration data and time with the following equation (Equation 2):

$$I\ = C1 + C2\sqrt{t} \quad \text{Eq. [2]}$$

Here, C1 (LT^{-1}) and *C*2 ($LT^{-1/2}$) represent parameters of hydraulic conductivity and soil sorptivity respectively, where *I* (L) denotes cumulative infiltration, and *t* (T) signifies time. The calculation of infiltration rate *K* follows this formula (Equation 3):

$$K = \frac{C1}{A} \qquad \text{Eq. [3]}$$

Where *C*1 is the slope of the curve of cumulative infiltration versus the square root of time, and *A* is a value linking the van Genuchten parameters for 12 soil texture classes to the radius of the disk and applied pressure head. The *A* parameter for the silty clay loam (α=0.01 and n=1.23) was obtained from a table provided in the User's manual. It's noteworthy that these A parameter values are derived from the USDA database encompassing soils from the United States.

Statistical Analyses

Data were tested for normal distribution and equal variances using the Shapiro-Wilk Test and Levene's Test to fulfill assumptions for ANOVA. Data were analyzed using a statistical model to account for the randomized complete block design and analyze the effect of Fallow management as a fixed factor. When analyzing bulk density data, Fallow management main effects were evaluated for each of the sampling depths. Block and Year (when sampling was conducted more than one year) were considered random effects. The effects of Fallow management were determined using analysis of variance (ANOVA), and main effects were considered significant at $\alpha = 0.1$. Both ANOVA and mean separations with pairwise comparisons were carried out using PROC GLIMMIX in the SAS® software (SAS Institute Inc, Cary, NC).

Results

Bulk density

Bulk density among the different Fallow management treatments is presented in Figure A.1. On average, the lowest bulk density values were nearer the soil surface, and they increased with depth. No major differences between Fallow treatments were observed at any depth (p-value > 0.1 at all depths). Bulk density increased from the 0-5 cm depth to the 10-15 cm soil layer, then it slightly decreased from the 10-15 cm depth to the 15-30 cm depth, and finally, it increased to its highest point in the deepest soil layer (30-60 cm).

Infiltration rate

Infiltration rates averaged across 2022 and 2022 sampling years are presented in Figure A.2. Although no major differences were observed between Fallow management treatments (p-value: 0.5), there was a tendency to lower infiltration rates in the CR treatment.

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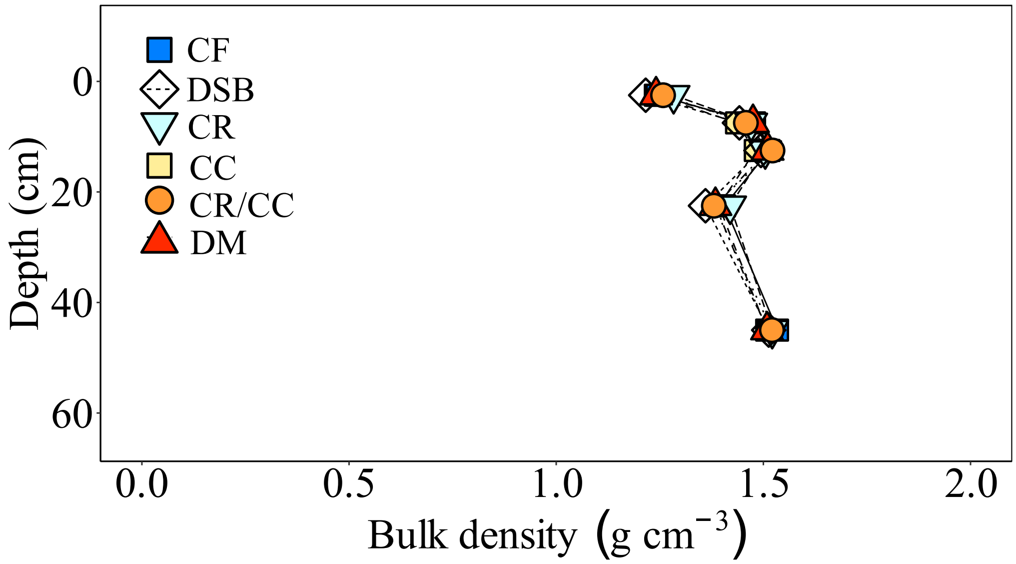

Figure A. 1 Bulk density vertical distribution in the soil profile as affected by Fallow management (CF: chemical fallow, DSB: double-crop soybean, CR: cereal rye, CC: crimson clover, CR/CC: cereal rye and crimson clover mix, DM: diverse mix).

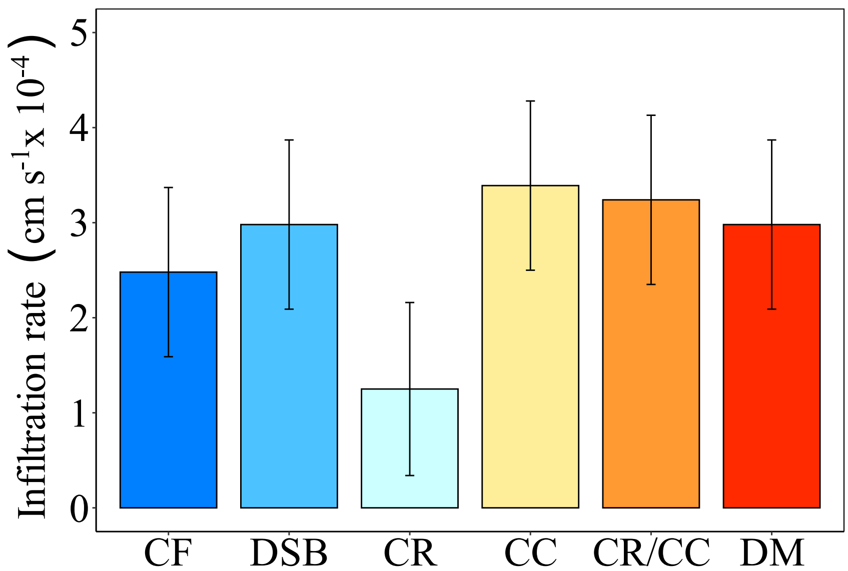

Figure A. 2 Infiltration rates as affected by Fallow management (CF: chemical fallow, DSB: double-crop soybean, CR: cereal rye, CC: crimson clover, CR/CC: cereal rye and crimson clover mix, DM: diverse mix).