

From microbial dynamics to agroecosystem resilience: Soil health responses to intensified and regenerative cropping systems in Kansas

by

Cesar Augusto Guareschi

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AN ABSTRACT OF A DISSERTATION

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Abstract

Sustaining agricultural production while preserving soil resources remains a central challenge for farming systems in rainfed agroecosystems in the face of climate variability, land degradation, and resource limitations. Soils are the foundations of agricultural systems, yet decades of intensive tillage management and monocropping have reduced soil organic matter levels, weakened soil structure, and disrupted microbial functioning across many dryland regions. These degradative trends are especially concerning in the U.S. Great Plains, where high variability in precipitation and frequent drought events constrain both crop yields and soil health stability. In this sense, regenerative agriculture (RA) practices, such as no-tillage, intensified crop rotations, and cover cropping, have gained recognition as a framework for improving soil function through the adoption of practices that enhance soil biological activity, increase organic matter inputs, and minimize soil disturbance. Understanding how these practices influence soil processes and the overall system's productivity over time is essential for designing productive, resilient, and sustainable farming systems.

This study investigates the interactions between soil biological processes, management intensity, and regenerative agriculture practices in rainfed cropping systems commonly used in Kansas. The overarching goal was to evaluate how management transitions toward greater crop intensification and diversification, reduced soil disturbance, and continuous soil cover influence soil health and overall performance at different spatial and temporal scales. Specifically, this research aimed to: i) examine the seasonal patterns in biological, chemical, and physical soil health indicators and identify the optimal sampling period for consistent soil health assessments; ii) assess the long-term effects of cropping systems intensification and diversification on

chemical, physical, and biological dimensions of soil health; and iii) evaluate the impacts of cover crop adoption on crop yield and yield stability across multiple on-farm research environments. Across the integrated studies, soil microbial biomass, soil microbial community composition, extracellular enzyme activities, soil organic carbon (SOC), and soil aggregate stability were measured as indicators of soil health.

Biological soil health indicators had strong temporal variability driven by crop growth stages, residue turnover, and climatic fluctuations. Soil microbial biomass and extracellular enzyme activities, especially β -glucosidase, were on average 30-100% higher during active wheat growth and peaked at flowering compared to post-harvest periods. Additionally, soil temperature and moisture were also identified as drivers of this variability. Among the sampling times, the fall sampling around winter wheat planting provided the most consistent and representative assessment of soil health. Moreover, in the five-year study, the transition from conventional tillage to continuous no-tillage significantly increased soil organic carbon by 8-12% and soil microbial biomass by 15-25% at 0-5 cm depth across all treatments. Extracellular enzyme activities and soil aggregate stability also displayed significant improvements after five years, especially at 0-5 cm depth. However, differences between crop rotations varying in crop intensity and diversity were not yet statistically significant, thus suggesting that measurable benefits from increased crop intensification and diversification require longer timeframes in these situations.

At the on-farm scale, cover crop adoption increased soil health. Notably, soil total microbial biomass and β -glucosidase increased by up to 40% and 30%, respectively, across sites. Soil organic carbon also increased modestly ($0.2 - 0.4 \text{ g kg}^{-1} \text{ yr}^{-1}$) in the cover crop treatments relative to the no cover crop treatments. Despite the improvements in soil health, crop yields of

corn, soybean, and winter wheat were statistically similar between treatments across locations and years. Crop yield variability was attributed to precipitation patterns associated with each location, with the cover crop treatments exhibiting a neutral or slightly positive effect under drier conditions, thus indicating a role in buffering yield losses during low-rainfall years rather than increasing average absolute yields.

Together, these findings demonstrate that biological soil health indicators are highly sensitive to short-term climatic and crop growth variation, and that improvements from regenerative agriculture practices accumulate progressively over time. The transition to continuous no-tillage systems rapidly enhances surface soil function, while the benefits of increased crop intensity and diversity, as well as cover cropping, emerge more gradually through sustained organic inputs and better soil function. Overall, this research provides a comprehensive, multi-scale assessment of soil health development under intensified, diversified, and regenerative cropping systems management. Through the link of seasonal microbial dynamics, long-term system evolution, and on-farm agronomic performance, this dissertation contributes to a deeper understanding of how soil processes modulate the resilience of rainfed agriculture in Kansas. These results highlight that the consistent application of regenerative agriculture practices and principles offers a viable pathway toward building more sustainable, profitable, efficient, and resilient farming systems in the U.S. Great Plains region.

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Approved by:

Major Professor
Dr. Charles W. Rice

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Dedication

I dedicate this dissertation to my family. To my grandparents Arlindo Quinto Guareschi, Avani Tonon Guareschi (*in memoriam*), Selvino Acadrolli (*in memoriam*), and Josefina Acadrolli. To my parents and sister, Cesar Vicente Guareschi, Lizete Maria Acadrolli Guareschi, and Maura Acadrolli Guareschi. To my wife, Ana Julia Paula Carcedo.

Chapter 1 - General introduction and literature review

Soils are natural bodies located at the interface between atmosphere, lithosphere (Lal, 2016), hydrosphere, and biosphere (Lehmann et al., 2020), being essential in the process of organic matter recycling, nutrient storage for plant uptake, carbon sequestration, climate moderation, and biodiversity's habitat (Lal, 2016). Soils are multicomponent, multifunctional, and complex systems (Kibblewhite et al., 2008). In addition, soils are also indispensable for ensuring food production and security, the functioning of global ecosystems, and the provision of ecosystem services (Paz-Ferreiro & Fu, 2016). Moreover, the provision of these ecosystem services is greatly affected through modifications of soil chemical, physical, and biological properties, especially in agroecosystems (Tilman et al., 2002; Power, 2010). However, agricultural practices and production are one of the main contributors of negative environmental impacts on the biosphere, mostly because of the increasing demand for food, fiber, and fuel production for an exponential population growth predicted over the next decades (Tilman et al., 2011; Alexandratos & Bruinsma, 2012; Hunter et al., 2017; Williams et al., 2020). In addition, predicted changes in climate pose the challenge of sustainably producing food, fiber, and fuel in a scenario with rising temperatures, higher frequency of extreme weather events, and uncertainties (Wheeler & von Braun, 2013).

Soil health is a concept defined as *“the continuous capacity of a soil to function as a living system within ecosystems, and land uses boundaries to sustain plant and animal production, maintain or enhance water and air quality, and promote plant, animal, and human health”* (Doran & Zeiss, 2000). Moreover, soil health is a widely used term in sustainable agriculture discussions to establish and describe the general state, condition, or quality of the soil

resource (Kibblewhite et al., 2008), where soil physical, chemical, and biological properties are assessed for determining and understanding different indices of soil health in an ecological perspective (Norris & Congreves, 2018). These soil properties, also known as soil health indicators, are correlated to relevant soil processes and affected by changes in environmental conditions and agricultural management practices (Yang, Siddique, and Liu 2020; Rodriguez et al., 2022). In addition, the aim for maximized crop production has led to human interventions which affected and changed, mostly negatively, the soil from its natural state, resulting in loss of other ecosystem functions (Kibblewhite et al., 2008). The health state of a soil can be determined by a set of indicators intended to provide key information regarding the soil's function, composition, and structure as well as integrate the complexities of the soil ecosystem (Paz-Ferreiro & Fu, 2016).

Soil health indicators are considered a set of measurable physical, chemical, and biological characteristics that are related to soil processes and used to evaluate and determine the soil health status (Allen et al., 2011). The use of these soil health indicators should be useful in the sense of i) correlating with ecosystem processes, ii) integrating soil physical, chemical, and biological soil characteristics, and iii) being sensitive to changes in management practices and variations in climate (Doran & Parkin, 2015b). Moreover, the use of soil health indicators in order to assess soil quality or health and its changes over time may be used as an indirect measure of soil functionality (Allen et al., 2011). Traditionally, chemical indicators such as soil organic carbon, total nitrogen, extractable phosphorus, cations (Ca^{2+} , Mg^{2+} , Na^+ , K^+), cation exchange capacity, and pH, physical indicators such as particle size distribution, bulk density, porosity, aggregate stability, and soil structure, and biological indicators such as microbial activity and community structure are the most widely used parameters on soil health

assessments. Overall, soil health indicators can be considered a set of measurable properties that allow the interpretation of the state of soil health on a relative scale (Dominati et al., 2010).

Nowadays, the most important limitation in the evaluation of soil health assessments in agroecosystems relies on the lack of a proper and healthy control aimed to be used as a reference for comparison purposes (Yang et al., 2020). Because of it, and because of the soil's spatial heterogeneity and temporal dynamics, trends over time are the most suitable way to quantify the effect of management practices on soil health (Karlen et al., 2011; Yang et al., 2020). The main goal and challenge of soil health maintenance and improvement is to maintain and ensure the long-term productivity and sustainability of cropping systems, using the evaluation of five vital functions: nutrient cycling, water relations, biodiversity and habitat, filtering and buffering, and physical stability and support (Hatfield et al., 2017).

The promotion, maintenance, and monitoring of soil health are fundamental factors for the sustainability of agroecosystems (Karlen & Rice, 2015; da Luz et al., 2019). In the midwestern U.S., the agricultural production systems are mainly focused on targeting high yields while ecological processes, such as nutrient and water cycling, and soil health, are deteriorating (Gliessman, 2014; Prokopy et al., 2020; Krupek et al., 2022). In addition, the use of intensive tillage systems and fallow-based systems has led to declining productivity of the agricultural systems over the years (Kirby et al., 2000). In this sense, the adoption of management practices aiming to minimize soil disturbance, maximize biodiversity and living roots, and maximize soil cover are considered opportunities to reverse soil degradation trends, as well as benefit ecological processes, overall productivity, and resilience of the agricultural systems (Delgado et al., 2011; Krupek et al., 2022).

Cropping intensification is defined as the substitution of fallow periods by periods with cash, or cover, crops in a determined timeframe. Crop periods are defined as the crop's growing seasons, and fallow periods are defined as the period between the crop's harvest and the planting of the subsequent crop (Farahani et al., 1998). Crop diversification is defined as the adoption of different crop species on a specific cropping system. Therefore, cropping systems intensification and diversification, in addition to no-tillage systems, are approaches intended to maximize crop productivity while preserving natural resources as well as the soil's ecosystem functions, processes, and services. These benefits have the potential to represent short and long-term benefits for both producers and the environment by stabilizing economic returns, enhancing the overall productivity of the agricultural systems, improving the efficiency, and increasing adaptability and mitigation to climate change effects (Kirby et al., 2000).

In this chapter, chemical, physical, and biological soil health indicators in agroecosystems are reviewed. Firstly, soil microbial community structure and activity, the importance of soil microbes for soil functioning in agroecosystems, and the methods and approaches to quantify soil microbial community structure and activity. In addition, chemical and physical soil health indicators are also reviewed, as well as their importance and effect on agricultural ecosystems. Secondly, the interactions between crops, soils, crop physiological stages, and soil health indicators are discussed in the following section, as these factors have a strong influence on the state of soil health. Finally, the effect of crop rotations as well as intense and diverse cropping systems is discussed since the intensification of crop rotations also influences chemical, physical, and biological soil health indicators.

Soil microbial properties

One of the major components of soil functioning is soil organisms (Bünemann et al., 2018), being one of the largest pools of biodiversity on earth, also affecting the ecosystem's stability (Yang et al., 2020). Microorganisms present in the soil are responsible for the link between plant roots and soil, nutrient cycling and recycling, organic matter decomposition, and also act as accurate indicators for specific soil functions as they quickly respond to changes that might occur in the soil ecosystem (Jacoby et al., 2017; Tahat et al., 2020). Therefore, soil microbial properties have the potential to indicate how well the soil ecosystem can support plant growth and thus the productive capacity of the soil environment (Sinsabaugh & Follstad Shah, 2011). In addition, soil microbial community, structure, and activity have been demonstrated to be responsive to agricultural management practices, such as tillage, crop rotation, and residue management (Biederbeck et al., 2005; Mohammadi, 2011). Likewise, the labile fraction of soil organic matter, mainly organic carbon, also has the potential to indicate microbial activity as well as indicate the overall soil productivity, mostly because of its capacity to provide nutrients for plant and soil microorganisms (García-Orenes et al., 2010).

Increased soil microbial diversity and activity have been shown to benefit soil functioning in agroecosystems, therefore increasing the overall sustainability of agricultural systems (Venter et al., 2016). However, the effects of environmental variability on soil microbial diversity and activity remain incompletely understood, particularly regarding the short-term responses of microbial communities to fluctuations in soil moisture and temperature, and their interactions with plant growth stages and root-derived carbon inputs.

Soil Microbial Activity

Soil extracellular enzymes play an essential role in biochemical processes occurring in the soil, such as organic matter recycling inside the soil ecosystem, and their activities are closely related to soil organic matter, microbial activity, and biomass, and soil physical properties (Ch et al., 2017). Enzymes are primarily derived from microbial origin (Ladd, 1978); however, they also originate from plants and animals (Tabatabai, 2018), catalyzing biochemical reactions and are a vital part of nutrient cycling in the soil environment (Bandick & Dick, 1999). Moreover, soil enzyme activities are also used as indicators in the evaluation of the degree of alteration of soil properties, either in natural or agroecosystems, having a strong correlation with soil health (Kandeler et al., 1999; Mohammadi, 2011). In the sense of soil microbial ecology, microorganisms produce enzymes intended to assimilate bioavailable nutrients immediately, which are considered intracellular enzymes, and secrete enzymes to the environment for further nutrient cycling and decomposition, which are known as extracellular enzymes. Extracellular enzymes can mediate the decomposition of complex organic compounds, such as soluble saccharides, organic acids, amino acids, cellulose, hemicellulose, and lignin (Baldrian & Šnajdr, 2011; Baldrian, 2014), reducing macromolecules into low-molecular-mass compounds that will be available for microbial assimilation and energy generation (CO₂) (Dick, 2002).

The extracellular enzyme activity in soil has been studied for several decades, and some of the most common extracellular enzymes include chitinase (N-acetyl- β -D-glucosaminidase, Enzyme Commission number [EC] 3.2.1.30), cellulase (EC 3.2.1.4), xylanase (EC 3.2.1.8), β -glucosidase (EC 3.2.1.21), aminopeptidases (EC 3.4.11.1), urease (EC 3.5.1.5), phosphatases (e.g., acid phosphatase, EC 3.1.3.2; alkaline phosphatase, EC 3.1.3.1), and oxidases (e.g., phenol oxidase, EC 1.10.3.2; peroxidase EC 1.11.1.7). Chitinase hydrolyzes chitin chains from the

terminal nonreducing end and releases simple N-acetylglucosamine units. Cellulase hydrolyzes the 1,4- β -glucosidic bond in cellulose and lichenin, xylanase hydrolyzes the 1,4- β -glucosidic bond from hemicellulose, and β -glucosidase hydrolyzes the terminal glucosidic bond, releasing β -D-glucose. Aminopeptidase cleaves N-terminal residues of peptides and proteins, where Leucine-aminopeptidase is the most widely measured aminopeptidase because of its potential to hydrolyze the most abundant substrate (Sinsabaugh & Follstad Shah, 2011). Urease catalyzes the hydrolysis of urea to CO_2 and NH_3 . Phosphatases catalyze the hydrolysis of both esters and anhydrides of H_2PO_4 , releasing inorganic P from phosphosaccharides and phospholipids. In addition, the predominant phosphatases are acid phosphatase and alkaline phosphatase, depending on soil pH. Phenol oxidase and peroxidase, as oxidases, are expressed for various purposes, including detoxification, oxidative stress, and acquisition of C from lignin.

The assessment of soil enzyme activity is based on the measurement of either the product appearance or substrate disappearance when the soil is incubated with specific substrates for each enzyme (Dick, 2020). In general, the measurement of a product's appearance is preferred. Extracellular enzyme activity assays do not quantify the concentration of an enzyme in soil, as it would involve the extraction of a specific protein from soil and subsequent quantification of this protein. From an ecological perspective, it is the specific enzyme potential activity that is most important (Dick, 2020). Through the addition of artificial substrates, linked with a colored compound (chromophore), into a diluted and homogenized soil slurry, it is possible to determine enzyme activities by measuring the increase in absorbance of visible light over a specific time of incubation. The resulting potential activity data from each specific soil enzyme are closely correlated with microbial activity and substrate decomposition rates, also used as an indicator of

environmental nutrient limitation for microbes (Zeglin et al., 2007; Sinsabaugh et al., 2009; German et al., 2011; Burns et al., 2013).

When measuring the appearance of a product in enzyme activity assays, several variables have the potential to affect results from soil enzyme activity measurements. The extraction efficiency of product or substrate from soil, the buffer pH, substrate concentration, the soil's drying technique, amount of soil, time span of reaction, temperature, the stoichiometry of the reaction, the storage or pretreatment of soil before assay, and the use of a proper control are factors that may influence whether the success or unsuccess of the enzyme activity assay (Dick, 2020).

Microbial diversity

Soil microbes represent the vast majority of life on Earth, being essential for the soil ecosystem functioning, catalyzing indispensable processes in the biogeochemical cycles in the biosphere (Whitman et al. 1998; van der Heijden et al. 2008; van der Heijden and Wagg 2013). Moreover, the soil microbial diversity is also considered an important index of soil ecosystem health, indicating that as the biodiversity of a soil ecosystem increases, the resilience and stability of this ecosystem should also increase (Torsvik & Øvreås, 2002; Garbeva et al., 2004; Entry et al., 2008). In contrast to studies commonly used to assess microbial activity, such as microbial respiration, biomass, and enzyme activity, less attention has been given to studies aiming to determine microbial community structures in the soil ecosystem, as well as their response to changes in soil properties or management (Hill et al., 2000).

To assess the soil microbial community structure, phospholipid fatty acid (PLFA) analysis methodology has been used. This methodology is also helpful in the sense of capturing

effects of soil management practices (Zelles et al., 1992), pollution (Frostegård et al., 1993), fumigation (Macalady et al. 1998), as well as changes in soil quality and health (Reichardt et al., 1997; Bossio et al., 1998; G. Peterson et al., 1998; Bardgett, 2002). Phospholipids fatty acid are found exclusively in the cell membranes and rapidly degraded and metabolized as the cells die, thus serving as an important indicator of active microbial biomass in the soil system (Hill et al., 2000). Therefore, the presence and abundance of signature fatty acids in soil indicate the presence and abundance of either particular or group of organisms belonging to those signatures (Hill et al., 2000). Some of the common PLFA signatures are: 1) *cy17:0* and *cy19:0* as indicators of Gram-negative and Gram-positive bacteria (Schouge et al., 2008), 2) *16:1 ω 5* as an indicator of the presence of arbuscular mycorrhiza (Olsson et al., 1995), 3) *18:2 ω 6,9* as an indicator for fungi (Frostegård et al., 2011), 4) branched-chain fatty acids as indicators for Gram-positive bacteria, and 5) cyclopropyl fatty acids as indicators of Gram-negative bacteria (Frostegård et al., 2011).

Soil physical properties

The physical characteristics of the soil reveal details about the flow and storage of air and water through the soil system, as well as the factors influencing seeds' germination, root development, and erosion processes. Therefore, many soil physical characteristics serve as the building blocks for other chemical and biological processes, which may be further influenced by the climate, topography, and land use (Allen et al., 2011). A wide range of soil physical properties are considered soil health indicators, such as aggregate stability, water infiltration, bulk density, and rooting depth (Allen et al., 2011). In this sense, soil physical health, or physical

quality, is manifest in various ways. Poor water infiltration, poor aeration, runoff, and erosion are characteristics of poor soil physical health and are closely correlated to a common cause – poor soil structure (Dexter, 2004). Farming practices and compaction have an important impact on soil structure, which corresponds to the distribution of the primary soil particles (sand, silt, and clay), and is the most significant soil physical property that is affected by organic matter (Dexter, 2004).

Aggregate stability

Soil aggregate stability is a soil physical characteristic that affects soil sustainability and crop production (Amézketa, 1999). Soil aggregation is the result of the rearrangement of its particles, and further flocculation (Duiker et al., 2003; Bronick & Lal, 2005). The aggregate stability of a soil is determined by its structure, management practices, as well as chemical and biological properties of the soil system, and it is measured by the resistance of the soil aggregates to external energy (Moebius et al., 2007; Allen et al., 2011). Moreover, the maintenance of a good soil structure, and therefore a high soil aggregate stability, is essential in order to preserve the soil productivity, minimizing negative effects such as soil erosion and environmental pollution (Amézketa, 1999). Soil aggregates occur in a wide range of sizes and are normally grouped by size (Bronick & Lal, 2005).

Aggregates are often classified by size, where macroaggregates are greater than 250 μm , and microaggregates are smaller than 250 μm . Because of its close correlation with soil organic carbon storage and other important ecosystem functions such as water infiltration capacity, movement and storage of water, and microbial community and activity, soil aggregate stability is considered a useful soil health indicator (Blanco-Canqui & Lal, 2004; Magdoff & Weil, 2004;

Moebius et al., 2007; Rimal & Lal, 2009; Allen et al., 2011; Lal, 2016), as well as an indicator of soil structure (Six et al., 2000). In addition, aggregate stability quickly responds to agricultural management changes, such as no-tillage and manure application (Six et al., 1999; Lupwayi et al., 2001; Mikha & Rice, 2004).

The selection of a method for determining aggregate stability within a soil system should be based on the purpose of the analysis, soil type, and the level of disruptive energy that those aggregates experience in field conditions (Herrick et al., 2001; Almajmaie et al., 2017).

Therefore, a wide array of protocols is used to test and quantify soil aggregate distribution and stability, including raindrop impact, dry sieving, wet sieving, ultrasonic vibration, and clay dispersion (Almajmaie et al., 2017; Nimmo & Perkins, 2018). The determination of water stable aggregates by wet sieving measures the stability of air-dried soil clods into smaller aggregates after slaking, the process of quickly wetting air-dried soil to disrupt air that is trapped inside the soil aggregates (Yoder, 1936; Kemper & Rosenau, 2018).

Soil chemical properties

Soil chemical properties have the potential to hold, supply, and cycle nutrients in the soil environment (Schoenholtz et al., 2000). Moreover, the bioavailability and amount of macro- and micronutrients to sustain crop production in agroecosystems can determine either deficiencies or toxicities that directly affect crop production and quality. In addition, soil chemical properties, or indicators, also influence microbial processes along with physical-chemical processes on the movement and availability of water in the soil (Powers et al., 2015). In this context, soil chemical indicators are mostly used as indices of nutrient supply (Powers et al., 2015). Some of the soil

chemical properties proposed as indicators of nutrient supplying capacity for agricultural, rangeland and forest soils are: soil organic carbon (SOC) (Doran and Parkin 1994), soil organic matter (SOM) (Karlen & Stott, 2015), soil nitrogen (N), phosphorus (P), and potassium (K), organic N (Doran and Parkin 1994), total N (Reganold & Palmer, 1995), extractable NH_4 and $\text{NO}_3\text{-N}$ (Harris & Clark, 1996), soil extractable P (Burger et al., 1994), cation exchange capacity (CEC) (Karlen & Stott, 2015), extractable K, Ca, and Mg (Reganold & Palmer, 1995), and pH (Doran and Parkin 1994).

Phosphorus

Most of the soil phosphorus (P) is derived from mineral weathering and organic matter decomposition (Schoonover & Crim, 2015). P occurs on soil in different forms, including inorganic and organic P, differing in their behavior and fate in the soil ecosystem (Hansen et al., 2004; Turner et al., 2007; Shen et al., 2011). Moreover, its bioavailability in soils is a key factor for determining agricultural yields as well as in assessments of soil health and soil quality (Cardoso et al., 2013). Although the use of synthetic sources of soluble P through fertilization can supply the plant's demand for this nutrient, it can be converted into insoluble forms over time (Schoonover & Crim, 2015). In addition, the crop's harvest characterizes the removal of P from the system, and for these reasons, P is often considered a limiting nutrient in soil (Schoonover & Crim, 2015).

Potassium

Potassium (K) is the most available macronutrient of any other soil macronutrient, yet existing in soil as a mineral form and is not readily available for plant uptake (Schoonover &

Crim, 2015). Sadusky et al., (1987) study revealed that in Delaware soils, mineral K represented around 98% of the total soil K. It is mainly derived from crystalline structures of soil minerals, such as feldspars and micas, contributing to K in four forms, exchangeable K, non-exchangeable K (Schoonover & Crim, 2015; Pratt, 2016), solution K, and mineral K (Mouhamad et al., 2016). Solution K generally has low levels in the soil, unless recent K amendments have been made to the soil, and is also the source from which plants and microbes uptake K. Moreover, solution K is the form most subject to leaching in soils (Mouhamad et al., 2016). Exchangeable K is bound to the surface of clay minerals and humic substances, being readily available for plant uptake or exchanged with other cations (Mouhamad et al., 2016). Non-exchangeable K is held between adjacent tetrahedral layers of clay minerals, making its release a slow and diffusion-controlled process (Sparks, 1987; Mouhamad et al., 2016).

Nitrogen

Nitrogen (N) is the macronutrient required in the greatest quantities by plants and considered the most limiting nutrient for plant growth, mostly because of the lack of its bioavailability for plant uptake in the soil (Schoonover & Crim, 2015). In soils, nitrogen occurs in both organic and inorganic forms; however, only the inorganic forms of N are available for plant uptake. Nitrate (NO_3^-) and ammonium (NH_4^+) are the main forms of N occurring in soils (Carter & Gregorich, 2008). Although nearly 95% of N found in soils occurs in the organic form, therefore unavailable for immediate plant uptake, soil microorganisms have the potential to convert organic N into inorganic forms (nitrate and ammonium) readily available for plant uptake through a process known as nitrogen mineralization (Schoonover & Crim, 2015). Nitrogen in nitrate form is water-soluble and highly susceptible to leaching or turning into

nitrous oxide (NO_2^-) in anaerobic conditions, being released into the atmosphere as a greenhouse gas (Bremner, 1997).

Soil pH

Soil pH is a useful measurement and indicative of soil chemical properties, having broad-reaching, possibly either positive or negative effects on plants' development, nutrient availability, and nutrient uptake by plants (Pessarakli, 1999). Moreover, it is closely related to soil biogeochemical processes (Bååth et al., 1995; Brady, n.d.; Falkengren-Grerup et al., 2006; Hong et al., 2019), influencing the structure and functioning of terrestrial ecosystems (Bi et al., 2007; Hong et al., 2019). Moreover, several factors have the potential to affect soil pH, including climate, soil buffer capacity, N fertilization, and vegetation type (Hong et al., 2019). Usually, values of soil pH less than 4 indicate the presence of free acids, which generally originate from oxidation of sulfides; pH values between 4 and 5.5 indicate the occurrence of exchangeable aluminum (Al), and pH values ranging from 7.8 to 8.2 indicate the presence of calcium carbonate in the soil system (Thomas, 2015). In the sense of spatial distribution, soils with pH ranging from 4 to 7 are extensively distributed within tropical and subtropical regions of the world (Thomas and Hargrove 1984). In addition, soil pH is generally lower in wetter environments (Slessarev et al., 2016), generally lower in low altitudes (Binkley & Fisher, 2019), and regulated by buffering systems (Hong et al., 2019). Along with the environmental factors that shaped soil pH and its spatial distribution, anthropogenic activities also played an important role in influencing changes in soil pH (Hong et al., 2018). The extensive rely on nitrogen fertilization has been one of the major causes of soil acidification (Guo et al., 2010; Yang et al., 2012, 2015), by damaging soil

buffering systems (Bowman et al., 2008) and enhancing the leaching loss of base cations (Matschonat & Matzner, 1996; Hong et al., 2019).

Soil Organic Carbon (SOC)

Soil organic carbon (SOC) represents approximately 58% of soil organic matter (SOM) by weight (Sikora et al., 1996), allowing the determination of SOM by the direct measurement of SOC. Soil organic matter is a complex soil component because its composition encompasses plant, microbial, and animal bodies in various stages of decomposition as well as mixtures of other heterogeneous organic substances closely linked to soil inorganic constituents (Christensen, 1992; Ramesh et al., 2019). Moreover, several pools and fractions of SOM are observed in the soil system, varying in degree of decomposition and stability, where these fractions are useful in determining short- and long-term effects of land use management on SOC dynamics. (Ramesh et al., 2019). Labile fractions of SOC, such as light fraction organic carbon (Six et al., 2001), particulate organic carbon (Cambardella & Elliott, 1992), readily oxidized carbon (Blair et al., 1995), and microbial biomass carbon (Jenkinson & Powlson, 1976) have shown to be sensitive to land use and management changes, thus being used as soil health and quality indicators (Ramesh et al., 2019). Therefore, SOM is considered a key indicator of soil health as its maintenance and restoration are vital for ensuring long-term soil functioning and productivity (Tisdale et al., 1985; Zhao et al., 2015; Ramesh et al., 2019). SOC can be determined through the loss of ignition method, where the organic matter content of a soil is determined by oxidizing organic matter to CO₂ at elevated temperature inside a muffle furnace, followed by a measurement of the weight loss (Zhang and Wang, 2014).

Cropping systems

Ensuring food security remains one of the biggest challenges for a world with an increasingly growing population, allied with climate change scenarios and the depletion of natural resources and environment (Bhardwaj et al., 2011). In addition, the expansion and intensification of agriculture over the last decades have brought substantial benefits in the sense of economic and social developments; however, it also has contributed to environmental degradation, mainly through biodiversity loss, greenhouse emissions, and soil and water conservation (MAE, 2005; Kirschenmann, 2010). Increasing crop productivity while maintaining environmental sustainability has been a big challenge for farmers and agricultural scientists recently. The attribution for the use of enhanced farming practices relies on soil conservation, lagging of productivity, and social pressures to decrease environmental concerns related to agriculture (Bergtold et al., 2017). In order to diminish these negative impacts, some alternatives, such as conservation agriculture (CA), started to be adopted (Holland, 2004). This concept heavily relies on three fundamental principles: i) the diversification of cropping systems, ii) the reduction of soil tillage and adoption of no-tillage systems, and iii) the permanent soil covering.

Cropping systems are defined as a cropping pattern used in an agroecosystem and its interactions with the farm resources, available technology, and other farm enterprises, which determine their makeup (Rana & Rana, 2011). The use of a specific cropping system is based on and will depend on climate and on the availability of resources necessary for crop production, such as soil and water (Rana & Rana, 2011). Moreover, the intensification and diversification of cropping systems are alternatives intended to optimize the utilization of natural resources, spread risks, enhance profitability, and maximize crop productivity per unit area. Besides, in addition to

these benefits, the intensification and diversification of cropping systems also have the potential to mitigate and reduce environmental impacts associated with agriculture and crop production (Gaba et al., 2015).

Cropping systems intensification and diversification

Crop intensification refers to the increase in the number of crops utilized in a cropping system, while crop diversification refers to the use of different crop species in a cropping system. Modern cropping systems need to be designed in a way to sustain and enhance crop production and increase yield stability under a changing climate scenario (Liu et al., 2019; Reckling et al., 2022). Some of the alternatives to increase crop production, without expanding the land utilization for agriculture, are to increase the productivity of a single crop per year and per unit area or increase the number of crops harvested in the same timeframe and area through crop intensification (Andrade, 2011; Gaba et al., 2015; Waha et al., 2020; Massigoge et al., 2023). However, such techniques are limited by a set of factors, including the water availability for crop production (Arvor et al., 2012; Liu et al., 2013; Cohn et al., 2016) and the length of the growing season (Monzon et al., 2014; Yu et al., 2015). In North America, however, most of the cropland suitable for crop intensification still relies on cereal or legume production utilizing a single crop per year rotation (Spiertz & Ewert, 2009; Siebert et al., 2010; Waha et al., 2020; Massigoge et al., 2023). Therefore, increasing the number of crops harvested per unit area per year poses as an alternative to increase crop production, maximize the efficiency in the use of resources, and enhance soil health (Andrade, 2011; van Opstal et al., 2011; Brooker et al., 2015).

In the U.S. Great Plains, there is a need for alternatives to commonly adopted cropping systems based on the utilization of year-long fallow periods, also known as summer-fallow,

which have traditionally characterized the dryland cropping systems used in the region (Hansen et al., 2012; Rosenzweig et al., 2018). Moreover, the utilization of summer-fallow periods is mainly intended to increase the storage of water in the soil for further utilization on the subsequent crop in the system. However, during the early adoption of this practice and with the addition of tillage practices, less than 20% of the precipitation received during the fallow period was efficiently stored in the soil for the following crop (Greb, 1979). With the adoption of no-tillage systems, significant improvements in soil water storage during summer fallow periods were observed; however, these numbers have remained stagnant at 40% since the 1970s (Peterson et al., 1998; Nielsen et al., 2005; Lyon et al., 2007). In addition, increases in N and P uptake by plants were observed in intensified cropping systems compared with single-crop systems, mainly due to changes in soil microbial communities (Rosenzweig et al., 2018). These changes were potentially mediated through carbon limitations imposed by the single-crop summer-fallow cropping systems on the microbial communities in the soil, which limited their growth and activity (Rosenzweig et al., 2018).

Besides intensification, the diversification of cropping systems plays an important role in the overall crop production, allied with environmental sustainability. Some studies suggest that more diverse cropping systems have the potential to provide a wider range of ecosystem functions and services, as well as more stability, over time when compared with less diverse agricultural systems (Swift et al., 2004; Lefcheck et al., 2015; Beillouin et al., 2021). Increasing attention has been given to crop diversification on cropping systems as a sustainable practice aimed to improve crop productivity and sustain agricultural development (Brooker et al., 2015; Tamburini et al., 2020; Cong et al., 2021; Wang et al., 2022). Moreover, the addition of different crop species, either cash or cover crops, into the cropping systems has shown to increase soil

organic carbon levels (Jin et al., 2020), total nitrogen (Cong et al., 2015), and phosphorus bioavailability (Hacker et al., 2015; Wu et al., 2019; Phoenix et al., 2020). These positive impacts of crop diversification on soil C, N, and P are closely related to the functionality and community structure of soil microbes, which benefit from a wider range of exudates released by different plant root systems (McDaniel et al., 2014; Denier et al., 2022). Thus, several soil attributes are potentially benefited from the increase of diversity in cropping systems, as well as the whole functionality of the soil ecosystem.

In this sense, diversifying cropping systems is not only a critical tool for maximizing crop yields and production but also vital for enhancing soil health, increasing soil biodiversity, and improving soil nutrient cycling and efficiency (Barbieri et al., 2019; Yang et al., 2020). The diversity of plant functional groups is an important factor to be considered in management strategies to be adopted, mostly because the overall richness of crop species utilized in a specific cropping system has the potential to be a driver of soil health (Milcu et al., 2013). One of the most common approaches of crop diversification consist in the mixture of different crop functional groups in order to achieve potential benefits from them, such as the use of legume crops aiming to fix atmospheric nitrogen (Vukicevich et al., 2016; Yang et al., 2020). In addition, higher diversity of plants with different functional attributes also benefits different niches of soil functional microbes, enhancing overall soil health, soil functionality, and the provision of ecosystem services (Vukicevich et al., 2016; Yang et al., 2020).

Plant-soil microbes Interactions

The interactions between plants, soil, and soil microorganisms have the potential to regulate nutrient cycling and losses in agroecosystems (Bohlen et al., 2001). Changes in the composition of soil microbial communities are often mediated by plants, which can feed back and affect the growth and development of other neighboring plants in a process known as plant-soil feedback (Bever, 2003; Ke et al., 2021). The plant's rhizosphere, which is defined as the zone of soil that is influenced and affected by the plant roots (Balandreau & Knowles, 1978), is considered a pool of microorganisms of either symbiotic or pathogenic nature (Akram et al., 2017). In addition, while symbiotic relationships between plants and soil microbes have the potential to stimulate plant development and productivity, antagonistic relationships between soil microbes and plants have the potential to limit plant development and therefore productivity. Symbiotic relationships potentially stimulate plant growth by conferring biotic and/or abiotic stress tolerance, while antagonistic, or pathogenic, relationships involve host-pathogenic interactions resulting in growth and productivity limitations (Abhilash et al., 2012; Akram et al., 2017). Rhizosphere microbial communities' composition is shaped through different soil traits, such as soil moisture, nutrient availability, texture, and pH (Schlemper et al., 2017), in addition to soil management practices (Barbosa Lima et al., 2015; Schlemper et al., 2017).

Several biotic and abiotic factors influence the makeup and activity of soil microbial communities as well as the connections with soil and plants, and these microbial communities in turn influence the environment's quality, plant development, and the generation of root exudates (Bever et al., 1997). For example, some beneficial rhizosphere bacteria affect plant performance by enhancing mineral solubilization for further plant uptake (Bar-Ness et al., 1991; Crowley et al., 1991; Derylo & Skorupska, 1992; Bever et al., 1997), fixing atmospheric nitrogen (Albrecht et al., 1981), suppressing plant pathogens (Thomashow et al., 1990; Hamdan et al., 1991), and

producing hormones that stimulate plant growth (Tien et al., 1979; Nieto & Frankenberger, 1989). Moreover, bacterial communities in the rhizosphere have been shown to respond in density, composition, and activity to the amount and diversity of organic root exudates, eventually creating plant-specific microbiotas that may also vary depending on the plant development stages (Mahaffee & Kloepper, 1997; Wieland et al., 2001; Curl & Truelove, 2012).

Crop development stages

The introduction of plants in soils affects the native microbial communities through the supply of inocula and root exudates that will either benefit or suppress the growth of native bacteria and fungi (Houlden et al., 2008). Studies have shown that plants have a significant effect on the rhizosphere microbial community structure, as the data suggest that the seasonal variations in microbial activity and community structure in the rhizosphere are plant-dependent (Dunfield & Germida, 2001; Garbeva et al., 2004). The crop's growing season is considered one of the main drivers of microbial community structure's variations in agricultural systems, as plant roots develop through the growing season, they have the potential to alter the temporal distribution and quality of exudates (Houlden et al., 2008; Lauber et al., 2013; Degruene et al., 2017). Additionally, diverse cropping systems offer a heterogeneity of decaying plant biomass residue, senescent roots, and, therefore roots exudates that will provide a better habitat for soil microorganisms (McDaniel et al., 2014; Hsiao et al., 2019). Soils are dynamic ecosystems, and understanding the variability of soil health indicators is an important factor in determining the applicability of soil health indices.

Study objectives

The main objective of this study is to quantify the effects of crop intensification and diversification on soil health indicators and crop yields under traditional cropping systems commonly used in Kansas. We understand that crop intensification and diversification are pathways to maximize crop production, enhance the efficiency of natural resources, and restore soil health while preserving the environment. Although several studies reported the effect of land use management practices on chemical, physical, and biological soil health indicators, the effect of intensified and diversified cropping systems on soil health, as well as the dynamics of soil health indicators over the crop's growing season remain unclear. This study was designed to explore the interactions between crop rotations, crop development stages, and soil health indicators. The objectives of each chapter are:

Chapter 2: Seasonal variability of soil health indicators under different rainfed cropping systems. The objective of this study was to assess the temporal and vertical dynamics of biological soil health indicators under six different rainfed cropping systems with varying levels of crop intensity and diversity.

Chapter 3: The effect of cropping systems intensity and diversity on soil health indicators in winter wheat-based systems in Kansas. The objective of this study was to assess the effect of intensification and diversification of cropping systems on chemical, physical, and biological soil health indicators over a period of 5 years of implementation.

Chapter 4: Understanding crop yields under regenerative agriculture practices in Kansas.

The objective of this study was to assess the effect of the use of regenerative agriculture practices, especially the use of cover crops replacing fallow periods between cash crops, on corn, soybean, and wheat absolute yield responses and yield stability metrics.

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Chapter 2 - Seasonal variability of biological soil health indicators under intensified and diversified rainfed winter wheat-based cropping systems

Abstract

Seasonal dynamics can substantially influence biological soil health indicators, yet the magnitude and drivers of such variability in rainfed winter-wheat-based systems remain unclear. This study aimed to i) quantify the seasonal variability and ii) evaluate treatment and time effects on biological soil health indicators, and iii) identify the most suitable timing for soil sampling to ensure consistent soil health assessments. Soil samples were collected in six rainfed cropping systems, ranging in crop intensity and diversity, from 2021 to 2023 at three depths (0-5, 5-15, and 15-30 cm) across nine sampling times linked to crop growth stages (planting, flowering, and harvest). Soil microbial activity was assessed through extracellular enzyme assays, while soil microbial biomass and structure were determined using phospholipid fatty acid (PLFA) analysis. Temporal patterns were evaluated using generalized additive mixed models (GAMMs) incorporating climatic covariates, soil depth, and treatment interactions. There was strong temporal variability across indicators, particularly in the 0-5 cm soil layer. Enzyme activities and microbial biomass increased during winter wheat development, peaking at flowering or harvest stages, though stabilizing or declining during summer crop growth. The amplitude of variation ranged from 30% to over 100% and was indicator specific. Soil temperature had a significant effect on microbial and enzymatic responses. Overall, soil sampling timing had a major influence

on soil health interpretation, with fall (winter wheat planting) identified as the most reliable period for consistent assessments of soil health in winter wheat-based systems.

Introduction

Soils support dynamic ecosystems capable of responding to fluctuations in climate, weather, management practices, and plant growth cycles (Hsiao et al., 2019). The concept of soil health integrates these dynamics by describing the overall quality of soil as a living ecosystem, capable of sustaining plant and animal production while maintaining water and air quality and promoting plant, human, and animal health (Doran and Zeiss, 2000; Kibblewhite et al., 2008). Soil health is a complex variable encompassing soil physical, chemical, and biological properties that are assessed to understand and determine the functional role of soils under both productive and ecological perspectives (Norris & Congreves, 2018). Assessments of soil health thus aim to capture soil functionality, resilience, and biological composition, integrating complex processes that occur within the soil ecosystem (Paz-Ferreiro & Fu, 2016). Additionally, soils encompass a large range of different environments containing significantly variable abiotic properties, microbial communities, activity, and abundance (Fierer, 2017), and understanding the variability within and among soil environments is fundamental. This is particularly relevant because the ecological functionality of soils is mediated largely by biological processes that regulate nutrient cycling, carbon turnover, and aggregate stability (Dick, 1992).

In agroecosystems, management practices such as crop rotation and the inclusion of cover crops can help maintain or improve soil health by promoting enhanced nutrient cycling and microbial diversity (Ashworth et al., 2017). Previous crop residue and its management

substantially influence soil microbial communities (Ashworth et al., 2017; DeBruyn et al., 2011), as residue quantity and quality directly affect soil microbial abundance, composition, and enzymatic activity (White and Rice, 2009). Wheat-based cropping systems commonly adopted in the US Southern Great Plains are characterized by continuous winter wheat (*Triticum aestivum* L.) and extended fallow periods lasting four to fourteen months, depending on the precipitation regime. Although these fallow periods aid soil moisture recharge for the next crop, they often exhibit low soil water storage efficiency (Farahani et al., 1998; Nielsen et al., 2005; Patrignani et al., 2019). Therefore, increasing the intensity (the number of crops utilized in the cropping systems) and diversity (different crop species used in the cropping systems) of the crop rotation opens an opportunity to better use soil moisture, increase crop production, but also enhance soil health by offering a wider range of crop residue quantity and quality, thus creating a more habitable and resourceful soil ecosystem (McDaniel et al., 2014; Hsiao et al., 2019).

The quality and composition of crop residues, particularly their carbon-to-nitrogen (C/N) ratio, lignin, and cellulose content, influence decomposition rates and nutrient mineralization, which in turn drive soil microbial responses in the soil (Beyaert and Voroney, 2011; McDaniel et al., 2014; Hsiao et al., 2019). Residues from cereal crops such as corn (*Zea mays* L.) and wheat are typically more recalcitrant and display a higher C/N ratio when compared to legumes like soybean (*Glycine max* L.). Therefore, the C/N ratio of plant tissues directly affects the decay rate of residues, where plant residues with a lower C/N ratio decompose faster than those with a higher C/N ratio (Beyaert and Voroney, 2011). Thus, the input of contrasting residue types may generate different trajectories of soil microbial abundance and activity (Wang et al., 2015). In addition to residue inputs, plants actively shape soil microbial ecology through root exudates that supply labile carbon and signal compounds to the rhizosphere (Haichar et al., 2014), with

quantities and composition of root exudates varying within plant species and growth stages (Zhang et al., 2017). In this sense, increasing plant diversity on crop rotations may benefit soil microbial community, structure, and activity (Hättenschwiler et al., 2005; McDaniel et al., 2014). Soil microbial communities are affected by plant presence and species (Mendes et al., 2013; Panico et al., 2020), and different plant developmental stages mediate the production of distinct root exudates and metabolites, with the potential to select specific microbial communities (Zhang et al., 2017). Furthermore, root exudates rich in substrates like sugars, carboxylic acids, and amino acids may regulate the abundance and activity of soil fungi and bacteria (De Graaff et al., 2010; Panico et al., 2020). Mandal et al. (2007) reported the effects of mineral and organic fertilization on soil microbial community and activity at the early development and flowering stages of wheat. Similarly, Oberholster et al. (2018) reported higher microbial diversity in the sorghum's rhizosphere at the flowering development stage compared to bulk soils. Plant root systems at different development stages have the potential to induce changes in soil structure and nutrient availability, thus also mediating changes in microbial community structure and activity, especially in the rhizosphere (Baumert et al., 2018; Marschner et al., 2002).

Although there are studies considering the temporal variability of soil properties, they are mainly focused on changes based on long-term crop rotations. Fewer studies have focused on the seasonal variability of selected soil health indicators on rainfed wheat-based cropping systems, where crop growth stages, climate variability, and crop intensity can substantially influence soil microbial and enzymatic processes. The objectives of this study were to measure and understand the seasonal and vertical variability of soil health indicators under rainfed, intense, and diverse wheat-based cropping systems and determine the optimal soil sampling time for soil health assessments under these conditions. Specifically, we aimed to i) evaluate the effect of cropping

systems intensity and diversity on biological soil health indicators, ii) characterize the temporal dynamics of soil extracellular enzyme activities and soil microbial community biomass across crop growth stages, iii) assess how these responses vary across soil depths and in relation to short-term climatic variables, and iv) determine the optimal period for soil sampling to minimize temporal variability. We hypothesized that biological soil health indicators would exhibit significant seasonal fluctuations linked to crop growth stages and residue turnover, with greater amplitude in more intense and diverse cropping systems. Additionally, we further hypothesized that biological soil health indicators would be most stable during the fall period, coinciding with the winter wheat planting, representing the most suitable timing for consistent soil health assessments. Soil extracellular enzyme activities were reported as indicators of soil microbial activity, and phospholipid fatty acids (PLFA) were used as an indicator of microbial community biomass and structure. Soil physicochemical properties were also measured across the crop development stages, including soil gravimetric water content, pH, soil organic carbon (SOC), total nitrogen (TN), plant-available nitrogen, plant-available phosphorus (P), and plant-available potassium (K). These physicochemical properties were assessed and described in this manuscript for characterization at each sampling moment.

Materials and Methods

Site and Experiment Descriptive

This study was conducted from October 2021 through July 2023 at the Kansas State University Ashland Bottoms Research Experiment Station (39.12408 N, 96.63514 W). The predominant soil type at the study location is classified as a Silty Clay Loam Wymore series, fine

mesic Aquertic Arguidoll (USDA-NRCS Soil Survey Geodatabase, 2014). Prior to the establishment of our study, the experimental field had a grain sorghum (*Sorghum bicolor* (L.) Moench)– soybean (*Glycine max* L.) – winter wheat rotation under conventional tillage (chisel plow, tandem disk, and field cultivator) system prior (~ 15 years) to the establishment of this study. Weather data, such as monthly observed precipitation and temperature, were obtained from the Kansas Mesonet weather station located in Manhattan, KS (Patrignani et al., 2020), located around 10 km from the study site. Historical weather (30 years) records for this location indicated an annual average precipitation of 791 mm, and an average temperature of 13.1 °C. The cumulative precipitation during the study period was 1337 mm, with most of the rainfall occurring in the spring and early summer (March-July) (Table 2.1) (Kansas Mesonet, 2023).

Table 2.1. Monthly mean temperature (T) and accumulated precipitation (PP) recorded from November 2021 to July 2023 in the study site. Values between brackets indicate the absolute deviation from the historical value (30 years). Source: Kansas Mesonet Station in Manhattan (2025).

Month	2021		2022		2023	
	T (°C)	PP (mm)	T (°C)	PP (mm)	T (°C)	PP (mm)
Jan	-	-	-1.1 (0)	6.9 (-7)	0.3 (+1.4)	56.4 (+42.5)
Feb	-	-	0.1 (-1.1)	2 (-19.2)	2.6 (+1.4)	26.7 (+5.5)
Mar	-	-	6.6 (-0.6)	56.6 (+11.4)	5.8 (-1.4)	23.6 (-21.6)
Apr	-	-	12.4 (-0.4)	25.6 (-53)	13.3 (+0.5)	51.3 (-27.3)
May	-	-	19 (+0.4)	230.6 (+109)	19.4 (+0.8)	131.8 (+9.9)
Jun	-	-	24.6 (+0.5)	154.4 (+30.4)	23.8 (-0.3)	82.8 (-41.2)
Jul	-	-	26.1 (-0.3)	128 (+33.9)	25.8 (-0.6)	81.5 (-12.6)
Aug	-	-	25.6 (+0.1)	33.3 (-72.1)	-	-
Sep	-	-	22 (+1.2)	58.2 (-18.2)	-	-
Oct	-	-	14.5 (+0.7)	29.9 (-26.1)	-	-
Nov	8.6 (+1.9)	28.9 (-3.5)	5.6 (-1.1)	90.7 (+58.3)	-	-
Dec	5.5 (+4.8)	8.1 (-13.7)	-0.9 (-1.6)	29.7 (+7.9)	-	-

The study compared six different crop sequences varying in intensity and crop type, including grain crops, cover crops, and forage crops (Fig. 2.1). The crop species utilized for this experiment were, specifically, alfalfa (*Medicago sativa* L.), corn (*Zea mays* L.), soybean, cowpea (*Vigna unguiculata* L.), moth beans (*Vigna aconitifolia*), grain sorghum, tepary bean (*Phaseolus acutifolius*), triticale (*×Triticosecale* Wittmack), and winter wheat.

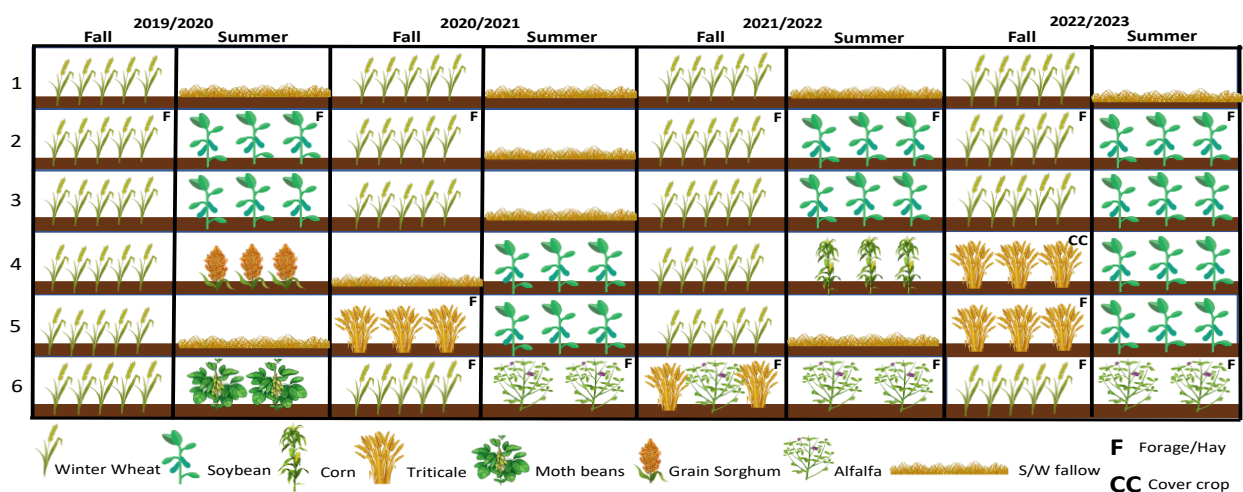


Figure 2.1. Schematic representation of the treatments assessed in the study. Treatment 1: Control; Treatment 2: Adaptive Forage; Treatment 3: Semi-Intensive; Treatment 4: Intensive; Treatment 5: Dual-purpose Semi-Intensive; Treatment 6: Dual-purpose Intensive. The sketch is not to scale and does not represent the actual duration of each crop in the rotation. Adapted from Massigoe et al. (2024).

The study was conducted in a randomized complete block design with four replications, with each experimental unit measuring 13 m wide by 15 m long. Treatments 1 (Control, T1), 4 (Intensive, T4), and 5 (Dual-purpose semi-intensive, T5) were managed under a standard

nitrogen application. Treatments 2 (Adaptive Forage, T2) and 3 (Semi-Intensive, T3) were managed under a progressive nitrogen management differing in application timing, placement, and rate when compared to the standard management. Treatment 6 (Dual-purpose intensive, T6) was conducted with green-nitrogen management, where no nitrogen fertilizer was applied but sourced from a legume crop residue (either moth bean or soybean). Nitrogen rates were determined using an in-season nitrogen-recommendation tool based on crop modeling (Massigoge et al., 2024). Treatments under a standard nitrogen application management received a full rate of broadcast 90 kg N ha^{-1} of UAN (Urea Ammonium Nitrate; 28-0-0 of N-P₂O₅-K₂O) at spring green-up in the 2021-2022 and 2022-2023 winter wheat seasons. Treatments under the progressive nitrogen application management received one nitrogen application at spring green-up at a rate of 45 kg N ha^{-1} , and a second application around the jointing stage at a rate of 30 kg N ha^{-1} in the 2021-2022 and 2022-2023 winter wheat seasons. The vegetative and reproductive stages of winter wheat were determined through field observations using the Zadoks scale (Zadoks et al., 1974).

For the winter crops, winter wheat and triticale for forage purposes were planted at early planting dates (late September to early October), before the optimum planting dates for the region. Winter wheat for grain purposes, when following summer fallow, forage soybean, and full-season soybean, was planted at optimum (mid-October) planting dates for the region. Winter wheat for grain purposes, when following double-cropped soybeans or corn, and triticale cover crop, was planted at late (November) planting dates for the region. Summer crops following winter fallow, triticale for forage purposes, and triticale as a cover crop were planted in mid-May as full-season crops. Summer crops following winter wheat for grain purposes were planted in early July, immediately after harvest, as double-cropped crops.

Winter crops were planted utilizing a 0.19 m row spacing with a 9-row Great Plains 506® no-till drill (Great Plains Manufacturing, Salina, Kansas, USA). Soybeans, corn, grain sorghum, and cowpea were planted utilizing a 0.762 m row spacing. Moth, tepary beans, and alfalfa utilized a 0.381 m row spacing. All the summer crops were planted using a 2-row John Deere 7000® planter (John Deere, Moline, Indiana, USA). Crops intended for forage purposes (alfalfa, triticale, and forage soybeans) were mowed at late bud, late boot, and R3 phenological stages (Fick & Mueller, 1989), with all shoot biomasses being removed from the plots after mowing. Triticale used as a cover crop was chemically terminated in mid-May. Diammonium phosphate (DAP; 18-46-0 of N-P₂O₅-K₂O) fertilizer was utilized in the subsurface at the planting of winter crops at the rate of 55 kg ha⁻¹. No phosphorus fertilizer was utilized in summer crops. The experiment was conducted under a no-tillage system and under rainfed conditions.

Soil Sampling

Soil samples were collected below the crop residue in one core that was split at 0-5 cm, 5-15 cm, and 15-30 cm depth using a manual probe with an inner diameter of 2.5 cm. The samples were taken in each plot at roughly every crop's planting, flowering, and harvest from November 2021 to July 2023. Detailed sampling dates, crop growth stages, and days after first sampling are provided in Table A.1 in Appendix A. The recommended timing of sampling for baseline was determined at winter wheat planting stages according to the soil sampling recommendation for soil health assessments from Karlen & Stott (2019). After collection, samples were accommodated in a cooler and transported to the Agronomy Department at Kansas State University. The soil from each plot was combined, homogenized, and subsampled for further chemical and biological analysis. Subsamples used for chemical properties were air-

dried, ground, and sieved through a 2-mm mesh. Subsamples for soil microbial properties were stored at -20 °C in a walk-in freezer before further processing and analysis.

Soil Physical and Chemical Measurements

Soil gravimetric water content was determined using the thermo-gravimetric method, which consisted of oven-drying soil samples at 48 °C until reaching constant mass. Soil pH was determined using a 1:10 soil/water slurry (Watson and Brown, 1998). Available macronutrients were determined using the 1 M KCl extraction for inorganic N (Keeney, 1982), Mehlich-3 for available phosphorus (Mehlich, 1984), and the standard ammonium acetate method for available potassium (Warncke and Brown, 1998) at the Soil Testing Laboratory at Kansas State University, Manhattan, KS. Results from soil gravimetric water content were reported as g H₂O kg⁻¹ soil, and results from available nitrogen, available phosphorus, and available potassium were reported as mg kg⁻¹ soil.

Soil organic carbon (SOC) and total nitrogen (TN) were measured and determined by a LECO CN828 Carbon/Hydrogen/Nitrogen combustion analyzer (LECO Corporation, St. Joseph, MI, 2020), following the methodology from Nelson & Sommers (1982) for SOC determination, and Bremner and Mulvaney (1982) for TN determination. The physiochemical characterization of the study site, containing measurements of soil pH, soil organic carbon (SOC), extractable P, and total N, is described in Table 2.1, and a detailed explanation of all physiochemical soil parameters is provided in Table A.2 in Appendix A.

Extracellular Enzyme Activities

Soil potential enzyme activities were measured as described by Tabatabai (2018), however, without the use of toluene (Acosta-Martínez and Tabatabai, 2011). The amount of soil and the volume of solution were reduced by half while maintaining the proportions of soil:solution used in the original assay methodology (Acosta-Martínez and Cotton, 2017; Acosta-Martínez et al., 2019). Individual assays included a carbon-acquiring enzyme (β -glucosidase [β G]), a phosphorus-acquiring enzyme (acid phosphatase [AP]), a sulfur-acquiring enzyme (arylsulfatase [AS]), and a nitrogen-acquiring enzyme (N-acetyl- β -D-glucosaminidase [NAG]). For each respective assay, 0.5 g of air-dried soil (sieved at <5mm) was incubated at 37 °C in solution with its appropriate buffer and 0.5 mL of its appropriate substrate at optimal pH for each enzyme for 1 h. For β G, the start buffer used was a MUB with pH 6, the substrate was p-Nitrophenol- β -D-glucopyranoside (0.05 M) (*Sigma Aldrich® catalog # N70006*), and the stop solution used was 0.1 M THAM at pH 12. For AP, the starting buffer was a MUB with pH 6.5, the substrate was p-Nitrophenol phosphatase Disodium Salt (0.05 M) (*Sigma Aldrich® catalog # 4876*), and the stop solution used was 0.5 N NaOH. For AS, the start solution used was 0.5 M Acetate Buffer at pH 5.8, the substrate was p-Nitrophenol sulfate (*Sigma Aldrich® catalog # N3877*), and the stop solution used was 0.5 N NaOH. Finally, for NAG, the start solution used was 0.1 M Acetate buffer at pH 5.5, the substrate used was p-Nitrophenol-N-acetyl- β -D-glucosaminide (0.01 M) (*Sigma Aldrich® catalog # N9376*), and the stop solution used was 0.5N NaOH. Additionally, 0.5 mL of 0.5M CaCl₂ was added as stop solution to the four enzymes after incubation. Each assay was performed with two replicates and one control, to which substrate was added after the incubation time. After the incubation and addition of stop solutions, the soil suspension was filtered using a Whatman® No. 2v filter, and the filtrate was measured at 400nm

wavelength on a Genesys 20 Visible spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA).

Absorbance values from the control samples were subtracted from those obtained for the two repetition samples before the calculation of the amount of *p*-nitrophenol released during the incubation time using a standard calibration curve. The calibration curve was developed using the same methodology for samples and with standards containing 0, 10, 20, 30, 40, and 50 µg of *p*-nitrophenol in MUB. In case of color intensity exceeded the highest *p*-nitrophenol standard solution, sample filtrates were diluted at 1:4 (1 mL of filtrate and 3 mL of stop solution) for βG, 1:10 (0.5 mL of filtrate and 4.5 mL of stop solution) for AP, 1:2 (1 mL of filtrate and 1 mL of stop solution) for AS, and 1:3 (1 mL of filtrate and 2 mL of stop solution) for NAG. Potential soil enzyme activities were reported as micromoles of *p*-nitrophenol per gram of dry soil per hour.

Soil microbial community structure and abundance

Soil phospholipid fatty acids (PLFA) were estimated following the modified methodology from the White and Ringelberg method (Hsiao et al., 2019; White et al., 1997). The total lipids were extracted from 5 g of freeze-dried soil with a 2:1:0.8 solution of methanol, chloroform, and phosphate buffer respectively. PLFA were isolated from total lipids through extraction using silicic acid chromatography columns (disposable SUPELCO Supelclean 6 mL columns, J.T. Sigma Aldrich®, St. Louis, Missouri, USA) and further methylated to fatty acid methyl esters (FAME) using a solution of 0.2 M methanolic potassium hydroxide. Resulting FAMES from each sample were analyzed using a Thermo Scientific Trace GC-ISQ 7000 (Thermo Fisher Scientific®, Waltham, Massachusetts, USA) equipped with an AI/AS 1310

column (27 cm x 27 cm x 17.7 cm; 12.9 L, Thermo Fisher Scientific®, Waltham, Massachusetts, USA). Internal standard 19:0 fatty acid methyl esters were used to determine concentrations (MilliporeSigma N5377; MilliporeSigma; Burlington, St. Louis, Missouri, USA).

The microbial groups present in the FAME were assigned based on biomarkers. FAME peaks were identified through a comparison with a total of 32 biomarkers, where 26 biomarkers were from a bacterial acid methyl esters mix (BAME; Matreya 1114; Matreya LLC, Pleasant Gap, Pennsylvania, USA) and 6 additional FAMEs custom-made for 10Me16:0 (Cayman Chemical 24823; Cayman Chemical Company; Ann Arbor, Mississippi, USA), 10Me18:0 (Larodan 21-1810; Larodan AB; Solna, Sweden), a17:0 (Matreya 1614; Matreya LCC, Pleasant Gap, Pennsylvania, USA), C16:1:11 (Matreya custom synthesis; Matreya LCC, Pleasant Gap, Pennsylvania, USA), C18:1:11:cis (MilliporeSigma 17264; MilliporeSigma, Burlington, St. Louis, Missouri, USA), and C20:0 (MilliporeSigma 10941; MilliporeSigma; Burlington, St. Louis, Missouri, USA). Resulting fatty acids were grouped into Gram-positive (+) bacteria (i15:0, a15:0, i16:0, i17:0, and a17:0), Gram-negative (-) bacteria (19:0 Δ 9,10; C18:1:11:cis; 17:0 Δ 9,10; C10:0:2-OH, C12:0:3-OH, C14:0:2-OH, C14:0:3-OH; C16:1:0:cis, C16:0:2-OH), actinomycetes (10Me16:0 and 10Me18:0), arbuscular mycorrhizal fungi (AMF) (C16:1:11), and fungi (C18:2:9,12) (White and Rice, 2009). The abundance of phospholipid fatty acids was reported as nmol of PLFA per gram of dry soil. Total microbial biomass was estimated by the sum of all PLFA biomarkers in microbes with FAME for C11:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, and C20:0. Fungi to bacteria ratio was calculated by dividing the sum of AMF and saprophytic fungi by the sum of Gram (+), Gram (-), and actinomycetes.

Data Analysis

All statistical analyses were conducted in R Studio (version 2024.4.1.748) (R Core Team, 2023), following a mixed modeling framework that accounted for the hierarchical and temporal structure of the experimental design (Wood, 2017; Pedersen et al., 2019). A generalized additive mixed model (GAMM) approach was used to assess the seasonal dynamics and treatment effects in biological and chemical soil health indicators using the *mgcv* (Wood, 2025) package on R Studio. This approach was chosen over traditional repeated-measures ANOVA to capture nonlinear temporal patterns, control autocorrelation, and incorporate climate variables (Simpson et al., 2012; Ogle et al., 2015). Each soil variable was analyzed separately, with the time variable (in days since the first soil sampling) used as a penalized spline smoother fitted for each treatment to model nonlinear temporal responses. Fixed effects included treatment-by-depth interactions, short-term climatic variables (air temperature, soil temperature, and cumulative precipitation aggregated in 7-to-60-day windows before sampling), and an autoregressive lag term to account for temporal dependency between successive sampling events. Random effects were included for block and plot ID to account for the nested experimental design. A continuous autoregressive correlation structure (CAR1) was fitted for irregularly distributed sampling dates using the *nlme* package (Pinheiro & Bates, 2000). Model residuals were inspected through visual diagnostics to assess normality, variance homogeneity, and independence. The best-fitting model for each variable was selected based on Akaike Information Criterion (AIC), Bayesian Information Criterion (BIC), and deviance explained. Model performance metrics included R^2 and Root Mean Squared Error (RMSE). Estimated marginal means (EMMs) were obtained from the selected models using the *emmeans* package (Lenth et al., 2025), followed by Holm-adjusted pairwise comparisons ($\alpha = 0.05$) to identify treatment differences at each soil depth. Temporal

trajectories were derived from the fitted smoothers to describe seasonal dynamics and to estimate rates of change and peak timing of each soil health indicator. Climate sensitivity was assessed by extracting and testing the significance of standardized regression coefficients associated with short-term air temperature, soil temperature, and precipitation covariates. Finally, to determine optimal sampling times for detecting treatment effects, variance ratios were computed for each sampling date and variable between- and within-treatment variance and ranked to identify periods maximizing discrimination among treatments. All visualizations were produced using the *ggplot2* (Wickham, 2016) package. The complete workflow, including model fitting, diagnostics, and model selection, is summarized in Tables A.3, A.4, and Equation A.1 in Appendix A.

Results

Biological soil health indicators had significant variability throughout the study period. Across indicators, temporal dynamics associated with sampling timing, crop growth stages, and environmental conditions represented the dominant drivers of variation, while treatment effects and soil depth contributed secondary differences in the magnitude of variation.

Precipitation and Temperature During the Study Period

Observed air temperature and total monthly precipitation during the study period had a substantial seasonal fluctuation typical of the climate of Kansas (Table 2.1). Mean monthly temperatures ranged from -1.1 °C in January 2022 to 26.1 °C in July 2022, with similar magnitudes observed in 2023. Compared with the 30-year historical average, temperatures generally deviated by less than ± 1 °C. Precipitation, however, had greater inter- and intra-annual

variability. The highest monthly precipitation was received in May 2022 (231 mm), exceeding the historical average by more than 100 mm, followed by June 2022 (154 mm) and July 2022 (128 mm). Conversely, several months had precipitation deficits relative to the 30-year historical average, notably April 2022 (-53 mm) and August 2022 (-72 mm). The early months of 2023 were relatively dry, except for January, which received around 60 mm above the long-term average. Overall, the first half of 2022 was characterized by warmer and wetter conditions, whereas the spring of 2023 presented near-average temperatures, but below-average monthly precipitation.

Chemical Soil Health Indicators

Average chemical soil health indicators varied moderately across sampling times and soil depth (Table 2.2). Soil pH values ranged between 6.2 and 6.6. At 0-5 cm depth, soil pH was nearly stable throughout the sampling dates, while at 5-15 and 15-30 cm, values averaged between 6.2 and 6.3. Soil organic carbon (SOC) decreased with depth. At 0-5 cm depth, SOC values ranged from 15.9 to 16.0 g C kg⁻¹, while deeper soil layers displayed SOC values ranging from 10.9 to 11.5 g C kg⁻¹, representing a reduction of approximately 30% relative to the topsoil layer. Within each depth, SOC remained relatively constant across sampling times.

Soil extractable phosphorus (P) exhibited substantial depth and temporal differences (Table 2.2). Soil extractable P at 0-5 cm was higher at planting (45.1 mg P kg⁻¹), decreased at the flowering stage (37.4 mg P kg⁻¹), and increased at harvest (43.1 mg P kg⁻¹). At 5-15 cm depth, soil extractable P values had a substantial decrease (~ 70%) relative to the 0-5 cm soil layer, though with similar seasonal fluctuations. At 15-30 cm depth, extractable P levels were low (2 – 3 mg P kg⁻¹) with substantially lower variation across sampling times. Soil total nitrogen (TN)

also decreased with soil depth, though exhibiting greater temporal variability than SOC. At 0-5 cm, soil TN increased by 20% from planting to flowering (12.6 mg N kg⁻¹ at planting and 15.1 mg N kg⁻¹ at flowering), and remained stable at harvest. At 5-15 cm depth, a similar trend was observed, with a 13% increase in soil TN between planting and flowering. In contrast, at 15-30 cm depth, limited variation across sampling times was observed for soil TN. A detailed assessment of soil gravimetric water content (GWC), pH, SOC, extractable potassium (K), P, TN, and inorganic nitrogen (In. N) at each sampling time is described in Table A.2 in Appendix A.

Table 2.2. Characterization of average soil physicochemical properties at the depths of 0-5, 5-15, and 15-30 cm from the study period at winter and summer crops planting, flowering, and harvest. SOC: soil organic carbon, P: plant-available phosphorus, TN: soil total nitrogen. Deviations are reported as standard errors.

Sampling Time	pH	SOC	P	TN
		g C kg ⁻¹	mg kg ⁻¹	
			0-5 cm	
Planting	6.5 ± 0.1	16 ± 0.3	45.1 ± 3	12.6 ± 0.3
Flowering	6.5 ± 0.09	15.9 ± 0.3	37.4 ± 2.8	15.1 ± 0.2
Harvest	6.6 ± 0.1	16 ± 0.3	43.1 ± 2.8	15 ± 0.2
5-15 cm				
Planting	6.2 ± 0.1	13.2 ± 0.2	17.8 ± 1.8	11.8 ± 0.1
Flowering	6.2 ± 0.1	13.4 ± 0.1	10.3 ± 1.1	13.3 ± 0.1
Harvest	6.3 ± 0.1	13.1 ± 0.1	9.8 ± 0.9	12.9 ± 0.1
15-30 cm				
Planting	6.3 ± 0.06	11 ± 0.2	3 ± 0.6	10.6 ± 0.1
Flowering	6.3 ± 0.05	10.9 ± 0.2	2.2 ± 0.3	11.5 ± 0.2
Harvest	6.3 ± 0.06	11.5 ± 0.2	2.7 ± 0.3	11.7 ± 0.2

Seasonal Dynamics of Biological Soil Health Indicators

Temporal variation in the biological soil health indicators across the sampling times was observed in this study, reflecting substantial seasonal dynamics (Fig. 2.2). Both soil extracellular enzyme activities and soil microbial biomass indicators had significant temporal effects (Table 2.3), though the magnitude and direction of effects varied between indicators and treatments. Across treatments, soil extracellular enzyme activities had greater seasonal responses than soil microbial biomass, and temporal patterns were consistent with fluctuations associated with major crop growth stages, especially between planting and harvest of both winter and summer crops.

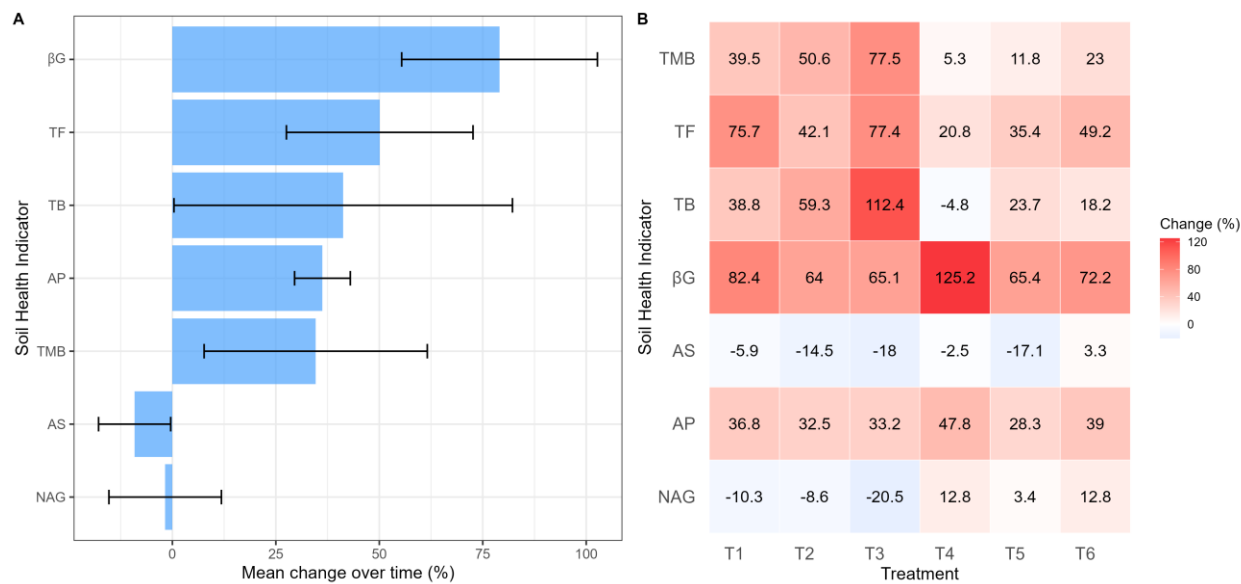


Figure 2.2. Overall percent change in soil health indicators relative to the initial values (A).

Percent change in soil health indicators relative to the initial value for each treatment throughout the study period at 0-5 cm depth (B). TMB: soil total microbial biomass; TB: soil total bacterial biomass; TF: soil total fungal biomass; β G: soil potential β -glucosidase activity; AP: soil

potential acid phosphatase activity; AS: soil potential arylsulfatase activity; NAG: soil potential N-acetyl- β -D-glucosaminidase activity.

Among the extracellular enzymes, soil β G had the most pronounced temporal variation (Fig. 2.3A), with mean activity changes exceeding 100% (Fig. 2.2), thus indicating a significant temporal effect on its activity (Table 2.3). β G increased after the winter crops planting in 2021 and reached its peak activity between the transition between the flowering and the harvest of winter crops in 2023 (day 530 after the first sampling; May 2023) for most of the treatments. The largest relative increase in activity was observed in treatment T4, where β G activity increased by approximately 125%, while the lowest relative increase in activity was observed in treatment T6 (72%). The highest rates of change for soil β G were observed during the transition between the summer crops harvest in 2022 (day 335 after the first sampling; October 2023) and the winter crops harvest in 2023 (day 609 after the first sampling; July 2023). Acid phosphatase (AP) also had substantial and significant temporal variability (Fig. 2.3B), though with less consistent magnitude across the treatments. Soil AP decreased after the winter crops flowering (day 152 after the first sampling; April 2022), and subsequently increased, reaching its maximum values around the winter crops harvest in 2023 (day 609 after the first sampling; July 2023). Relative increases in soil AP ranged from 32% to 48%, with the highest temporal change observed in treatments T4 and T1.

Table 2.3. Summary of the temporal dynamics of biological soil health indicators across treatments over the study period. Values represent the percentage change in each indicator relative to its initial level, the mean timing (in days relative to the first sampling) of the observed

peak response, and the corresponding dominant crop growth stage. Reported *p*-values indicate the significance of temporal variation as determined from the generalized additive mixed models (GAMMs). TMB: soil total microbial biomass; TB: soil total bacterial biomass; TF: soil total fungal biomass; β G: soil potential β -glucosidase activity; AP: soil potential acid phosphatase activity; AS: soil potential arylsulfatase activity; NAG: soil potential N-acetyl- β -D-glucosaminidase activity.

Indicator	Av. Change (%)	Mean peak time (days)	Crop Stage at Peak	<i>p</i> value
TMB	34.6	445	WP/WF – 2023	< 0.001***
TB	41.3	430	WP/WF – 2023	< 0.001***
TF	50.1	609	WH – 2023	< 0.001***
βG	79.1	530	WF/WH – 2023	< 0.001***
AP	36.3	609	WH – 2023	< 0.001***
AS	-9.1	102	WP/WF – 2022	< 0.01**
NAG	-1.7	305	SF – 2022	0.08

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

Soil AS had moderate temporal variability (Fig. 2.4C), with relative changes ranging from -20% to 13% across the treatments, with an average 9% reduction in activity throughout the sampling times (Fig. 2.2). The peak in soil AS was observed between the planting and flowering of winter crops in 2022 (Table 2.3), after which AS decreased steadily. Even though the temporal variability of AS was significant, the overall magnitude of changes was smaller than that observed for soil β G and AP over the same period. Soil NAG exhibited minimal temporal variation (Fig. 2.3D), with a non-significant overall change of -1.7%. Across all treatments, soil NAG remained relatively stable throughout the sampling period, peaking at the summer crop flowering stage in 2022. The amplitude of temporal variability in soil NAG varied across treatments, ranging from -20% to 13%, with the largest relative decrease in treatment T3 (Semi-

Intensive) and the largest relative increase in treatments T4 (Intensive) and T6 (Dual-purpose Intensive) (Fig. 2.2).

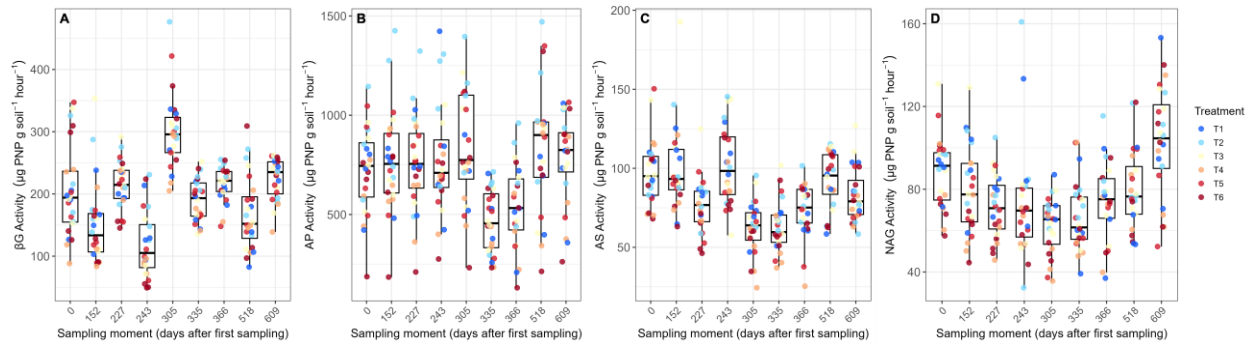


Figure 2.3. Distribution of soil βG (A), acid phosphatase (B), arylsulfatase (C), and NAG (D) activities across sampling times and treatments. Boxplots represent the distribution of observed values at each sampling moment (expressed in the x-axis as days after the first sampling). Winter crops planting (2021); day 0; Winter crops flowering 2022: day 152; Winter crops harvest (2022): day 227; Summer crops planting (2022): day 243; Summer crops flowering (2022): day 305; Summer crops harvest (2022): day 335; Winter crops planting (2022): day 366; Winter crops flowering (2023): day 518; Winter crops harvest (2023): day 609.

Soil microbial biomass indicators had significant temporal variation (Fig. 2.4), though with a smaller magnitude than the observed variability in extracellular enzyme activities. Total microbial biomass (TMB) increased throughout the study period, exhibiting an average increase of approximately 35% across treatments (Table 2.3). Temporal variation of soil TMB declined between the winter crops flowering in 2022 (day 152) and summer crops flowering in 2022 (day 305), with a peak at the transition between the winter crops planting and flowering in 2023 (day 446) (Fig. 2.4A). The amplitude of temporal variability in soil TMB varied between treatments,

ranging from approximately 5% to 78%, with the highest relative increase observed in treatment T3 and the smallest in treatment T4 (Fig. 2.4).

Soil total bacterial biomass (TB) showed significant variation across sampling times (Table 2.3). Soil TB had an overall increase of approximately 41% relative to initial levels with a peak between the winter crops planting and flowering in 2023 (day 430) (Fig. 2.4B). Soil TB decreased between the winter crops flowering and summer crops flowering in 2022 (days 152 and 305, respectively), followed by an increase towards the winter crops season in 2023. Between treatments, soil TB temporal variability ranged between -5% and 112%, with the largest relative increase observed in treatment T3 (Semi-intensive) and the smallest observed in treatment T4 (Intensive) (Fig. 2.2). Soil total fungal biomass (TF) had the most consistent and continuous temporal increase among the soil microbial indicators, with an average relative increase of 50% (Table 2.3). Soil TF increased after the winter crops planting in 2021 (Day 0) and reached its maximum values at the winter crops harvest in 2023 (Day 609) (Fig. 2.4C). The amplitude of temporal variability in soil TF varied between treatments, ranging from 21% to 77%, with the highest relative increase in treatment T3 (Semi-intensive) and the smallest relative increase in treatment T4 (Intensive) (Fig. 2.2).

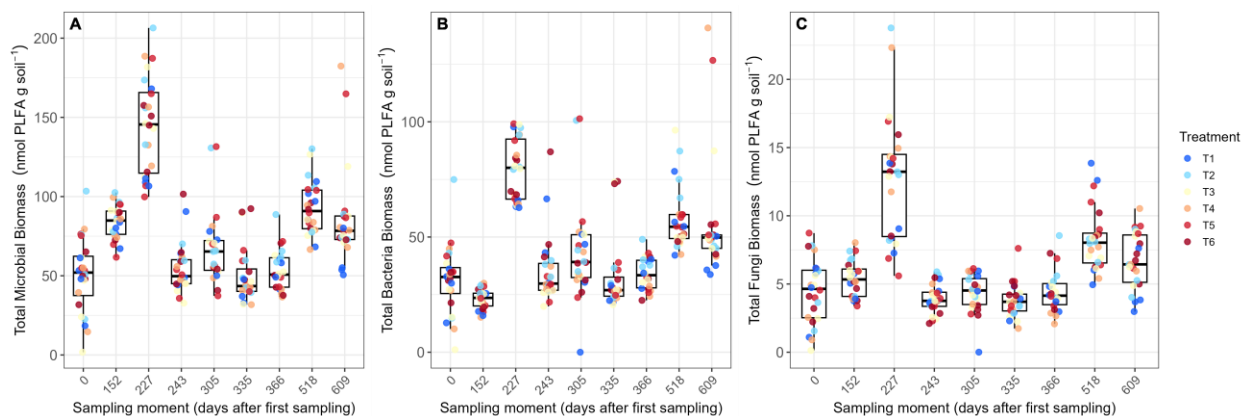


Figure 2.4. Distribution of soil total microbial (A), total bacteria (B), and total fungi (C) biomass across sampling times and treatments. Boxplots represent the distribution of observed values at each sampling moment (expressed in the x-axis as days after the first sampling). Winter crops planting (2021); day 0; Winter crops flowering 2022: day 152; Winter crops harvest (2022): day 227; Summer crops planting (2022): day 243; Summer crops flowering (2022): day 305; Summer crops harvest (2022): day 335; Winter crops planting (2022): day 366; Winter crops flowering (2023): day 518; Winter crops harvest (2023): day 609.

Vertical Stratification of Biological Soil Health Indicators

Substantial vertical stratification occurred across the biological soil health indicators, with consistent declines in values among the properties from surface soil (0-5 cm depth) to deeper soil layers (5-15 and 15-30 cm depth; Table 2.4). The decline in biological soil health indicators as a function of soil depth ranged from approximately ~30% to ~90%. Soil β G activity had the greatest vertical gradient among the extracellular enzyme activities, declining nearly 70%, from 0-5 cm to 15-30 cm. The rate of decline in soil β G activity, expressed as the mean slope in Table 2.6, was -6.3, with moderate variability between treatments (CV ~ 12%). Soil AP and AS decreased by ~54% and ~49%, respectively, across the three depths with mean slopes of -11 and -1.5, respectively. Soil NAG had the lowest decline, which decreased by 33%. However, NAG had higher variability among treatments (CV ~43%), indicating a less consistent depth effect across treatments.

Table 2.4. Summary of the vertical stratification of biological soil health indicators across the three soil depths (0-5, 5-15, and 15-30 cm). Values represent the average percentage change of

each indicator relative to the 0-5 cm soil layer, standard deviation (SD), minimum and maximum percent range, and the mean slope describing the rate of decline across depth layers. The coefficient of variation (C.V. slope) of the slope indicates the variability of the depth-related responses between the treatments. Negative values denote decreases with increasing soil depth. TMB: total microbial biomass; TF: total fungal biomass; TB: total bacterial biomass; β G: potential β -glucosidase activity; AP: potential acid phosphatase activity; AS: potential arylsulfatase activity; NAG: potential N-acetyl- β -D-glucosaminidase activity.

Indicator	Depth effect	SD	Min. Change	Max. change	Mean slope	C.V. slope
	(%)		(%)	(%)		(%)
TMB	-76.2	2.8	-79.0	-72.5	-2.1	16.4
TF	-92.4	0.8	-93.4	-91.4	-0.2	13.2
TB	-64.0	4.4	-68.9	-58.4	-1.1	18.9
βG	-70.0	2.2	-72.1	-65.7	-6.3	11.7
AP	-53.6	3.6 e ⁻¹⁴	-53.6	-53.6	-11.3	7.6
AS	-48.7	2.2 e ⁻¹⁴	-48.7	-48.7	-1.4	8.2
NAG	-32.8	10.2	-43.2	-20.2	-0.9	42.8

Soil depth had a substantial effect on soil microbial community indicators (Table 2.4). The overall decline in soil microbial community indicators as a function of soil depth ranged between 64% and 92%. Among these indicators, soil TF had the most pronounced depth effect, decreasing by 92% across the soil profile, with a relatively low slope (-0.2) and low variability between treatments (CV ~13%). Soil TMB and TB had less decline with depth (76% and 64%, respectively), greater slopes (-2.1 and -1.1, respectively), though with higher variability between treatments (CV ~16-19%) compared with soil TF.

Climate Drivers of Seasonal Variability in Biological Soil Health Indicators

Climate variables exerted significant, but indicator-specific, effects on soil biological properties (Table 2.5). Soil temperature had the strongest influence on the temporal variability across all variables. Among the soil extracellular enzyme activities, soil β G activity had the most pronounced sensitivity to weather variables, with a positive association with air temperature. These effects were expressed over a short temporal window (7 days), indicating responsiveness to short-term temperature fluctuations. Soil AP activity responded negatively to soil temperature. The response occurred over a 30-day period, indicating an influence over sustained periods. Soil AS and NAG had a positive response to soil temperature. Additionally, soil AS and NAG were influenced over larger time windows (60 days), thus indicating that their activities were regulated by soil temperature and precipitation regimes over an extended period rather than short-term variations. Precipitation had lower (coefficients < 0.001) effects on soil extracellular enzyme activities.

Table 2.5. Summary of weather covariates influence and response windows for biological soil health indicators. Values represent standardized regression coefficients for air temperature, soil temperature, and precipitation effects on each indicator. Response windows indicate the time period (7, 14, 30, or 60 days) over which weather covariates exerted the strongest influence on biological soil health indicators. TMB: soil total microbial biomass; TB: soil total bacterial biomass; TF: soil total fungal biomass; β G: soil potential β -glucosidase activity; AP: soil potential acid phosphatase activity; AS: soil potential arylsulfatase activity; NAG: soil potential N-acetyl- β -D-glucosaminidase activity; *n.s.*: no significant.

	Soil Temperature	Precipitation	Response Window	Effect Size
Indicator	(Days)			

TMB	-0.17	0.004	30	0.12
TF	-0.25	0.006	30	0.17
TB	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
βG	20.3	0.6	7	14.7
AP	-0.24	0.0003	30	<i>n.s.</i>
AS	0.03	0.0007	60	0.02
NAG	0.21	0.0009	60	0.17

Soil microbial community indicators were moderately sensitive to weather, primarily associated with soil temperature (Table 2.5). Soil TMB decreased in response to increasing soil temperature. Additionally, this effect was expressed over a 30-day period, suggesting that soil TMB dynamics were not influenced by short-term temperature fluctuations but rather by sustained monthly temperature patterns. Similarly, soil TF had a negative association with soil temperature. The effects of soil temperature on soil TF were expressed over a 30-day period, indicating a responsiveness over extended periods rather than short-term fluctuations in temperature. In contrast, soil TB had no significant weather associations, without soil temperature or precipitation covariates emerging as significant predictors. Similar to the extracellular enzymes, precipitation effects were low for the soil microbial community indicators, suggesting that soil temperature was the main weather drivers of soil microbial dynamics over the study period.

Optimal Sampling Window for Soil Health Assessments

Biological soil health indicators had substantial variability in both the magnitude and consistency of responses across sampling times (Fig. 2.5). Across all the assessed biological indicators, coefficients of variation (C.V.) ranged between 15 and 50%, with substantial fluctuations corresponding to distinct crop growing stages. During planting of winter crops in

2022 (WP-2022), microbial biomass indicators (TMB, TF, TB) exhibited C.V. of 15 and 35%, reflecting comparatively stable estimates of these biological soil health indicators. However, lower temporal stability was observed during the summer crops harvest (SH-2022) and winter crops harvest in 2023 (WH-2023), especially for soil TF and β G activity, where C.V.s exceeded 45%. Moreover, among the extracellular enzymes, β G and AP had lower average variability throughout the study period, whereas AS and NAG displayed greater temporal variability (Fig. 2.5A).

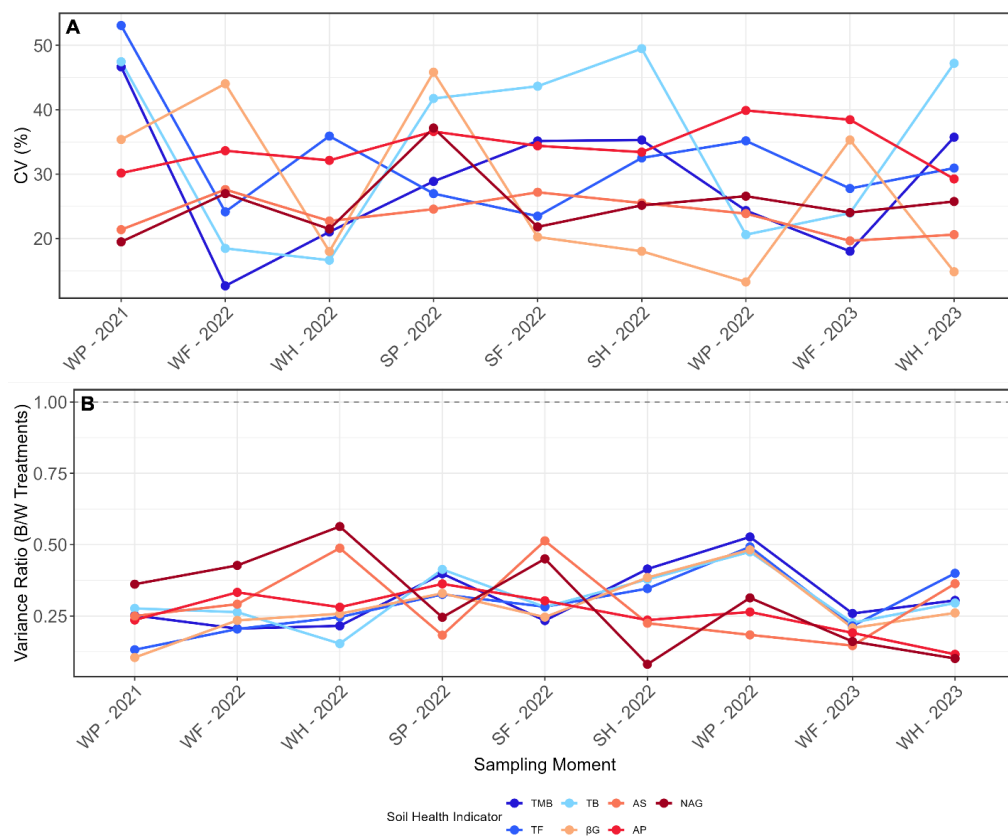


Figure 2.5. Temporal patterns of (A) coefficient of variation (C.V. %) and (B) between-to-within treatment variance ratio for biological soil health indicators across sampling moments. Lower CV values indicate higher measurement precision, while higher variance ratios reflect greater

treatment discrimination. WP: winter planting; WF: winter flowering; WH: winter harvest; SP: summer planting; SF: summer flowering; and SH: summer harvest.

Temporal changes in the between-to-within treatment variance ratio revealed differences in the stability and discriminative capacity of the biological indicators across the sampling times (Fig. 2.5B). The variance ratio ranged between 0.2 and 0.55, with the highest ratio for the winter crops harvest in 2022 (WH-2022) and summer crops flowering (SF-2022). During these periods, soil β G, AP, and TMB had greater temporal differentiation, with variance ratios exceeding 0.5, thus indicating substantial variation across sampling times. Conversely, during the winter crops flowering in 2023 (WF-2023) and winter crops planting in 2021 (WP-2021), variance ratios were lowest, reflecting lower temporal differentiation between treatments and more uniform indicator values. For the whole study period, soil β G had the largest fluctuation in variance ratio (0.10 to 0.50), while soil TF and AS had the lowest temporal variance ratio, remaining below 0.30.

The combined temporal behavior of both precision (C.V.) and variance ratio metrics revealed that biological soil health indicators varied considerably throughout the sampling times, with clear differences in the magnitude of their temporal instability. The lowest combined variability and moderate discrimination was observed at winter crops planting in 2021 (WP-2021; 0.23) and summer crops planting (SP-2022; 0.32) (Fig. 2.5; Fig. 2.6), when most biological soil health indicators had lower coefficients of variation and therefore more consistent measurements across indicators. In contrast, sampling times corresponding with mid-summer and late-harvest had the highest observed variability in both extracellular enzymes and microbial community indicators, thus increasing the potential for over- or underestimation of soil health conditions. Across all sampling times, the mean variance ratio calculated across indicators was greater during the winter crops planting in 2022 (WP-2022; 0.39), followed by the summer crops

flowering (SF-2022; 0.33) and summer crops planting (SP-2022; 0.32), indicating greater ability to discriminate treatment differences during these periods. Therefore, these sampling times were associated with greater treatment discrimination among cropping systems. Conversely, the lowest mean variance ratios were observed during the later stages of the study, especially during the winter crops flowering in 2023 (WF-2023; 0.20) and winter crops planting in 2021 (WP-2021; 0.23), indicating periods of reduced treatment discrimination among the assessed indicators (Fig. 2.6).

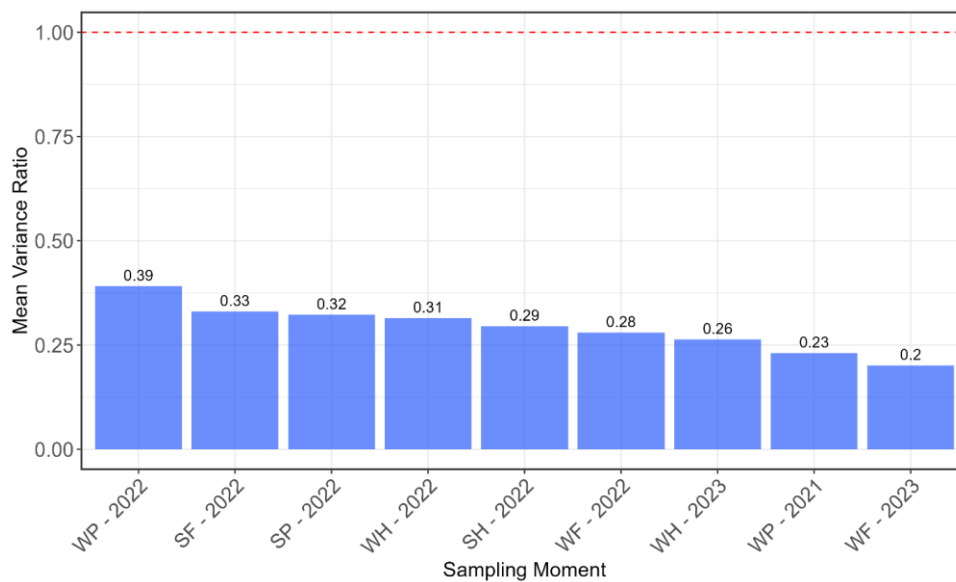


Figure 2.6. Overall ranking of sampling moments for soil health assessments based on the mean between-to-within treatment variance ratio across all indicators. Higher values indicate stronger overall discrimination among treatments. The red dashed line denotes the reference ratio of 1, representing equality between- and within-treatment variance.

Relative Contribution of Cropping Systems

Although the variability between sampling times represented the dominant source of variation across biological soil health indicators, treatment differences were observed when averaged across sampling times at 0-5 cm depth (Table 2.6). Overall, the proportion of significant pairwise contrasts ranged from 0 to 50%, indicating that treatment effects were present but generally lower than seasonal fluctuations.

Extracellular enzymes had significantly greater treatment effects among the cropping systems than soil microbial biomass indicators. Among the extracellular enzymes, soil β G had the greatest treatment contrasts, with seven significant pairwise comparisons and an average effect size of $28 \mu\text{mol PNP g}^{-1} \text{ soil h}^{-1}$ (Table 2.6), and with the greatest difference occurring between treatments T4 and T2 ($68 \mu\text{mol PNP g}^{-1} \text{ soil h}^{-1}$). Soil AS had moderate treatment differentiation, with four significant contrasts and an average effect size of approximately $8 \mu\text{mol PNP g}^{-1} \text{ soil h}^{-1}$. In contrast, soil NAG had only two significant contrasts and a mean effect size of $9 \mu\text{mol PNP g}^{-1} \text{ soil h}^{-1}$. Soil AP had no significant differences among treatments. Soil microbial biomass indicators, on the other hand, had lower treatment contrasts compared with extracellular enzymes. Soil TMB had two significant pairwise comparisons with an average difference of $12 \text{ nmol PLFA g soil}^{-1}$. Soil TF had four significant contrasts; however, the magnitude of these differences was relatively small, with an average effect size of $1 \text{ nmol PLFA g soil}^{-1}$. Soil TB had no significant treatment differences. A graphical representation of the relative treatment differences among the biological soil health indicators assessed in this study is provided in Figure A.1 in Appendix A.

Table 2.6. Summary of significant pairwise treatment contrasts for biological soil health indicators at 0-5 cm depth. Estimates represent the difference between contrast treatments. Standard error (SE) and associated p-values indicate the statistical significance of treatment effects. Significant pairs indicate the total number of treatment comparisons exhibiting significant differences for each indicator. Mean effect represents the average absolute difference across significant pairs. Maximum difference represents the largest observed contrast. AS: soil arylsulfatase activity; NAG: soil N-acetyl- β -D-glucosaminidase activity; β G: soil β -glucosidase activity; TMB: soil total microbial biomass; TF: soil total fungal biomass.

Indicator	Contrast	Estimate	SE	p value	Sig. Pairs	Mean effect	Max. Diff.
AS	T4-T2	15.5	6.05	< 0.05*	4	7.7	15.5
AS	T6-T2	14.8	6.02	< 0.05*			
AS	T4-T3	12.9	6.1	< 0.05*			
AS	T6-T3	12.2	6.1	< 0.05*			
NAG	T4-T2	19.0	7.6	< 0.05*	2	8.9	19.0
NAG	T6-T2	18.5	7.6	< 0.05*			
β G	T4-T2	68.4	15.8	<0.001***	7	28.2	68.4
β G	T5-T2	54.8	15.8	<0.001***			
β G	T2-T1	-50.8	15.7	<0.01**			
β G	T4-T3	35.1	15.6	< 0.05*			
β G	T6-T2	34.9	15.8	< 0.05*			
β G	T6-T4	-33.5	15.5	< 0.05*			
β G	T3-T2	33.3	15.8	< 0.05*			
TMB	T6-T3	-23.8	11.2	< 0.05*	2	12.1	24.7
TMB	T3-T2	24.7	12.0	< 0.05*			
TF	T4-T1	-1.8	0.62	<0.01**	4	0.8	1.8
TF	T2-T1	-1.5	0.64	< 0.05*			
TF	T4-T3	-1.4	0.64	< 0.05*			
TF	T5-T1	-1.3	0.63	< 0.05*			

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

Discussion

Seasonal variability of biological soil health indicators was assessed with different levels of cropping system intensity and diversity. This may have implications for sampling timing decisions linked with the different crop growth stages among the different cropping systems (Hsiao et al., 2019). These findings reinforce the hypothesis that the presence of living plants significantly affects soil health indicators, in addition to the significant effects climatic variability on these indicators (Cavaglieri et al., 2009; McDaniel and Grandy, 2016; Hsiao et al., 2019). Although seasonal dynamics represented the dominant source of variability in biological soil health indicators, moderate differences between treatments were also observed. Cropping systems with continuous living cover and legume inclusion (e.g., T4 and T6) had higher enzyme activities and microbial biomass compared to less diverse crop sequences. These findings are consistent with studies implying that diversified crop rotations maintain higher soil biological activity due to continuous organic inputs and associated rhizosphere processes (Bowles et al., 2020). In contrast, research in perennial grasslands and forest systems emphasized that greater plant diversity has the potential to stabilize enzyme activity rather than enhance it (French et al., 2021). This divergence likely stems from differences in disturbance regimes and resource turnover rates, with annual cropping systems responding more dynamically to management practices and residue cycling. Overall, the magnitude of seasonal fluctuations across sampling times exceeded the differences observed among treatments, emphasizing the dominant role of crop phenology and environmental conditions in regulating soil biological processes in this study.

Across the study period, the most responsive indicators to the presence of living plants were those directly linked to carbon turnover and microbial biomass activity, specifically β G, AP, and TMB. These indicators had clear peaks in activity and biomass during periods of active crop growth and rhizosphere development, particularly during crop flowering. This synchronization between crop growth stages and soil biological activity is consistent with rhizosphere-driven processes, including root exudation, that have been shown to influence short-term microbial and enzymatic responses (Kuzyakov & Blagodatskaya, 2015; McDaniel & Grandy, 2016). Root-derived carbon inputs and signaling compounds are known to serve as immediate energy sources for microbes, also triggering the increased synthesis of extracellular enzymes, such as β G and AP, that facilitate organic carbon and phosphorus turnover (Chaparro et al., 2014). In contrast, biological soil health indicators associated with other nutrient cycling pathways, such as NAG and AS, had lower temporal variability, suggesting that not all biological soil health indicators are equally sensitive to crop growth stages. Enzymes involved in nitrogen and sulfur cycling typically reflect longer-term organic matter turnover rather than short-term inputs of labile carbon associated with rhizosphere activity (Wang et al., 2023). This functional difference aligns with previous studies demonstrating that soil β G and phosphatases generally respond within days to shifts in labile carbon availability, while NAG and AS require sustained substrate changes and supply (Bastida et al., 2019). Conversely, previous studies have also emphasized less pronounced seasonal patterns for soil β G and AP activities under dry and warm weather conditions. For example, (Burns et al., 2013) reported declines in β G activity during warm and dry summer months, likely due to enzymatic denaturation and substrate limitation. Additionally, the observed variability of these biological soil health indicators may also suggest influences of plant residue retention from the previous crops (Dennis et al., 2010;

Hu et al., 2018). Our findings in this study reinforce that cropping systems with crop residue retention and labile carbon inputs sustained enzyme activity even under warmer conditions, where management and residue inputs can buffer weather effects on soil health indicators (French et al., 2021).

Soil depth strongly modulated the magnitude and the variability of soil biological activity, with a clear stratification observed in both extracellular enzyme activity and soil microbial biomass. While the 0-5 cm soil layer had the highest absolute values of biological soil health indicators, it also had greater temporal fluctuations compared to the 5-15 and 15-30 cm layers. This behavior can be attributed to higher soil organic matter concentrations and residue accumulation near the soil surface, thus leading to more pronounced interactions between abiotic factors, plant inputs, and microbial communities (Bell et al., 2010; Sun et al., 2016). Although the 5-15 cm depth layer may still be considered part of the surface soil, the near-surface 0-5 cm layer represents a dynamic zone highly sensitive to fluctuations in soil moisture, temperature, and carbon inputs. Moreover, these differences in variation among the soil layers are likely stronger in no-tillage systems, driven by the lack of soil physical disturbance and increased root density at 0-5 cm (Chassot et al., 2001; Baker et al., 2007; Wendt and Hauser, 2013). The results found in this study support the conclusion that the 0-5 cm depth soil layer receives greater amounts of plant residue inputs, root exudates, and senescent root inputs, therefore reflecting greater variations across seasons (Hsiao et al., 2019). The observed pattern in this study suggests, therefore, that the 0-5 cm soil layer was not only the most sensitive to management and environmental drivers, but also the most variable.

Winter wheat was the main crop in the systems evaluated, and the fallow period after winter wheat harvest was replaced with double-cropped soybean for most of the treatments. The

alternating sequence of winter wheat and soybean established a system characterized by contrasting residue quality and decomposition dynamics, which may have driven part of the observed temporal variation in the assessed biological soil health indicators. Winter wheat residue, with a C/N ratio of approximately 50%, decomposes substantially more slowly than soybean residue (Havlin et al., 1990; Aher et al., 2017). Studies have shown that approximately 47% of wheat residue is decomposed after 32 days due to its high C/N ratio and high lignin content, compared with 68% of soybean residue under the same conditions (Broder and Wagner, 1988; Beyaert and Voroney, 2011). This sustained input of high-lignin, high-cellulose wheat residue likely extended the substrate supply for soil β G and other carbon-degrading enzymes throughout the subsequent growing seasons, explaining the sustained β G activity observed from the summer crops harvest through the following winter wheat cycle. Even though winter wheat produces more residue in terms of quantity, its lower quality slows microbial turnover but stimulates enzyme systems specialized in decomposing complex carbohydrates (Hsiao et al., 2019b; Shahbaz et al., 2017; Zhang et al., 2017). On the other hand, the summer crop phase, mainly dominated by soybean, supplied more labile carbon and nitrogen inputs that likely fueled microbial biomass accumulation and AP activity early in the winter wheat growing season. These findings support the assumption that both the quantity and quality of crop residue directly modulate microbial community size and structure and soil enzymatic function, leading to the observed seasonal fluctuations observed in this study (Kramer et al., 2013; Hsiao et al., 2019). Moreover, rhizosphere-derived labile carbon, including root exudates during winter wheat development, may have contributed to enhanced microbial biomass and enzyme activities, as the rhizodeposition has been shown to provide readily available carbon sources that fluctuate with plant phenology (Carvalhais et al., 2011; Li et al., 2014; Hsiao et al., 2019; Panico et al., 2020).

The synchronous increase in β G and AP activities during wheat flowering and grain filling supports this interpretation, consistent with studies implying that rhizosphere processes intensify as plants reach reproductive stages (Bastida et al., 2019; French et al., 2021; Hsiao et al., 2019b). Overall, these results emphasize the importance of residue quality, decomposition rates, root exudates, and how they interact to control the magnitude and timing of microbial and enzymatic responses.

Furthermore, weather variables also exerted a substantial influence on the biological soil health indicators assessed in this study. Soil temperature was a dominant driver of the observed seasonal variation, while precipitation played a secondary role. The magnitude and direction of responses were, however, indicator specific. Soil enzyme activities, particularly β G and AP, had strong and opposite sensitivities to soil temperature. Soil β G declined in activity under elevated soil temperatures, suggesting a differential thermal regulation of extracellular enzyme synthesis and turnover. This observed pattern aligns with studies showing that moderately higher temperatures enhance C-degrading enzyme production due to greater microbial demand for labile carbon, while excessive soil temperatures can inhibit enzyme stability and microbial synthesis (Allison et al., 2010). Furthermore, NAG and AS were less responsive to weather conditions, displaying overall weak temperature dependence and modest positive associations with precipitation. These relationships reflect the longer substrate turnover rates of N- and S-related processes, which have a better response to moisture-driven substrate diffusion than to short-term thermal fluctuations (Birch, 1958; German et al., 2012). Similarly, soil microbial biomass indicators displayed different climatic responses. Soil TMB, TB, and TF decreased under high soil temperature conditions. This pattern is consistent with the trade-off between enhanced metabolic rates and potential thermal or moisture stress at the soil surface (Nottingham

et al., 2016). Precipitation effects, even though weaker in magnitude, were consistently positive for soil microbial biomass, emphasizing that transient wetting events stimulate microbial growth and enzymatic activity by alleviating drought-induced dormancy and denaturation (Schimel, 2018). Moreover, the analysis of optimal climate aggregation windows indicated that short-term fluctuations (7-30 days) were most predictive of soil microbial and enzymatic responses, highlighting the rapid adaptation of soil biota to recent environmental conditions. These results emphasize that soil biological activity responses in wheat-based systems integrate recent temperature and moisture dynamics, with soil β G and AP activities being more weather-sensitive indicators, while microbial biomass indicators exhibited a buffered, but consistent, response to soil thermal regimes.

The pronounced sensitivity of biological soil health indicators to both crop growth stages and weather fluctuations highlights the importance in the selection of sampling time for soil health assessments. Several factors are currently used as criteria for the selection of soil health indicators. Karlen and Stott (2019) argued that effective soil health indicators should be sensitive to changes in management practices, and these changes should be sensed in a short time (1-3 years). Because soil extracellular enzymes and microbial communities respond to temperature and moisture regimes, sampling at suboptimal times can lead to over- or underestimation of biological functioning, thereby compromising the interpretation of management effects. The results from this study indicated that biological soil health indicators, especially soil β G, AP, TMB, and TF, had substantial temporal variation across sampling times, with greater stability and treatment differentiation observed at planting and flowering stages rather than harvest stages. These findings align well with studies implying that soil enzymatic activities and microbial communities are more temporally stable under active root growth and moderate climatic

conditions (Franzluebbers et al., 2001; Hsiao et al., 2019b). Periods immediately following the crops planting coincide with increased rhizosphere activity, which has been associated with enhanced microbial colonization; however, preceding the strong seasonal temperature and moisture fluctuations at later stages of crop development (Kuzyakov & Blagodatskaya, 2015). As a result, they represent an optimal window in which biological soil health indicators capture both biological activity and management-induced differences without the confounding effects of climatic extremes. Conversely, samples collected at late-season senescence had higher within-treatment variability and lower variance ratios, thus reflecting increased environmental heterogeneity and transient microbial responses to residue decomposition and environmental stress. This pattern reinforces, therefore, that the interpretability and reproducibility of soil health indicators data depend not only on the analytical methodology but also on the timing of sampling relative to climatic and crop growth cycles (Karlen & Stott, 2015). Based on the variance ratio and coefficient of variation analyses in this study, the planting and flowering stages of both winter and summer crops, particularly WP-2022 and SF-2022, emerged as the most reliable timings for soil health assessments in winter wheat-based cropping systems. Soil sampling during these time periods would minimize the risks of misinterpretation due to transient biological or environmental fluctuations, thus ensuring that soil health measurements better reflect sustained management effects rather than short-term perturbations.

The significant variability observed in biological soil health indicators throughout this study highlights the strong influence of crop residue quantity and composition, as well as plant growth stages, on soil microbial community dynamics and activity. The increase in soil microbial biomass and extracellular enzymes during the wheat growing season likely reflected the combined effects of residue inputs from the preceding soybean crop and enhanced

rhizosphere carbon inputs intensified with crop development. Although root exudation and rhizosphere-derived processes were discussed as potential drivers of the observed variability in biological soil health indicators, these were not directly measured in this study and were therefore interpreted as plausible mechanisms based on previous literature. These findings reinforce the role of residue quality and root-derived substrates as primary regulators of soil microbial and enzymatic processes in agroecosystems. Nevertheless, the variability detected across sampling times also highlights the inherent challenge of assessing soil health indicators that are highly responsive to transient biological and environmental factors. Based on the evidence from this study, soil sampling conducted during the fall, coinciding with winter wheat planting, represented the most appropriate timing for soil health assessments in winter wheat-based cropping systems of the U.S. Great Plains, as it captures stable biological activity while minimizing climatic and phenological confounding effects. Despite its robust temporal coverage, this study was limited by its scope in characterizing the underlying labile organic pools that may drive biological variation. Measurements of particulate organic matter (POM), dissolved organic carbon, and microbial biomass turnover would provide critical insight into the seasonal dynamics of substrate availability to the soil microbiome. Similarly, the quantification and biochemical characterization of root exudates, such as low molecular weight organic acids (LMWOA), would strengthen understanding of how rhizosphere carbon inputs regulate microbial and enzymatic responses through the growing season. Future research integrating these labile carbon pools with biological and weather data will be essential to understand the coupled effects of residue quality, root exudation, and environmental conditions variation on biological soil health indicators across seasons.

Conclusion

This study demonstrated that biological soil health indicators exhibited significant temporal variability, with significant changes occurring over short-term periods. The temporal dynamics of these indicators were strongly influenced by soil sampling timing, substrate availability, climatic conditions, and interactions with plants across their growth stages. The results from this study highlight that both the intensity and diversity of cropping systems influenced the magnitude and temporal variability of biological soil health indicators, reflecting the responsiveness of soil biological processes to plant-driven and environmental factors. Depth-dependent differences further revealed that the 0-5 cm layer was the most sensitive layer to seasonal fluctuations, emphasizing the influence of surface residues and rhizosphere dynamics in driving soil biological activity. When integrated across temporal, depth, and climatic dimensions, these patterns indicated that the timing of soil sampling has the potential to substantially affect the interpretation of soil health assessments. Among the evaluated soil sampling times, the fall season coinciding with the winter wheat planting represented the most stable and representative timing for soil sampling, minimizing transient effects associated with crop growth stages and residue turnover. Future research suggests a deeper understanding of the plant and root exudates' effects on the temporal dynamics of soil microbial community and activity. Moreover, a more detailed resolution of soil carbon composition would provide a better understanding of the soil carbon dynamics across crop seasons, especially the most labile soil carbon fraction.

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Chapter 3 - The effect of cropping systems intensity and diversity on soil health indicators in winter wheat-based systems in Kansas

Abstract

The replacement of summer fallow periods with additional summer crops in winter wheat-based cropping systems has been proposed as a strategy to improve soil health in rainfed agroecosystems. However, the responses of chemical, physical, and biological soil health indicators to increased crop intensity and diversity remain unclear. This study evaluated the effects of six rainfed, no-tillage cropping systems varying in crop intensity, diversity, and purpose on chemical, physical, and biological soil health indicators over five years in Kansas. Treatments included a winter wheat-summer fallow control and intensified and diversified crop sequences incorporating grain, forage, and dual-purpose crops. Soil health indicators were measured at the beginning of the experiment in 2019 and again in 2024, and included soil organic carbon (SOC), water-stable aggregates, soil microbial biomass and composition, and extracellular enzyme activities at three soil depths: 0-5, 5-15, and 15-30 cm. Across all treatments, measures of SOC, microbial biomass, and selected enzyme activities increased over time at 0-5 cm, whereas aggregate stability exhibited modest, variable changes. However, treatment-level differences were generally not observed after five years. Grain-based systems produced substantially greater cumulative plant biomass than forage and dual-purpose systems, yet these differences did not yield consistent soil health improvements at the treatment level. Biological soil health indicators revealed improvements in greater magnitude than chemical and physical indicators, reflecting a substantial effect of the transition from conventional tillage to

continuous no-tillage. Overall, the results from this study indicate that soil health responses during the first five years were dominated by the legacy effect associated with reduced soil disturbance rather than by differences in crop intensity and diversity. These findings suggest that measurable soil health benefits from cropping systems intensification and diversification may require longer timeframes, sustained organic inputs, and reduced soil disturbance.

Introduction

The exponential growth of the world's population poses significant challenges for ensuring food security and for the provision of feed, fuel, and fiber (Bhardwaj et al., 2011). These challenges are amplified by climate change, natural resources depletion, and land degradation, which collectively threaten the long-term sustainability of crop production systems worldwide (Foley et al., 2011; IPCC, 2023). Although the expansion and intensification of agriculture over recent decades have contributed to economic and social development, they have also driven biodiversity loss, greenhouse gas emissions, soil degradation, and water pollution (MEA, 2005; Kirschenmann, 2010). Increasingly, soils have been considered a vulnerable resource whose degradation directly undermines crop yield stability, climate resilience, and environmental quality in agroecosystems. In addition to physical and chemical degradation, the biological dimension of soil health, largely governed by the soil microbiome, sustains the regulation of nutrient cycling, carbon stabilization, and aggregate formation (Lehman et al., 2015; Fierer, 2017). Soil microbial communities act as both engineers and indicators of soil functioning, linking management practices and ecosystem resilience (Fierer, 2017; Trivedi et al., 2020). Consequently, agricultural systems must be redesigned to sustain crop production and yield

stability under increasingly variable climatic conditions while preserving soil and environmental resources (Liu et al., 2019; Reckling et al., 2022).

Crop production in the U.S. Central Plains region is characterized by cereal crops (e.g., wheat (*Triticum aestivum* L.)) in rotation with fallow periods to conserve soil water for subsequent crops (Peterson et al., 2020; Obour et al., 2021). Although winter wheat-fallow systems have historically provided stable crop production under water-limited conditions, they are also associated with extended periods of bare soil, reduced carbon inputs, and suppressed biological activity, thus increasing the potential for soil physical, chemical, and biological degradation (Unger & Vigil, 1998; Nielsen & Vigil, 2010). Moreover, fallow periods, which can range from 4 to nearly 14 months depending on the precipitation regime, are not efficient for water storage for the subsequent cash crop (Nielsen and Vigil 2010; Patrignani et al., 2019; Massigoge et al., 2024). Precipitation regimes in the region are highly variable, not only in total amount but also in seasonal distribution, creating mismatches between water availability and crop demand (Hansen et al., 2012; Easterling et al., 2017). Furthermore, recent climate projections suggest increasing interannual variability and more frequent extreme and adverse events, reinforcing the need for cropping systems that improve soil-mediated buffering capacity rather than relying solely on water conservation through fallow periods (Obour et al., 2021; IPCC, 2023).

Cropping systems intensification and diversification, defined as increases in crop frequency and functional diversity within a crop rotation, are alternatives to increase crop production without expanding land use (Andrade et al., 2017; Gaba et al., 2015; Waha et al., 2020; Massigoge et al., 2024). Replacing summer fallow periods with grain, forage, or dual-purpose crops has the potential to increase plant-derived carbon inputs, reduce erosion, stimulate

microbial processes, and improve nutrient cycling efficiency (Bowles et al., 2020; Brooker et al., 2015). From a microbiological perspective, increased crop functional diversity extends the range of root exudates and residue composition entering the soil system, thereby fostering enzymatic niche differentiation and stimulating resource complementarity within microbial communities (Delgado-Baquerizo et al., 2016; Luo et al., 2023). This microbial functional diversity enhances nutrient turnover efficiency and supports the emergence of soil multifunctionality beyond simple increases in biomass input. However, recent evidence increasingly implies that soil health benefits from cropping systems intensification and diversification are not universal, and responses depend on crop functional traits, residue fate, disturbance regime, and the balance between carbon inputs and outputs (Peterson et al., 2020; Poeplau et al., 2020). In forage and dual-purpose systems, for example, aboveground biomass removal can fundamentally alter carbon allocation pathways. (Kuzyakov & Domanski, 2000; Hirte et al., 2018; Kong & Six, 2010; Yang et al., 2023). With less carbon retained in surface residues, a larger proportion of assimilated carbon is directed belowground to roots and rhizosphere exudates (Bardgett & van der Putten, 2014). This shift reduces the input of structural carbon that supports SOC accrual and aggregates formation (Blanco-Canqui & Lal, 2008a), while also limiting the organic substrates that sustain soil microbial communities and extracellular enzyme activities (Sinsabaugh et al., 2008). Therefore, the intensification of cropping systems may increase crop complexity without necessarily improving soil health metrics over short timeframes. In this sense, this conditionality highlights the need for system-specific evaluations that move beyond assumptions about linear improvements in soil health with increased crop intensity and diversity.

In this context, however, the intensification and diversification of cropping systems can improve soil health through multiple, interconnected mechanisms that regulate soil carbon

cycling, aggregation, nutrient dynamics, and biological activity. Increasing crop frequency and functional diversity can enhance the temporal continuity of plant cover, increase rhizosphere inputs, diversify residue quantity and quality, as well as promote complementary resource use among crops (Finney and Kaye, 2017; Yousefi et al., 2024). From a mechanistic perspective, diversified crop rotations can stimulate microbial activity through the provision of a broader range of carbon substrates, increase aggregate stability through larger inputs of particulate organic matter, and improve nutrient cycling efficiency by synchronizing residue decomposition with microbial demand (Lehman et al., 2015; Rillig et al., 2023). Furthermore, recent syntheses indicate that diversifying crop rotations may increase soil multifunctionality and resilience by strengthening biological feedback that regulates carbon stabilization and soil structure (Bünemann et al., 2018; Norris et al., 2020). Consequently, detectable improvements in soil physical, chemical, and biological health may require sufficient time, sustained organic inputs, and favorable interactions among management components, thereby reinforcing the importance of soil health evaluations across multiple indicators and over time, particularly during the early phases of system transition.

Despite growing interest in soil health-oriented cropping systems among producers, researchers, and policymakers (Augarten et al., 2023), substantial uncertainty remains regarding the soil health outcomes of intensification and diversification in rainfed winter wheat-based cropping systems in the U.S. Great Plains. Few studies have simultaneously evaluated soil chemical, physical, and biological indicators during the early years following both cropping systems diversification and intensification, and the transition from conventional tillage to no-tillage. Moreover, limited empirical evidence exists comparing grain-based systems with forage and dual-purpose systems that involve biomass removal under otherwise similar environmental

conditions. This study addresses these gaps by evaluating soil health indicators over five years of implementation of six rainfed, no-tillage cropping systems in Kansas. The objectives of this study were to (i) quantify temporal changes in chemical, physical, and biological soil health indicators after five years of cropping systems intensity and diversity implementation, and (ii) determine whether increased crop intensity and diversity resulted in detectable soil health benefits beyond those associated with the transition to continuous no-tillage. We hypothesized that cropping system intensification and diversification would enhance chemical, physical, and biological soil health indicators by increasing plant-derived carbon inputs and substrate diversity. Chemical soil health indicator assessed was soil organic carbon (SOC). Water-stable aggregates was used as the physical soil health indicator, and soil microbial community abundance and composition and extracellular enzyme activities were assessed as biological soil health indicators. Cumulative observed aboveground and estimated belowground plant biomass for each treatment over the course of the study was calculated as a source of plant-derived carbon for the soil ecosystem.

Material and Methods

Experimental Site and Experiment Description

This study was conducted at the Kansas State University Ashland Bottoms Research Field (39.12408 N, 96.63514 W) located in Manhattan, Kansas, USA, from October 2019 to October 2024. The predominant soil type at the study location was classified as a silty clay loam (Wymore series, fine mesic Aquertic Arguidoll; USDA (2014)). Before the study was established, the experimental site was managed with a sorghum–soybean–winter wheat rotation

under a conventional tillage system (chisel plow, tandem disk, and field cultivator). Weather data, such as monthly average precipitation and temperature, were obtained from the Kansas Mesonet weather station in Manhattan, KS (Patrignani et al., 2020), which was approximately 10 km from the study site. Based on historical weather records (30 years), the annual average precipitation was 791 mm, and the average temperature was 13.1 °C. Monthly precipitation during the study period, the 30-year average precipitation is displayed in Table B.2 in Appendix B.

The study compared six treatments, as crop sequences, varying in crop intensity and diversity, including grain crops, forage crops, and cover crops (Figure 3.1). The crop species were alfalfa (*Medicago sativa* L.), corn (*Zea mays* L.), soybean (*Glycine max* L.), cowpea (*Vigna unguiculata* L.), moth bean (*Vigna aconitifolia*), grain sorghum (*Sorghum bicolor* (L.) Moench), tepary bean (*Phaseolus acutifolius*), triticale ($\times$ *Triticosecale* Wittmack), and winter wheat (*Triticum aestivum* L.). The study was conducted as a randomized complete block design with four replications, where each experimental unit was 12.9 m by 15.24 m. Treatments 1 (Control, T1), 4 (Intensive, T4), and 5 (Dual-purpose semi-intensive, T5) had a standard nitrogen application of 90 kg N ha⁻¹ at spring green-up in the 2019-2020, 2020-2021, 2021-2022, 2022-2023, and 2023-2024 seasons. Treatments 2 (Adaptive Forage, T2) and 3 (Semi-intensive, T3) had a progressive nitrogen application management differing in application timing, placement, and rate compared to the standard management, and received 45 kg N ha⁻¹ at spring green-up, followed by 30 kg N ha⁻¹ at jointing stage. The vegetative and reproductive stages of winter wheat were determined through field observations using the Zadoks scale (Zadoks et al., 1974). Treatment 6 (Dual-purpose Intensive, T6) was a green-nitrogen management, receiving no synthetic nitrogen, with nitrogen provided by a legume residue, such as moth beans, soybean,

and alfalfa. Nitrogen rates were determined using an in-season nitrogen recommendation tool based on crop modeling (Massigoge et al., 2024).

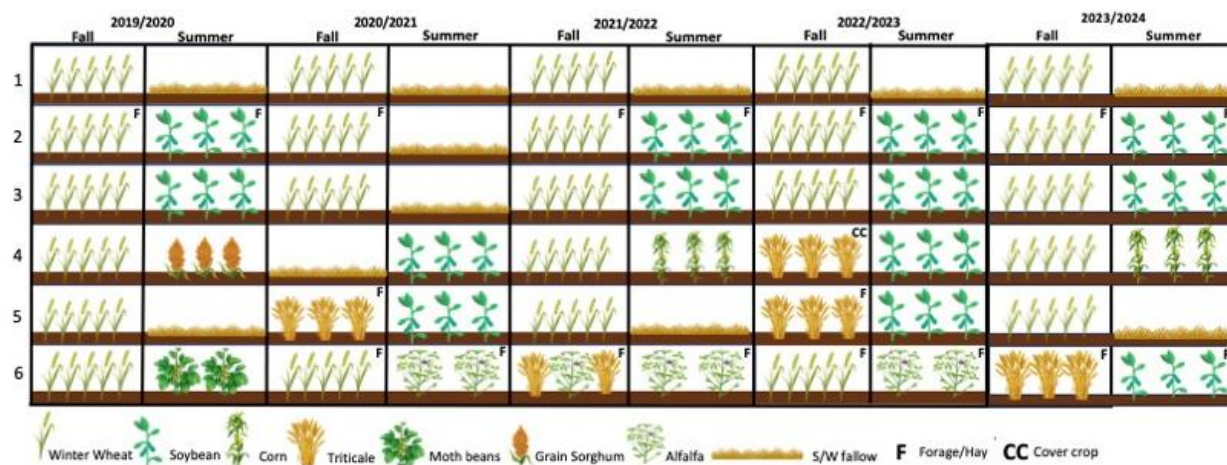


Figure 3.1. Schematic representation of the treatments assessed in the study. Treatment 1: Control; Treatment 2: Adaptive Forage; Treatment 3: Semi-Intensive; Treatment 4: Intensive; Treatment 5: Dual-purpose Semi-Intensive; Treatment 6: Dual-purpose Intensive. The sketch is not to scale and does not represent the actual duration of each crop in the rotation. Adapted from Massigoge et al. (2024).

For the winter crops, winter wheat and triticale for forage were planted at early planting dates (late September to early October), prior to the region's optimum planting dates. Winter wheat for grain, when following summer fallow, forage soybean, and full-season soybean, was planted at the region's optimum (mid-October) planting dates. Winter wheat for grain, when following double-cropped soybean or corn, and triticale cover crop, was planted at late (November) planting dates for the region. Summer crops following winter fallow, triticale for forage purposes, and triticale as a cover crop were planted in mid-May as full-season crops. Summer crops following winter wheat for grain were planted in early July, immediately after

harvest, as a double-crop. A detailed description of crop management for each one of the treatments is provided in Table B3 in Appendix B.

Winter crops were planted utilizing a 0.19 m row spacing with a 9-row Great Plains 506® no-till drill (Great Plains Manufacturing, Salina, Kansas, USA). Soybean, corn, grain sorghum, and cowpea were planted with a 0.762 m row spacing. Moth, tepary, and alfalfa was a 0.381 m row spacing. All the summer crops were planted using a 2-row John Deere 7000® planter (John Deere, Moline, Indiana, USA). Crops intended for forage purposes (alfalfa, triticale, and forage soybeans) were mowed at late bud, late boot (Zadoks et al., 1974), and R3 phenological stages (Fick & Mueller, 1989) with all shoot biomass removed from the plots after mowing. Triticale as a cover crop was chemically terminated in mid-May. Diammonium phosphate (DAP; 18-46-0 of N-P₂O₅-K₂O) was placed subsurface at the planting of winter crops at 55 kg ha⁻¹. No phosphorus fertilizer was added for summer crops. The experiment was conducted under a no-tillage system and under rainfed conditions.

Soil Sampling

Soil samples were collected within each experimental unit using a Giddings probe (Giddings Machine Company, Windsor, Colorado, USA) with a 5 cm-diameter metal tube in October 2019 and October 2024. Two soil cores were taken in each experimental unit, with the first used for soil physicochemical measurements at depths of 0-5, 5-15, and 15-30 cm. The second soil core was used for soil biological measurements collected at 0-5, 5-15, and 15-30 cm. Soil samples for aggregate stability were taken in each plot using a shovel at a depth of 0-5, 5-15, and 15-30 cm. After sampling, soil samples were placed in a cooler and transported to the Department of Agronomy at Kansas State University. Soil samples for physicochemical

measurements were stored at -4°C before processing and analysis. Soil samples for soil microbial properties were stored at -20°C before processing and analysis. The samples for physicochemical measurements were sent for chemical analysis in the Kansas State University Soil Testing Laboratory. Soil samples for aggregate stability were air-dried at room temperature and sieved through an 8 mm sieve before analysis.

Soil Physical and Chemical Measurements

Soil organic carbon (SOC) was determined by a LECO CN828 Carbon/Hydrogen/Nitrogen combustion analyzer (LECO Corporation, St. Joseph, MI, USA 2020), following methodology from Nelson & Sommers (1982). Soil water-stable aggregates were determined using the methodology described by Kemper & Rosenau (1986) and modified by Mikha & Rice (2004). Soil was weighed (100 g) and placed in a Yoder wet-sieving apparatus modified for the recovery of all particle fractions. Each soil sample was separated into four aggregate-size classes (8-2mm, 0-250-2 mm, 0.053-0.25 mm, and 0.02-0.053 mm diameter). Air-dried soil was placed in the top sieve (>2 mm), and water was added to submerge the soil for 10 min with an oscillation time of 10 min, stroke length at 4 cm, and a frequency of $30\text{ cycles min}^{-1}$. After the wet-sieving process, the sieves were poured into tins, and the oscillation container was poured into the finer sieves of 0.053- and 0.02-mm diameter, then into tins. Each particle fraction was dried at 70°C until all water was removed, then weighed to determine the percent aggregate size. Soil aggregates were fractioned into macroaggregates (>2 and 0.25-2 mm) and microaggregates (0.053-0.25 and 0.02-0.053 mm) size classes. Mean weight diameter (MWD) was determined through the sum of the aggregate mass from each sieve multiplied by the mean aperture of adjacent sieves and was expressed in mm.

Soil Biological Measurements

Extracellular Enzyme Activities

Soil potential enzyme activities were measured using the bench-scale colorimetric method described by Tabatabai (2018), however, without the use of toluene (Acosta-Martínez and Tabatabai, 2015). The amount of soil and the volume of solution were reduced by half while maintaining the proportions of soil:solution used in the original assay methodology (Acosta-Martínez & Cotton, 2017; Acosta-Martínez et al., 2019). Individual assays included a carbon-acquiring enzyme (β -glucosidase [β G]), a phosphorus-acquiring enzyme (acid phosphatase [AP]), a sulfur-acquiring enzyme (arylsulfatase [AS]), and a nitrogen-acquiring enzyme (N-acetyl- β -D-glucosaminidase [NAG]). For each respective assay, 0.5g of air-dried soil (sieved at <5mm) was incubated at 37 °C in solution with its appropriate buffer and 0.5 mL of its appropriate substrate at optimal pH for each enzyme for 1 h. For β G, the start buffer was a MUB at pH 6, the substrate was p-Nitrophenol- β -D-glucopyranoside (0.05 M) (Sigma Aldrich® catalog # N70006), and the stop solution was 0.1 M THAM at pH 12. For AP, the starting buffer was a MUB with pH 6.5, the substrate was p-Nitrophenol phosphatase Disodium Salt (0.05 M) (Sigma Aldrich® catalog # 4876), and the stop solution used was 0.5 N NaOH. For AS, the start solution used was 0.5 M Acetate Buffer at pH 5.8, the substrate was p-Nitrophenol sulfate (Sigma Aldrich® catalog # N3877), and the stop solution used was 0.5 N NaOH. Finally, for NAG the start solution used was 0.1 M Acetate buffer at pH 5.5, the substrate used was p-Nitrophenol-N-acetyl- β -D-glucosaminide (0.01 M) (Sigma Aldrich® catalog # N9376), and the stop solution used was 0.5N NaOH. Additionally, 0.5 mL of 0.5M CaCl₂ was also added as stop solution to the four enzymes after incubation. Each assay was performed with two replicates, plus one control, to which

substrate was added after the incubation time. After incubation and addition of stop solutions, the soil suspension was filtered using a Whatman® No. 2v filter, and the filtrate absorbance was measured colorimetrically at 400nm on a Genesys 20 Visible spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA).

Absorbance values from the control samples were subtracted from those obtained for the two repetition samples before the calculation of the amount of *p*-nitrophenol released during the incubation time using a standard calibration curve. The calibration curve was developed using the same methodology as for samples and with standards containing 0, 10, 20, 30, 40, and 50 ug of *p*-nitrophenol in MUB. When color intensity exceeded the highest *p*-nitrophenol standard solution, sample filtrates were diluted at 1:4 (1 mL of filtrate and 3 mL of stop solution) for β G, 1:10 (0.5 mL of filtrate and 4.5 mL of stop solution) for AP, 1:2 (1mL of filtrate and 1 mL of stop solution) for AS, and 1:3 (1 mL of filtrate and 2 mL of stop solution) for NAG. Potential soil enzyme activities were reported as micromoles of *p*-nitrophenol per gram of dry soil per hour.

Phospholipid Fatty Acids (PLFA)

Soil phospholipid fatty acids were estimated following the modified methodology from the White and Ringelbeg method (White et al., 1997; Hsiao et al., 2019). The total lipids were extracted from 5 g of freeze-dried soil using a 2:1:0.8 (v/v) methanol:chloroform:phosphate buffer solution. PLFA were isolated from total lipids through extraction using silicic acid chromatography columns (disposable SUPELCO Supelclean 6 mL columns, J.T. Sigma Aldrich®, St. Louis, Missouri, USA), and further methylated to fatty acid methyl esters (FAME) using a solution of 0.2 M methanolic potassium hydroxide. Resulting FAMEs from each sample

were analyzed using a Thermo Scientific Trace GC-ISQ 7000 (Thermo Fisher Scientific®, Waltham, Massachusetts, USA) equipped with an AI/AS 1310 column (27 cm x 27 cm x 17.7 cm; 12.9 L, Thermo Fisher Scientific®, Waltham, Massachusetts, USA). Internal standard 19:0 fatty acid methyl esters were used to determine concentrations (MilliporeSigma N5377; MilliporeSigma; Burlington, St. Louis, Missouri, USA).

The microbial groups present in the FAME were assigned based on biomarkers. FAME peaks were identified through a comparison with a total of 32 biomarkers, where 26 biomarkers were from a bacterial acid methyl esters mix (BAME; Matreya 1114; Matreya LLC, Pleasant Gap, Pennsylvania, USA) and 6 additional FAMEs custom-made for 10Me16:0 (Cayman Chemical 24823; Cayman Chemical Company; Ann Arbor, Mississippi, USA), 10Me18:0 (Larodan 21-1810; Larodan AB; Solna, Sweden), a17:0 (Matreya 1614; Matreya LCC, Pleasant Gap, Pennsylvania, USA), C16:1:11 (Matreya custom synthesis; Matreya LCC, Pleasant Gap, Pennsylvania, USA), C18:1:11:cis (MilliporeSigma 17264; MilliporeSigma, Burlington, St. Louis, Missouri, USA), and C20:0 (MilliporeSigma 10941; MilliporeSigma; Burlington, St. Louis, Missouri, USA). Resulting fatty acids were grouped into Gram-positive (+) bacteria (i15:0, a15:0, i16:0, i17:0, and a17:0), Gram-negative (-) bacteria (19:0 Δ 9,10; C18:1:11:cis; 17:0 Δ 9,10; C10:0:2-OH, C12:0:3-OH, C14:0:2-OH, C14:0:3-OH; C16:1:0:cis, C16:0:2-OH), actinomycetes (10Me16:0 and 10 Me18:0), arbuscular mycorrhizal fungi (AMF) (C16:1:11), and fungi (C18:2:9,12) (White & Rice, 2009). The abundance of phospholipid fatty acids was reported as nmol of PLFA per gram of dry soil. Total microbial biomass was estimated by the sum of all PLFA biomarkers in microbes with FAME for C11:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, and C20:0. Fungi to bacteria ratio was calculated by dividing the sum of AMF and saprophytic fungi by the sum of Gram(+), Gram(-), and actinomycetes.

Plant Biomass

Shoot samples were taken for biomass determination. Shoot biomass for each crop was collected at the respective harvest stage for grain-crops and at mowing stages for forage/dual-purpose crops. Total biomass accumulation during the period of the study was calculated using the shoot biomass produced by each crop in the system, in addition to root biomass estimations from each respective crop. Root biomass was estimated using root-to-shoot biomass ratios for the crops according to (West et al., 2010). Root-to-shoot biomass ratios used were: 0.2 for winter wheat, 0.15 for soybean, 0.18 for corn, 0.08 for grain sorghum, 0.85 for alfalfa, 0.20 for cowpea, and 0.18 for triticale (Bolinder et al., 1997). The cumulative biomass for each treatment during the study period is described in Table 3.1.

Data Analysis

To assess the treatments and temporal effect on soil health indicators, linear mixed-effect models were used using the *lmer()* function from the *lme4* package in R Studio (version 2024.4.1.748) (R Core Team, 2020). Individual soil health indicators were used as response variables and analyzed by depth. Changes in soil health indicators were calculated between the 5th sampling year (2024) and the baseline year (2019) ($\Delta = 2024 - 2019$), and these values were used as response variables. Treatments (TRT) were considered fixed variables. Random effects were included for the experimental design structure, with replicates (blocks) and plots (ID) nested within replicates to control for repeated measures. The normality of the response variables was tested using Shapiro-Wilk tests, and when not exhibiting normal distributions, response variables were transformed to meet this assumption. Logarithmic transformations were applied to

right-skewed variables, while square root transformations were used for response variables exhibiting moderate skewness. Response variables with acceptable residual distributions were analyzed on their original scale. Model assumptions were evaluated using residual diagnostics. Estimated marginal means were obtained using the *emmeans* package to compare treatment effects on changes in soil health indicators. Post-hoc contrasts were performed using TRTs vs. Control to evaluate differences in changes relative to the control treatment, and pairwise comparisons among treatments were also performed. Because the analysis was based on changes over time, direct comparisons between years were not performed. To control for multiple comparisons, Bonferroni-adjusted *p*-values were used, suitable for one-to-many comparisons. All visualizations were produced using the *ggplot2* package (Wickham, 2016). The statistical significance level was set at $\alpha = 0.05$.

Results

Weather

Mean annual temperatures and precipitation varied among years between 2019 and 2024, capturing a wide range of climatic conditions during the study period (Fig. 3.2). Average annual temperatures ranged approximately from 12 to 15 °C, while cumulative precipitation varied from about 600 to over 1,100 mm. When plotted relative to the 30-year site averages for mean temperature and precipitation, the study years were distributed across all climate quadrants, indicating a substantial interannual variability in both temperature and moisture regimes. The 2019 season was characterized by relatively cool temperatures and high precipitation. In contrast, 2020 and 2023 were closer to the historical site average temperatures, though exhibiting lower cumulative precipitation. The 2021 and 2024 seasons were warmer than the historical average

and received above-average precipitation, and the 2022 season was near the long-term mean temperature, with above-average precipitation.

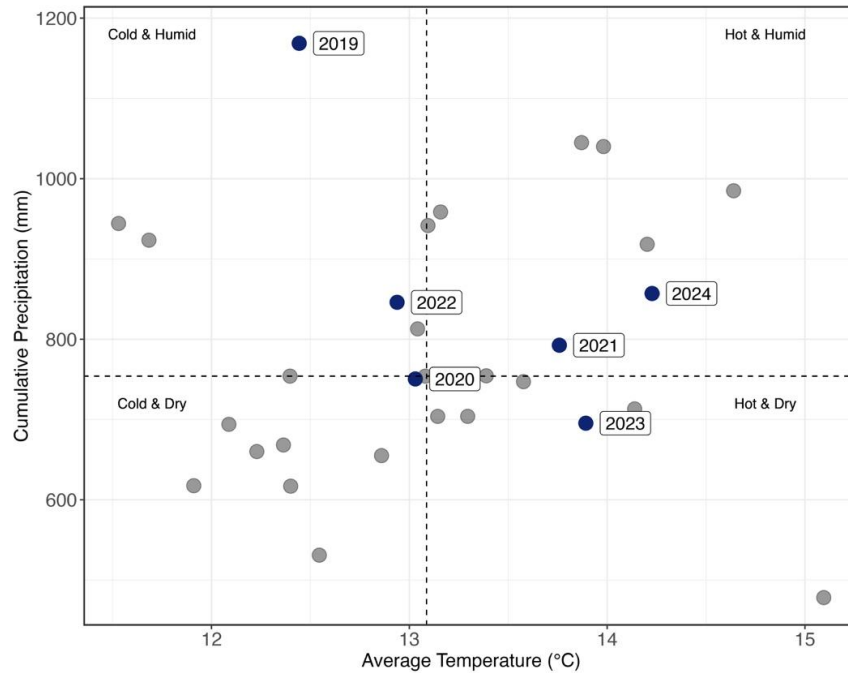


Figure 3.2. Interannual variation in average annual temperature and cumulative precipitation during the study period. Blue points represent individual study years, and gray points indicate the precipitation and temperature means from 1994 to 2025. Dashed vertical and horizontal lines represent the 30-year means for temperature and precipitation, respectively, dividing conditions into cold-humid, cold-dry, hot-humid, and hot-dry climate quadrants.

Biomass production

Cumulative crop biomass varied substantially among seasons and between winter and summer crops (Fig. 3.3). Winter crops (winter wheat, mostly) consistently produced greater total biomass than summer crops across all season-years. In winter seasons, total biomass production ranged from 3.5 to 16 Mg ha⁻¹, with the highest production during the 2019-2020 season and a

general decline in subsequent years. Across all winter seasons, aboveground biomass accumulated the majority of total biomass produced, while belowground biomass contributed a smaller but consistent proportion.

In contrast, summer crop biomass was substantially lower than the biomass produced by winter crops and more variable across years (Fig. 3.3). Total biomass production from the summer crops ranged from 0.5 Mg ha⁻¹ in 2020 to 3 Mg ha⁻¹ in 2022 and 2024. Similar to winter crops, aboveground biomass dominated total summer crop biomass, although the relative contribution of belowground biomass increased in years with higher total production.

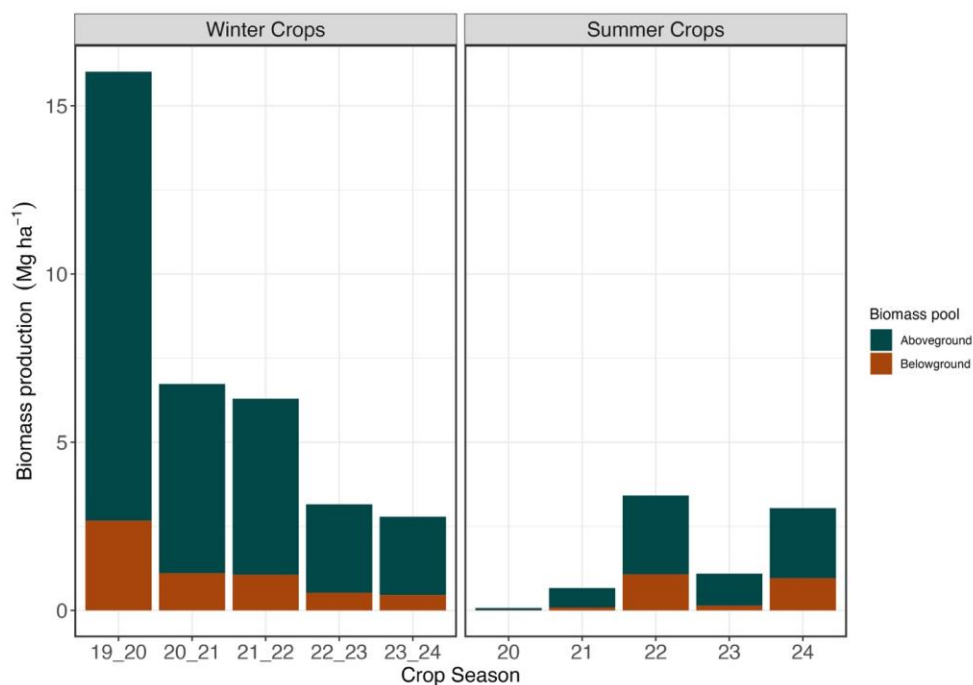


Figure 3.3. Seasonally measured dry aboveground and estimated belowground biomass production by winter and summer crops from 2019 to 2024. Bars represent total biomass partitioned into aboveground and belowground components. Data are shown separately from winter crops (left panel) and summer crops (right panel), highlighting seasonal differences in biomass magnitude patterns.

Cumulative biomass production from 2019 to 2024 differed markedly among treatments (Table 3.1). Grain-purpose treatments (T1, T3, and T4) consistently produced greater (82 %) cumulative aboveground and belowground biomass than the forage-based and dual-purpose treatments (T2, T5, and T6). Total cumulative biomass in grain systems ranged from 200 Mg ha⁻¹y⁻¹ to 250 Mg ha⁻¹y⁻¹ over 5 years, with the intensive cropping system (T4) exhibiting the highest production. In contrast, forage and dual-purpose treatments produced substantially lower total biomass, ranging from 93 Mg ha⁻¹y⁻¹ to 160 Mg ha⁻¹y⁻¹. Moreover, across all treatments, aboveground biomass constituted the largest proportion of total biomass.

Table 3.1. Cumulative observed aboveground and estimated belowground biomass produced by each treatment from 2019 to 2024. T1 : Control; T2 : Adaptative Forage; T3 : Semi-intensive; T4 : Intensive; T5 : Dual-purpose Semi-intensive; T6 : Dual-purpose Intensive.

Treatment	Aboveground	Belowground	Total
	Mg ha ⁻¹		
T1	165.7	33.1	198.8
T2	68.5	24.6	93.1
T3	177.5	34.2	211.7
T4	209.3	39.2	248.5
T5	136.2	26.1	162.3
T6	87.1	37	124.1

Soil physicochemical properties

Soil organic carbon (SOC) ranged from 13 to 19 g C kg⁻¹ across treatments at 0-5 cm depth. Changes in SOC from 2019 to 2024 did not differ among treatments (Fig. 3.4). At 5-15 cm and 15-30 cm depths, SOC did not significantly differ across sampling years and were not

significantly different between treatments and the control (Table B.4 in Appendix B). The proportion of water-stable macroaggregates at 0-5 cm depth was highly variable across treatments, ranging from 20 to 55 g kg⁻¹ soil, and there were no overall significant changes from 2019 to 2024 (Fig. 3.4). Similarly, the mean weight diameter of water-stable aggregates at 0-5 cm depth had substantial variability among treatments but remained relatively stable between 2019 and 2024 (Fig. 3.4). Mean Weight diameter (MWD) values ranged from 0.4 to 1.7 mm, but changes over time were not significantly different among treatments.

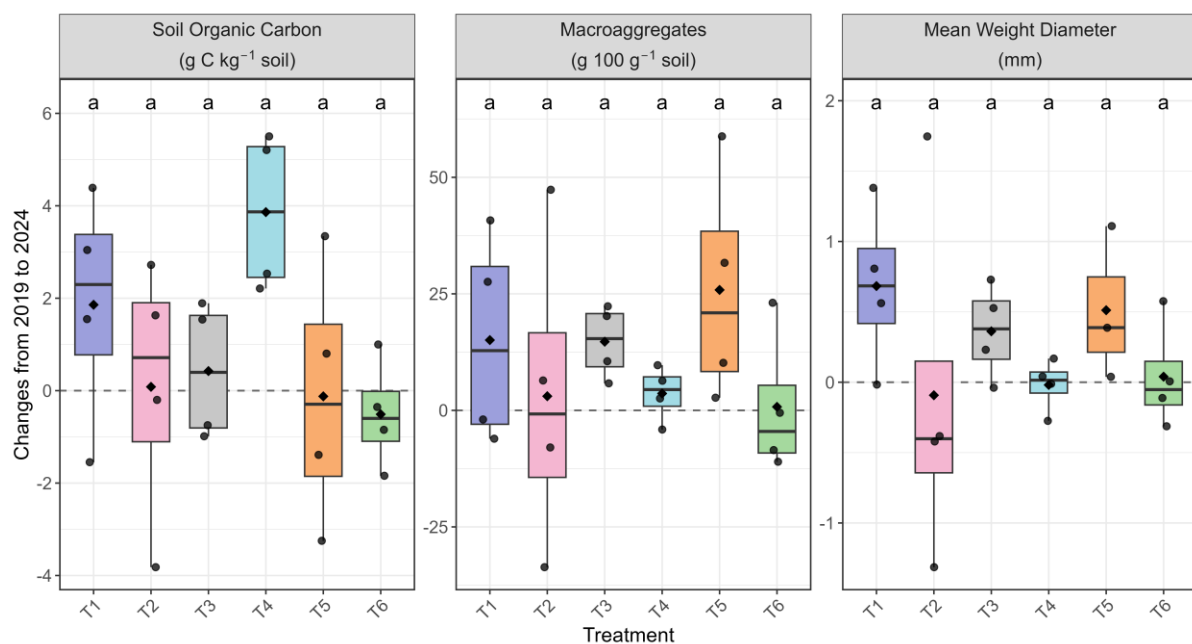


Figure 3.4. Changes from 2019 to 2024 in soil organic carbon (SOC), water-stable macroaggregates, and mean weight diameter (MWD) at 0–5 cm depth across treatments. Values represent treatment-specific changes ($\Delta = 2024 - 2019$). Letters indicate differences among treatments in their rate of change; treatments sharing the same letter are not significantly different ($p > 0.05$). T1 : Control; T2 : Adaptive Forage; T3 : Semi-intensive; T4 : Intensive; T5 : Dual-purpose Semi-intensive; T6 : Dual-purpose Intensive.

Contrasts comparing each treatment to the control (T1) indicated no treatment effect on changes in the separate fractions at 0-5 cm depth (Table 3.2). Although numerical differences were observed among treatments, none of the contrasts were statistically significant. For the >2.0 mm fraction, treatment T4 had a lower change in this aggregate proportion relative to the control; however, this difference was not significant. Likewise, no significant differences were detected for the other water-stable aggregate classes. Overall, changes in aggregate-size distributions at 0-5 cm depth were limited and did not significantly differ among treatments over the study period. Similarly, no overall significant treatment effects on changes in WSA fractions were observed for water-stable aggregates at 5-15 cm and 15-30 cm depth (Table B.3 in Appendix B).

Table 3.2. Contrast estimates of treatment effects on changes (2019-2024) in water-stable aggregates (WSA) fractions at 0-5 cm depth, relative to the control. Positive values indicate greater increases in WSA mass relative to the control from 2019 to 2024, while negative values indicate smaller increases or decreases.

Contrast	WSA > 2.0 mm	WSA 2 – 0.25 mm	WSA 0.25 – 0.053 mm	WSA 0.053 – 0.02 mm
	g kg soil ⁻¹			
	0-5 cm			
T2 – T1	-16.9	4.9	10.6	2.1
T3 – T1	-8.3	8.0	3.8	-1.1
T4 – T1	-15.2	3.7	9.7	2.9
T5 – T1	- 4.3	1.2	-1.4	3.2
T6 – T1	-13.1	-1.3	13.4	3.6

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

Extracellular Enzyme Activities

Soil β -glucosidase (β G) activity had substantial variability across treatments at 0-5 cm depth, with changes from 2019 to 2024 ranging approximately from 30 to 150 $\mu\text{g PNP g soil}^{-1} \text{hr}^{-1}$ across the treatments (Fig. 3.5). Although numerical increases were observed throughout the study period, particularly in treatments T3 (Semi-Intensive) and T6 (DP Intensive), changes in β G activity did not significantly differ among treatments. Soil acid phosphatase (AP) activity had even greater variability at 0-5 cm depth, with changes ranging from decreases of approximately 400 $\mu\text{g PNP g soil}^{-1} \text{hr}^{-1}$ to increases exceeding 600 $\mu\text{g PNP g soil}^{-1} \text{hr}^{-1}$. However, despite this wide range, changes in soil AP activity from 2019 to 2024 did not significantly differ among treatments. At 5-15 cm depth, changes in β G activity relative to the control ranged from approximately 8 to 40 $\mu\text{g PNP g soil}^{-1} \text{hr}^{-1}$, while changes in soil AP activity ranged from -343 to 143 $\mu\text{g PNP g soil}^{-1} \text{hr}^{-1}$ (Table B.6 on Appendix B). At 15-30 cm depth, soil β G and AP activities did not display significant changes from 2019 to 2024.

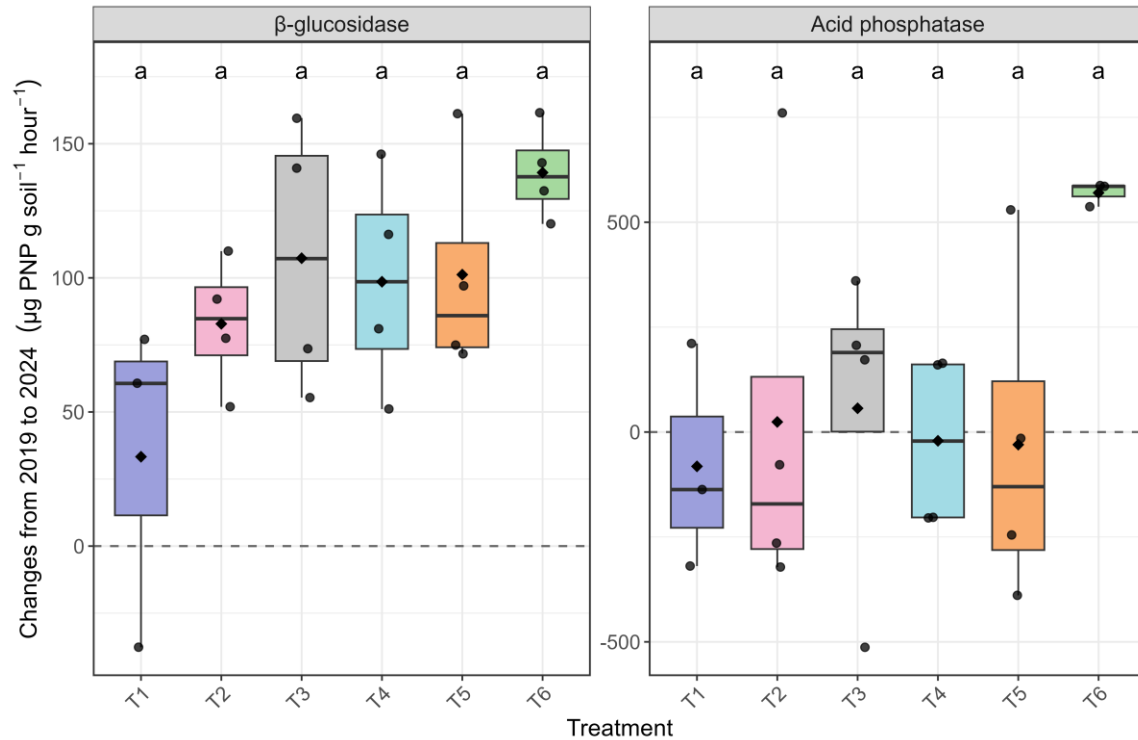


Figure 3.5. Changes from 2019 to 2024 in potential β G activity and potential AP activity at 0–5 cm depth across treatments. Values represent treatment-specific changes ($\Delta = 2024 - 2019$).

Letters indicate differences among treatments in their rate of change; treatments sharing the same letter are not significantly different ($p > 0.05$). T1 : Control; T2 : Adaptive Forage; T3 : Semi-intensive; T4 : Intensive; T5 : Dual-purpose Semi-intensive; T6 : Dual-purpose Intensive.

Arylsulfatase (AS) activity had consistent positive changes across treatments from 2019 to 2024 at 0-5 cm depth (Fig. 3.6), with increases ranging from 30 to 100 $\mu\text{g PNP g soil hour}^{-1}$. Despite this overall increase, changes in soil AS activity had significant differences between treatments, with treatment T3 (Semi-Intensive) showing greater increases when compared to treatments T2 (Adaptive Forage) and T4 (Intensive). Soil NAG activity had more variable responses at 0-5 cm depth, with changes from 2019 to 2024 ranging from approximately -120 to 90 $\mu\text{g PNP g soil hour}^{-1}$. Although some treatments (T2, T3, and T5) had positive changes, while

T6 had negative changes, these differences were not statistically significant among treatments. At 5-15 cm depth, changes in soil AS activity relative to the control ranged from approximately 6 to 21 $\mu\text{g PNP g soil hour}^{-1}$, with significant positive contrasts observed for T2 and T3 (Table B.6 on Appendix B). In contrast, changes in NAG activity at 5-15 cm depth were small and did not significantly differ among treatments. At 15-30 cm depth, changes in soil AS activity were minimal (-2 to 8 $\mu\text{g PNP g soil hour}^{-1}$) and did not significantly differ between treatments. Changes in NAG activity ranged from -51 to 29 $\mu\text{g PNP g soil hour}^{-1}$, but no significant treatment differences in changes were observed (Table B.6 on Appendix B).

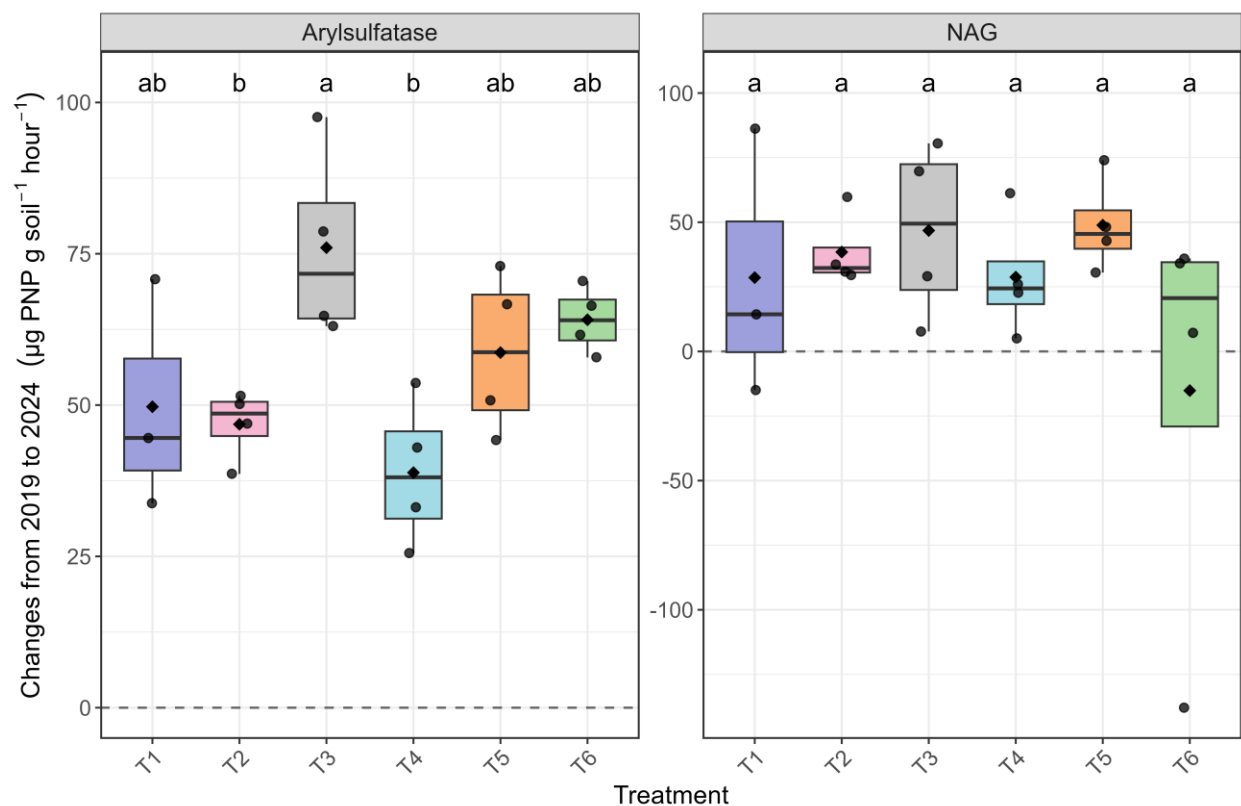


Figure 3.6. Changes from 2019 to 2024 in potential AS activity and potential NAG activity at 0–5 cm depth across treatments. Values represent treatment-specific changes ($\Delta = 2024 - 2019$). Letters indicate differences among treatments in their rate of change; treatments sharing the same

letter are not significantly different ($p > 0.05$). T1 : Control; T2 : Adaptive Forage; T3 : Semi-intensive; T4 : Intensive; T5 : Dual-purpose Semi-intensive; T6 : Dual-purpose Intensive.

Soil Microbial Community

Soil total microbial biomass (TMB), estimated through phospholipid fatty acid (PLFA) analysis, had large positive changes from 2019 to 2024 across treatments at 0-5 cm depth. Increases in soil TMB ranged from approximately 140 to 450 nmol PLFA g soil⁻¹ (Fig. 3.7). However, despite this substantial increase, changes in soil TMB did not significantly differ between treatments. Soil total bacteria (TB) also had consistent positive changes across treatments from 2019 to 2024, ranging from 80 to 350 nmol PLFA g soil⁻¹. Although differences were observed between treatments, particularly a higher increase in treatment T6, these changes were not significantly different between treatments at 0-5 cm depth. Similarly, soil total fungal biomass (TF) had positive changes from 2019 to 2024, ranging from 5 to 25 nmol PLFA g soil⁻¹. While lower increases in soil TF were observed in treatment T2, and higher increases were observed in treatment T5, changes in soil TF did not significantly differ between treatments at 0-5 cm depth. Moreover, no significant differences in changes from 2019 to 2024 were observed for soil TMB, TB, and TF at 5-15 and 15-30 cm depth (Table B.4 on Appendix B).

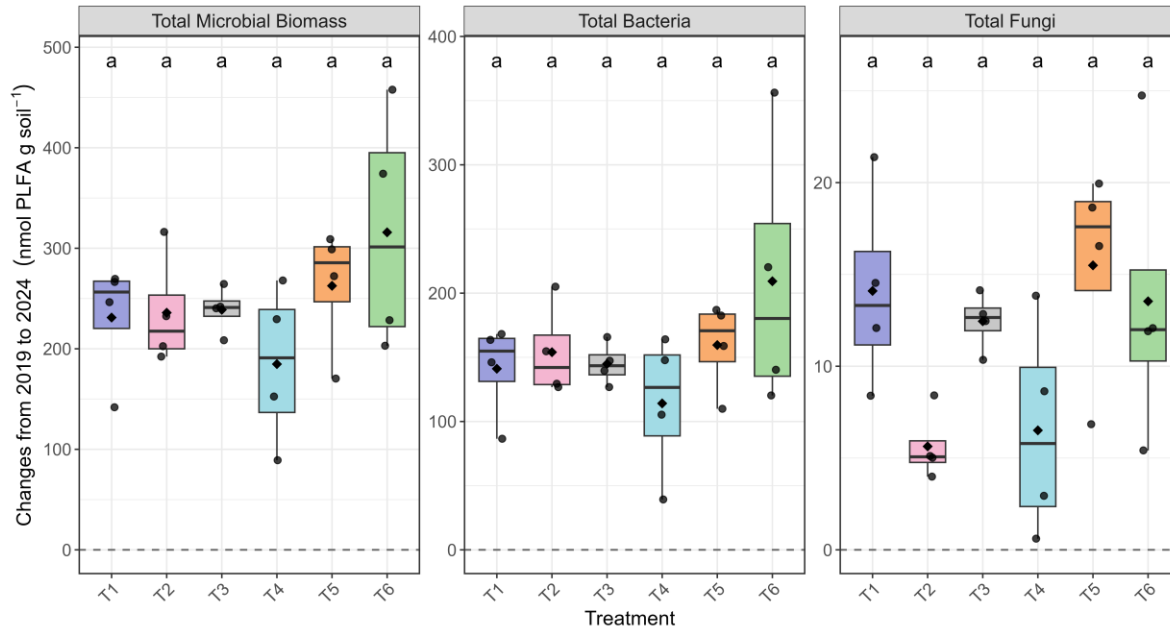


Figure 3.7. Changes from 2019 to 2024 in soil total microbial biomass, total bacterial biomass, and total fungal biomass at 0–5 cm depth across treatments. Values represent treatment-specific changes ($\Delta = 2024 - 2019$). Letters indicate differences among treatments in their rate of change; treatments sharing the same letter are not significantly different ($p > 0.05$). T1 : Control; T2 : Adaptive Forage; T3 : Semi-intensive; T4 : Intensive; T5 : Dual-purpose Semi-intensive; T6 : Dual-purpose Intensive.

Looking further into the soil microbial community and abundance, results from 0-5 cm depth had positive changes observed for gram-positive bacteria, gram-negative bacteria, and actinomycetes, while AMF had variable responses, including some decreases in selected treatments (Table 3.5). changes in gram-positive bacteria ranged from approximately -13 to 11 nmol PLFA g soil⁻¹, gram-negative bacteria from -12 to 56 nmol PLFA g soil⁻¹, and actinomycetes from -2 to 3 nmol PLFA g soil⁻¹. Changes in soil AMF were generally small, ranging from -0.1 to 1.3 nmol PLFA g soil⁻¹. Despite these differences, changes in soil microbial

community composition were not significantly different between treatments, with the exception of a significant increase in treatment T6. At 5-15 cm depth, changes in gram-positive bacteria ranged from -5 to 11 nmol PLFA g soil⁻¹, gram-negative bacteria from -21 to 32 nmol PLFA g soil⁻¹, actinomycetes from -0.5 to 2 nmol PLFA g soil⁻¹, and AMF from -0.2 to 0.5 nmol PLFA g soil⁻¹ (Table B.5 on Appendix B). At 15-30 cm depth, changes in microbial community composition ranged from -2 to 3 nmol PLFA g soil⁻¹ for gram-positive bacteria, from 3 to 26 nmol PLFA g soil⁻¹ for gram-negative bacteria, -0.5 to 0.1 nmol PLFA g soil⁻¹ for actinomycetes, and -0.2 to 1.3 nmol PLFA g soil⁻¹ for AMF. No significant differences in changes were observed between treatments at 5-15 and 15-30 cm depth.

Table 3.3. Contrast estimates of treatment effects on changes (2019-2024) in soil organic carbon and soil microbial community composition at 0-5 cm depth. relative to the control. Positive values indicate greater increases in greater Gram +, Gram -, Actinomycetes, and AMF biomass from 2019 to 2024, while negative values indicate lower Gram +, Gram -, Actinomycetes, and AMF biomass from 2019 to 2024. Gram +: gram-positive bacteria; Gram -: gram-negative bacteria; AMF: arbuscular mycorrhizal fungi.

Contrast	Gram +	Gram -	Actinomycetes	AMF
	nmol PLFA g soil ⁻¹			
	0-5 cm			
T2 – T1	5.1	5.2	2.6	-0.1
T3 – T1	-1.2	5.0	-0.1	0.6
T4 – T1	-12.8	-12.1	-2.1	-0.1
T4 – T1	8.1	8.1	2.2	0.4
T6 – T1	11.0	55.5	1.6	1.3*

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

Discussion

The intensification and diversification of winter wheat-based systems through the replacement of summer fallow periods with grain, forage, or dual-purpose crops is promoted as a strategy to improve soil health by increasing plant carbon inputs, stimulating microbial activity, and enhancing soil structure (Bourgault et al., 2022; Garba et al., 2022; Obour et al., 2021; Thapa et al., 2022; Zhu et al., 2024). In this study, soil health indicators, including soil organic carbon (SOC), large water-stable aggregates, microbial biomass abundance, and selected extracellular enzyme activities, had mainly positive changes from 2019 to 2024, although differences among cropping systems were mostly not statistically significant. These uniform improvements across treatments likely reflect the strong influence of the management transition from conventional tillage to continuous no-tillage initiated in 2019. The absence of soil disturbance enhances aggregate stability, reduces SOC exposure to decomposition, and provides more stable habitats for microbial communities, resulting in rapid improvements in soil biological and structural properties (Karlen et al., 2013; Six et al., 2000). As a result, these positive temporal shifts, shared across treatments, can emerge during the early phase of no-tillage adoption and potentially mask treatment-level differences attributable to increased crop intensity or diversity alone. This interpretation is supported by the consistent positive changes in microbial biomass and enzyme activity across treatments revealed in this study. Such early responses are typically associated with a reassembly of the microbial community toward copiotrophic taxa capable of fast growth and high carbon turnover rates under improved habitat conditions (Degrune et al., 2016),

However, the effectiveness of cropping systems intensification and diversification is strongly constrained by water availability, crop productivity, and legacy effects, especially in

regions such as the U.S. Great Plains (Garba et al., 2022). In this sense, improvements in soil health through these strategies may be dependent on whether the added crops will generate enough above- and below-ground biomass inputs beyond what is achieved under winter wheat-summer fallow management, and whether these inputs persist long enough to positively change soil health indicators. Studies in the U.S. Central Great Plains and other semi-arid regions implied that the addition of cover crops and short-season summer crops is frequently constrained by limited water availability and high evaporative demand, thus resulting in highly variable and often low biomass production (Blanco-Canqui et al., 2013; Nielsen et al., 2015; Schlegel & Havlin, 1997). Likewise, soil responses to these conditions are thereby dependent on these constraints, with effects towards the negative side when biomass production is insufficient (Blanco-Canqui & Lal, 2008b; Nielsen & Vigil, 2010; Unger & Vigil, 1998).

Soil Organic Carbon

SOC accumulation and stabilization are typically slow in rainfed conditions, constrained by limited carbon inputs and the need for physical protection within aggregates (Prairie et al., 2023; Zhou et al., 2024). In this study, changes in SOC did not differ among treatments. At the 0–5 cm depth, SOC increased on average by 14.5% in the grain-based systems (11.8% on T1, 2.8% on T3, and 29% on T4). In contrast, SOC displayed an overall decline of 1.4% in forage and dual-purpose systems (0.5 % of increase on T2, and a decrease of 0.85% on T5 and 3.8% on T6) over the same period. These contrasting trajectories suggest that intensification through the replacement of summer fallow did not uniformly enhance SOC across cropping systems within the five-year time frame. In forage-based and dual-purpose systems, partial or complete removal of aboveground biomass reduces surface residue returns, which can shift the relative importance

of belowground inputs for SOC formation and stabilization (Yang et al., 2023; Stanley et al., 2024; Li et al., 2025). However, root-derived carbon alone may be insufficient to offset reduced surface residue returns in semiarid environments, limiting or impeding SOC accumulation over short time frames (Kong & Six, 2010; Hirte et al., 2018), a trend observed in this study. The magnitude of SOC change observed, however, is consistent with previous studies in the U.S. Great Plains, where replacing fallow with cover crops or summer crops often results in small (5-10%) or inconsistent SOC responses over short time frames, particularly in near-surface soils and under inconsistent precipitation regimes (Blanco-Canqui et al., 2013; Nielsen et al., 2015). While SOC increased by 6.4% at the overall systems level, the additional carbon inputs associated with more diversified and intensified rotations were likely insufficient to exceed the threshold required for clear treatment-level differences after five years.

These findings align with global and regional syntheses demonstrating that SOC accumulation in rainfed agroecosystems is typically slow and strongly constrained by productivity, with responses to changes in tillage and crop rotation being both time-dependent and contingent on biomass inputs (West & Post, 2002; Conant et al., 2007; Paustian et al., 2016). Mechanistically, no-tillage enhances SOC preservation by reducing aggregate disruption and increasing the physical protection of organic matter within macro- and microaggregates (Six et al., 2002; Six et al., 2004; Denef et al., 2007). However, recent studies also highlight that SOC accrual is dependent on microbial carbon use efficiency and the conversion of residue inputs into mineral-associated organic matter, processes that do not respond rapidly to changes in cropping systems alone (Cotrufo et al., 2013; Kallenbach et al., 2016; Lavallee et al., 2020). In the present study, the transition from conventional tillage to continuous no-tillage in 2019 likely acted as a dominant, shared driver of SOC change across all treatments, potentially masking smaller

differences attributable to crop sequence or rotation intensity (Obour et al., 2021). Moreover, beyond total biomass production, differences in residue quality and carbon allocation pathways may have further influenced SOC responses among treatments. Residues with higher C:N ratios, such as corn and wheat residue, can slow decomposition and nutrient cycling, whereas more labile residues, such as soybean residue, decompose rapidly and contribute to SOC stabilization primarily through microbial processing pathways (Cotrufo et al., 2013). Furthermore, emerging evidence also emphasizes the role of microbial necromass as a precursor of mineral-associated organic matter (Liang et al., 2019), and the changes in microbial biomass across the study years should therefore represent transient pools that have not yet been transformed into stabilized carbon fractions. This interpretation aligns with our observed microbial biomass increase across all treatments, supporting the hypothesis that soil biological recovery precedes measurable SOC accrual and further stabilization.

Water-stable Aggregates

Soil aggregate stability is strongly influenced by both soil disturbance and organic inputs and is often expected to improve when following the adoption of no-tillage systems and diversified crop rotations (Tisdall & Oades, 1982; Six et al., 2004; Bronick & Lal, 2005). In this study, changes in water-stable aggregate fractions did not differ among treatments from 2019 to 2024. Furthermore, foundational studies describe soil aggregate formation as a hierarchical process in which macroaggregates formation depends on sustained inputs or particulate organic matter (POM) and microbial binding agents, while microaggregates formation is slower and within macroaggregates, contributing to long-term stabilization (Tisdall & Oades, 1982; Six et al., 2004; Bronick & Lal, 2005). Aggregate stabilization in no-tillage systems is increasingly

attributed to fungal hyphae and microbial extracellular polysaccharides that act as biological binding agents, integrating organic matter into physical structures (Rillig et al, 2015; Lehman et al, 2020). However, recent studies further show that aggregate turnover rates and mineral associations can delay significant treatment effects, even when management changes are substantial (Lavallee et al., 2020; Angst et al., 2021).

In our study, the replacement of fallow periods with summer crops produced mixed aggregate responses, mainly due to the assumption that organic inputs provided especially by the summer crops were constrained by water availability. This is consistent with studies in the U.S. Great Plains reporting variable changes in macroaggregate fractions in intensified wheat-based cropping systems, particularly when crop biomass production is inconsistent (Mikha and Rice, 2004; Blanco-Canqui and Lal, 2008). Additionally, the forage-based and dual-purpose treatments may have induced an additional constraint on aggregate formation due to partial biomass removal. Through this biomass removal, the reduction of surface residue limits the supply of POM required for aggregate formation and stabilization (Poeplau et al., 2020; Stanley et al., 2024), as previous studies in dryland systems imply that macroaggregate formation and stability can be reduced even under no-tillage management when organic inputs are exported (Blanco-Canqui et al., 2013; Ruis et al., 2019). In this context, the muted responses of micro- and macroaggregates in the forage-based treatments of this study likely reflect a balance between reduced soil disturbance and constrained organic inputs, also highlighting that soil structural improvements lag biological and chemical improvements.

Extracellular Enzyme Activities

In the context of extracellular enzyme activities, responses from β G, AP, AS, and NAG activities to cropping systems intensification and diversification were mostly neutral relative to the control treatment T1 after five years. This observed pattern indicates that soil enzymatic responses were also driven by system-wide factors, such as no-tillage adoption, rather than treatment-level differences in crop intensity and diversity. Similar responses have been reported in the transition from conventional tillage to no-till systems, where the lack of soil disturbance, greater surface residue retention, and improved microbial habitat were able to promote enhanced extracellular activity responses, especially in surface soils (Bandick & Dick, 1999; Franzluebbers, 2002; Burns et al., 2013). The substantial positive changes in β G and AS activities observed across treatments (71% for β G and 124% for AS) suggested an enhanced microbial processing of labile soil carbon and sulfur pools over time, as these extracellular enzymes are closely linked to the microbial access to organic substrates and are among the most responsive indicators to changes in management (Bandick and Dick, 1999; Acosta-Martínez et al., 2007; Sinsabaugh et al., 2008; Sapsford and Dickie, 2023). However, these responses may be constrained by the availability of labile substrates and by soil moisture dynamics, especially in dryland/rainfed systems (Calderón et al., 2016; Mikha et al., 2006).

Responses from AP and NAG activities, which reflect microbial demand for phosphorus and nitrogen, had a similar but not consistent pattern as β G and AS. From an enzyme stoichiometry perspective, the observed increase in β G relative to NAG and AP activities indicates a shift toward microbial carbon acquisition strategies, consistent with transient carbon limitations following the transition from a conventional tillage to a no-tillage system (Sinsabaugh et al., 2013). In this sense, enzyme stoichiometry theory predicts that such adjustments reflect the

capacity of microbial communities to rebalance C-, N-, and P-acquiring enzyme production in response to altered organic matter inputs and nutrient availability (Sinsabaugh et al., 2008). In our study, these changes may be addressed by increases in soil microbial community and activity over time as a response to no-tillage adoption after 2019. Conversely, previous studies reported moderate responses for AP and NAG in dryland systems, unless enough substrate is provided (Acosta-Martínez et al., 2007). In these systems, aboveground biomass removal likely further constrained enzymatic responses through the reduction of surface residue that typically stimulates microbial activity and enzyme production (Sapsford & Dickie, 2023). Surface residue retention is a key factor controlling enzyme activity and stability in surface soils, where partial or complete biomass removal has reduced enzymatic activity (Stroo & Jencks, 1982; Lehman et al., 2015). This pattern was observed in this study, where AP and NAG activities in forage and dual-purpose systems remained lower relative to grain-based rotations. Therefore, the incremental effects of replacing summer fallow with summer crops were likely constrained by summer water availability in some years, variable biomass production, and residue removal in forage and dual-purpose systems.

Soil Microbial Community Biomass and Abundance

Soil microbial biomass had large positive changes from 2019 to 2024 across all treatments, suggesting that these temporal responses in the soil microbial community were primarily driven by system-wide changes rather than differences in crop intensity and diversity. This observed pattern is consistent with previous studies, implying that soil microbial communities respond positively to reductions in soil disturbance and improvements in surface soil habitat after the conversion from conventional tillage to no-tillage (Franzluebbers et al.,

1995; Doran et al., 1998; Chen et al., 2020). In rainfed systems, soil microbial dynamics are strongly regulated by interannual climate variability and nutrient availability (Acosta-Martínez et al., 2007; Schlöter et al., 2018), and the responses to crop intensification and diversification are highly variable and often constrained by resource limitations (Mikha et al., 2006; Calderón et al., 2016). Bacterial and fungal communities had similar temporal trends, increasing from 2019 to 2024, with no significant treatment differences.

Soil bacteria are often closely linked to labile carbon inputs and short-term changes in residue availability, responding rapidly to improved surface soil conditions under no-tillage systems (Franzluebbers et al., 1995; Bossio et al., 2006; Creamer et al., 2016). The transition to continuous no-till likely enhanced soil bacterial habitat and carbon accessibility across all treatments, contributing to the observed increase (456% from 2019 to 2024). However, in dryland systems, treatment-level effects in soil bacteria may be constrained by limited soil moisture and residue inputs (Carpenter-Boggs et al., 2003; Mikha et al., 2006; Helgason et al., 2010), as well as by the functional redundancy capacity of bacterial communities to rapidly adjust to disturbance reduction irrespective of crop sequence differences (Carpenter-Boggs et al., 2003; Helgason et al., 2010). Similarly, soil fungal biomass increased over time (403% from 2019 to 2024), but exhibited minimal treatment differentiation after five years. Fungal communities are generally more sensitive to tillage than bacteria due to the vulnerability of hyphal networks to physical disturbance. Long-term no-tillage systems often promote fungal recovery as well as greater fungal contribution to microbial biomass (Six et al., 2006; Vries et al., 2012; Chen et al., 2020). Under this assumption, the transition to the no-tillage system in 2019 likely created favorable conditions for fungal growth across all treatments. Conversely, forage removal in the forage and dual-purpose systems may have limited resources that promote fungal

dominance and hyphal development, thus reducing the potential for significant treatment differences. Previous studies have reported that the sustained increase of soil fungal biomass and fungi-to-bacteria ratios is modulated by continuous residue inputs, which may not be achieved in situations of water limitation or aboveground biomass removal (Bailey et al., 2002; Nivelle et al., 2016). Taken together, the observed results in soil microbial community biomass and composition in this study suggested that the responses were mainly governed by the absence of soil disturbance and climatic constraints, rather than differences in cropping systems in the short term (Buyer et al., 2010; Schlöter et al., 2018).

Overall, this study demonstrated that after five years of the implementation of more intensified and diversified winter wheat-based cropping systems through the replacement of summer fallow by either grain, forage, or dual-purpose crops, did not yield clear treatment-level improvements in key soil health indicators relative to a winter-wheat – summer fallow control treatment. Some soil health indicators, such as SOC, water-stable aggregates, soil microbial biomass abundance and composition, and extracellular enzyme activities generally had consistent positive changes from 2019 to 2024. These changes were largely consistent across all treatments. These results reflect a dominant response to the management practice change that started in 2019, rather than any treatment-specific effects after 5 years of implementation. The most coherent explanation for the observed responses in this study is the strong legacy influence of the transition from a conventional tillage to a no-tillage system, which likely promoted the observed improvements in soil health in surface soil structure, microbial community and activity, as well as biochemical function. These, in turn, may have overshadowed more subtle differences that could arise from different, more intense, and diverse cropping systems. At the same time, biomass removal from forage-based and dual-purpose treatments may have further limited

significant differences between the treatments assessed in this study, particularly for soil health indicators linked to SOC stabilization and aggregate formation. Despite these overall improvements, the absence of a stronger treatment differentiation after 5 years highlights some important limitations in this study. The five-year assessment period may be insufficient to capture significant differences between cropping systems, especially following a major shift in soil disturbance regime. Evidence from long-term no-tillage systems within the Kansas Soil Health Network indicated that measurable improvements in soil health indicators following the introduction of cover crops were detected within a similar five-year timeframe, especially when soils have already been managed under no-tillage for over a decade (Pires, 2023). In addition, the functional redundancy within soil microbial communities, the slow aggregate turnover, and the strong influence of climatic variability in rainfed systems likely constrained treatment differences in this study. Future research should extend this type of soil health assessment beyond the early no-tillage recovery phase, incorporate fraction-specific measurements of soil organic carbon, and link microbial function traits, potential extracellular enzyme activities, and nutrient cycling dynamics to SOC stabilization pathways. Such integrated, long-term approaches will be essential for better capturing cropping systems responses and for determining whether intensified and diversified systems can deliver measurable soil health benefits under rainfed winter wheat-based production systems.

Conclusion

This study evaluated whether increasing cropping systems intensity and diversity through the replacement of summer fallow periods with grain, forage, or dual-purpose crops would result

in measurable improvements in soil health indicators under rainfed, no-tillage conditions in a winter-wheat system in Kansas. Across all treatments, substantial increases in measured soil health indicators were observed between the baseline year (2019) and the fifth year (2024), including increases in soil microbial biomass, extracellular enzyme activities, and selected physical indicators in surface soil (0-5 cm depth). However, these improvements were largely consistent across all treatments evaluated, including the control, and did not translate into clear or consistent treatment-level differences attributable solely to increased cropping systems intensity and diversity. These results indicate that the dominant driver of soil health changes across the evaluated years was the transition from conventional tillage to no-tillage, which exerted a legacy effect across all the cropping systems evaluated. Despite the substantial difference in cumulative biomass production among treatments, soil organic carbon and aggregate stability exhibited limited response to the intensity of cropping systems after five years. Biological soil health indicators responded more rapidly than chemical and physical indicators. Overall, these findings indicate that cropping systems intensification and diversification do not necessarily provide short-term soil health benefits beyond those achieved through the adoption of continuous no-tillage.

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Chapter 4 - Understanding crop yields under regenerative agriculture practices in Kansas

Abstract

The adoption of regenerative agriculture practices, such as cover cropping, has been widely promoted as a strategy to improve soil health and enhance the sustainability of cropping systems. Although numerous studies report positive soil health responses to cover crops, uncertainty remains regarding whether these improvements translate into consistent crop yield responses across diverse environments. The objective of this study was to assess the effects of cover crop adoption on crop yields and yield stability across contrasting environments in Kansas. A multi-year, three-location on-farm research network was used to evaluate corn, soybean, and winter wheat grown with cover crop and no-cover treatments in no-tillage systems. Chemical and biological soil health indicators were measured to characterize soil health responses to cover cropping. Crop yield responses were analyzed using linear mixed-effects models, precipitation-based analysis, and Finlay-Wilkinson regressions to quantify yield adaptability and stability across environments. Cover crops adoption improved soil health indicators across locations; however, no differences in absolute crop yields were observed across years, treatments, and locations. Yield variability was primarily driven by environmental conditions, rather than by cover crop use. Stability analysis indicated proportional yield responses for corn and wheat, while soybean exhibited evidence of potential yield buffering under less favorable conditions. These findings indicate that the primary agronomic value of cover crops lies in soil health improvements rather than enhancing short-term yield potential. Neutral responses in crop yield,

when combined with improved soil function, may support more resilient and resource-efficient cropping systems.

Introduction

Conventional agriculture has been characterized by decades of monoculture, soil tillage, and heavy reliance on synthetic inputs, and has contributed to a range of environmental issues, soil degradation, biodiversity losses, and greenhouse gas emissions (Saliu et al., 2023; Foley et al., 2011; Tilman et al., 2011). During the past 50 years, the advancements in agricultural technology, allied with the constantly growing world population, have significantly increased the strain on the world's soil resources (Panda et al., 2024). These processes are particularly concerning in areas characterized by high evaporative demand (Dewes et al., 2017) and limited annual average precipitation (Deines et al., 2019), such as the U.S. Great Plains (Massigoge et al., 2024). The widespread adoption of intensive tillage and often simplified crop rotations has led to significant declines in vital soil biological, chemical, and physical functions (Lal, 2016), which, when combined with limited water availability, pose a significant challenge to crop growth and production in this region. More recently, the adoption of regenerative agriculture practices, such as no-tillage (NT) and cover cropping, allowed agricultural systems to be more sustainable due to increases in soil organic carbon (SOC) and fertility (Obour et al., 2021), and increases in soil water infiltration, storage, and availability (Hansen et al., 2012; Peterson et al., 2020).

In this context, regenerative agriculture practices have gained prominence as a framework for enhancing soil function through minimized or absent soil disturbance, diversified crop

rotations, and permanent soil cover. Among these key principles, cover cropping (CC) is as a widely recognized practice for replacing fallow periods between cash crops in crop rotations, while improving soil structure, increasing SOC inputs, and stimulating soil microbial activity and nutrient cycling (Blanco-Canqui et al., 2015; Bowles et al., 2020). Additionally, cover crops have the potential to enhance water infiltration, reduce evaporative losses, and thus improve water storage and availability dynamics across variable climatic conditions (Abdalla et al., 2019; Basche & DeLonge, 2017). Therefore, cover cropping practices provide physical protection against erosive losses, promote additional biomass input to soil ecosystems, stimulate soil microbial activity and function, and enhance nutrient cycling (Blanco-Canqui et al., 2012; Blanco-Canqui & Ruis, 2020; Saba & Christy, 2021). However, despite the benefits of cover cropping for soil health, its capacity to translate these improvements into crop yield gains remains variable across environments, management systems, and time scales (Marcillo & Miguez, 2017; Nielsen et al., 2015; Tautges et al., 2016). This variability encompasses different precipitation regimes, soil characteristics, and management decisions such as cover crop species selection across regions, thus influencing the balance between soil health gains and crop yield trade-offs (Holman et al., 2018; Liebigh et al., 2019). These complexities highlight, therefore, the need for evaluations not only to understand how cover crops impact soil functioning, but also how these changes interact with crop performance across contrasting environments (Basche & DeLonge, 2017; Schipanski et al., 2014).

The impact of cover crop use on crop yields may be either positive or negative (Blanco-Canqui et al., 2012; Olson et al., 2010) and significantly context-dependent. Crop yield benefits from under-cover cropping management are often associated with improved soil health, which directly impacts water infiltration, storage, and availability (Wang et al., 2021). Moreover,

legume cover crops can supply a significant amount of nitrogen to subsequent cash crops, often reducing rates of synthetic nitrogen fertilizer applied (Schomberg et al., 2007). These changes, reportedly induced by cover crop use, have the potential to concomitantly improve subsequent crop yields, however, short-term yield reductions have also been documented (Cassman et al., 2025; Deines et al., 2023). In the U.S Great Plains context, especially in dryland systems, Nielsen et al. (2016) and Garba et al. (2022) reported winter wheat yield penalties when following spring-planted cover crops, mainly due to soil water use during the CC phase of the rotation in low-rainfall environments. Conversely, studies encompassing cover crop mixtures in corn – soybean – wheat rotations revealed more stable yields under low C:N ratio cover crop biomass, and corn yield penalties under high C:N ratio cover crop biomass (Hunter et al., 2019; Martinez-Feria et al., 2016). Therefore, these effects may vary considerably in response to weather conditions, management practices, crop rotations, cover crop species utilized, cover crop biomass quantity and quality, and termination time (Deines et al., 2023). These assumptions suggest that crop yield outcomes under cover crop management must be interpreted within specific environmental and management contexts rather than assumed to be universally positive or negative.

Despite intensive research on the soil health benefits of cover crops, a major knowledge gap remains in understanding how these improvements manifest in cropping systems across variable environments. Much of the existing literature is derived from controlled research stations, leaving uncertainties about the extent to which cover crop benefits on soil health translate into crop yield outcomes under climatic variability, different crop rotations, and soil heterogeneity. Furthermore, few studies simultaneously evaluate soil health indicators and crop yields over multiple years and locations, often limiting the ability to identify consistent trends

and pathways through which cover crops influence crop yield (Reckling et al., 2021; van Es & Karlen, 2019; Wood & Bowman, 2021). To address these gaps, this study uses a multi-year, three-location on-farm research network to understand how cover crops affect soil health and crop yields across diverse environments. The objectives of this study were to (i) quantify the effects of cover crops on crop yields and (ii) determine the effect of CC on yield stability across the environments. We hypothesized that cover crop adoption will positively affect crop yields and result in more stable crop yields, especially in environments with low water limitation for crop growth. Soil health indicators were assessed in this study and are reported for each site's characterization. Soil total microbial biomass (TMB), total bacteria (TB), and total fungi (TF) were reported as indicators of soil microbial community biomass and composition; soil extracellular enzyme activities were reported as indicators of soil microbial activity. Other soil physiochemical characteristics reported in this study include soil organic carbon (SOC), pH, and water-stable aggregates. Crop yields include corn, soybean, and winter wheat.

Materials and Methods

Study Site

This study was conducted at three on-farm research sites across Kansas as part of the Kansas Soil Health Network, including Beloit, KS, Bucyrus, KS, and Solomon, KS. The study was conducted in Bucyrus from 2018 to 2025, and from 2019 to 2025 in Beloit and Solomon. The coordinates, elevation, average annual precipitation, average annual temperature, and soil taxonomy for the three on-farm research sites are described in Table 4.1. Observed annual

precipitation, 30-year average annual precipitation, and deviation from the 30-year average annual precipitation for each one of the on-farm research sites are described in Table 4.2.

Table 4.1. Site name, coordinates, elevation, soil type, average annual precipitation, and average annual temperature.

Site	Coordinates	Elevation	Soil type	Average annual precipitation	Average annual temperature
		m		mm	°C
Beloit	39.3226N -98.1545W	460	Fine, smectitic, mesic Typic Argiustoll	700	12
Bucyrus	38.7395N -94.7068W	341	Fine, smectitic, mesic Aquertic Argiudoll	998	13
Solomon	38.8406N -97.3928W	381	Fine, mixed, superactive, mesic Pachic Argiustoll	848	13

Table 4.2. Site name, 30-year average annual precipitation, observed annual precipitation, and deviation from 30-year average annual precipitation for the Beloit, Bucyrus, and Solomon on-farm research sites (Source: Kansas Mesonet Stations Mitchell, Olathe, and Gypsum for Beloit, Bucyrus, and Solomon research sites, respectively).

Site	Year	30-year average annual precipitation	Observed annual precipitation	Deviation
			mm	
Beloit	2019	700	742	+ 42
Beloit	2020 ¹	700	635	- 46
Beloit	2021	700	494	- 205
Beloit	2022	700	472	- 227

Beloit	2023 ¹	700	459	- 240
Beloit	2024 ¹	700	443	- 256
Beloit	2025	700	609	- 90
Bucyrus	2018	998	838	- 159
Bucyrus	2019	998	1236	+ 238
Bucyrus	2020	998	940	- 57
Bucyrus	2021	998	1075	+ 77
Bucyrus	2022	998	940	- 57
Bucyrus	2023	998	909	- 88
Bucyrus	2024	998	912	- 85
Bucyrus	2025	998	846	- 151
Solomon	2020	848	716	- 131
Solomon	2021	848	590	- 257
Solomon	2022	848	630	- 217
Solomon	2023 ¹	848	577	- 270
Solomon	2024 ¹	848	647	- 200
Solomon	2025	848	835	- 12

¹ indicates years with no measured yield data.

Experimental design and treatment descriptions

The experiments were established in randomized strips laid out as a randomized complete block design (RCBD) with two treatments and four replications. Treatments were the presence of cover crops (CC) or the absence of cover crops (NC), replacing fallow periods between cash crops, at each study location. All on-farm research fields were managed under a no-tillage system. Strip sizes were designed to match field and equipment sizes at each location. At Beloit, the experimental field was 8.8 ha, with a strip size of 1.1 ha and a sampling grid of 0.37 ha. At Bucyrus, the experimental field was 21.6 ha, with a strip size of 2.7 ha and a sampling grid of 0.45 ha. In Solomon, the experimental field was 13.6 ha, with a strip size of 1.7 ha and a sampling grid of 0.43 ha.

In Beloit, the cropping system utilized was a winter wheat (*Triticum aestivum*) - soybean (*Glycine max*) - grain sorghum (*Sorghum bicolor*) rotation, and the cover crops used were winter pea (*Pisum sativum*), barley (*Hordeum vulgare*), radish (*Raphanus sativus*), triticale ($\times$ *Triticosecale* Wittmack), and oat (*Avena Sativa*). In Bucyrus, the cropping system was a corn (*Zea mays*) - soybean rotation, and cover crops utilized were cereal rye (*Secale cereale*), oat, radish, hairy vetch (*Vicia Sativa*), rapeseed (*Brassica napus*), and winter pea. At Solomon, the cropping system utilized was a winter wheat – soybean – grain sorghum rotation, and the cover crops utilized were pearl millet (*Pennisetum glaucum*), sudangrass (*Sorghum bicolor* $\times$ *S. sudanese*), sunflower (*Helianthus annuus*), radish, turnip (*Brassica rapa*), flax (*Linum usitatissimum*), and triticale. Cover crop use varied by year in each location. A schematic representation of the cropping systems at each location is presented in Table C.1 in Appendix C.

Soil Sampling

Soil samples were collected at each of the locations biannually at 0-5, 5-15, and 15-30 cm depth in geo-referenced sampling points using a shovel. At Beloit, soil samples were collected in the spring of 2019 at the winter wheat green-up stage, and in 2021, 2023, and 2025 after the termination of the cover crops and prior to the planting of the cash crops. At Bucyrus, soil samples were collected in the spring of 2018, 2020, 2022, and 2024 after the cover crops termination and prior to the planting of the cash crops. At Solomon, soil samples were collected in 2019 before winter wheat planting in the fall, and after the spring termination of cover crops in 2021, 2023, and 2025. Furthermore, the first year of sampling at each location was considered the baseline.

After sampling, soil samples were placed in a cooler and transported to the Department of Agronomy at Kansas State University. Soil samples for physicochemical measurements were stored at -4 °C before further processing and analysis, and soil samples for soil microbial properties were stored at -20 °C before further processing and analysis. The samples for physical and chemical measurements were composited, homogenized, subsampled, and oven-dried at 40 °C for chemical analysis at the Kansas State University Soil Testing Laboratory. Soil samples for aggregate stability measurements were air-dried at room temperature and sieved through an 8 mm sieve before analysis.

Soil chemical and biological analysis

Soil pH was determined using a 1:10 soil/water slurry (Watson and Brown, 1998). Extractable nutrients were determined using Mehlich-3 for extractable phosphorus (P) (NCR221, 2015). Furthermore, the sand, silt, and clay soil fractions for each site were determined by the hydrometer method using a solution of 50 g L⁻¹ of sodium hexametaphosphate as the dispersing solution (Gavlak et al., 2005). Soil water stable aggregates were determined using the methodology described by Kemper and Rosenau (1986) and modified by Mikha and Rice (2004). 100 g of soil was weighed and placed in a Yoder wet-sieving apparatus modified for the recovery of all particle fractions. Each soil sample was separated into four aggregate-size classes (8-2mm, 0.25-0.053 mm, 0.053-0.02 mm, and 0.02-0.002 mm diameter). Soil aggregates were fractioned into macroaggregates (>2 and 0.25-2mm) and microaggregates (0.053-0.25 and 0.02-0.053 mm) size classes. Mean weight diameter (MWD) was determined through the sum of the aggregate mass from each sieve multiplied by the mean aperture of adjacent sieves and was expressed in mm. Results from soil pH, sand, silt, and clay fractions, extractable P, water-stable macroaggregates,

and water-stable microaggregates for the three study locations at 0-5 cm depth are described in Table 4.3.

Table 4.3. Characterization of pH, texture, extractable P, macroaggregates, and microaggregates at Beloit, Bucyrus, and Solomon at the baseline soil health assessment year and the last soil health assessment across treatments at 0-5 cm depth. P: extractable phosphorus; Macro: water-stable macroaggregates; Micro: water-stable microaggregates.

Treatment	Year	pH	Sand	Silt	Clay	P	Macro	Micro
			%			g kg ⁻¹	g 100 g ⁻¹	
			0-5 cm					
			Beloit					
NC	2019	5.8	20.2	57.4	22.4	80.4	27.8	49.5
CC		5.6	18	56.5	25.6	82.3	29.1	50.5
NC	2025	6	-	-	-	97	39.5	47.2
CC		6.1	-	-	-	93.5	42.3	44.1
			Bucyrus					
NC	2018	6.6	13.3	63.3	23.4	42.3	43.1	46.7
CC		6.7	12.5	63	24.4	44.3	44.3	39.6
NC	2024	7.2	-	-	-	62.7	72.7	19.9
CC		7.2	-	-	-	48.2	76.7	15.5
			Solomon					
NC	2019	5.3	13.5	60	26.5	53	25.9	61.1
CC		5.2	13.6	58	28.6	56	27.5	59.6
NC	2025	5.5	-	-	-	68	50.9	38.7
CC		5.4	-	-	-	66	57.1	32

Soil organic carbon (SOC) was determined by the dry combustion method using a LECO CN828 Carbon/Hydrogen/Nitrogen combustion analyzer (LECO Corporation, St. Joseph, MI, USA, 2020), following methodology from Nelson and Sommers (1982). Soil phospholipid fatty acids (PLFA) were measured as an estimate of the soil microbial biomass community and

composition, following a modified methodology based on the original procedure (White & Ringelberg, 1996). Total lipids were extracted from 5 g of freeze-dried soil with a 2:1:0.8 solution of methanol, chloroform, and phosphate buffer, respectively. PLFA were isolated from total lipids through extraction using silicic acid chromatography columns (disposable SUPELCO Supelclean 6 mL columns, J.T. Sigma Aldrich®, St. Louis, Missouri, USA), and further methylated to fatty acid methyl esters (FAME) using a solution of 0.2 M methanolic potassium hydroxide. Resulting FAMES from each sample were analyzed using a Thermo Scientific Trace GC-ISQ 7000 (Thermo Fisher Scientific®, Waltham, Massachusetts, USA) equipped with an AI/AS 1310 column (27 cm x 27 cm x 17.7 cm; 12.9 L, Thermo Fisher Scientific®, Waltham, Massachusetts, USA). The microbial groups present in the FAME were assigned based on biomarkers' characteristics. Resulting fatty acids were grouped into Gram-positive (+) bacteria (i15:0, a15:0, i16:0, i17:0, and a17:0), Gram-negative (-) bacteria (19:0 Δ 9,10; C18:1:11:cis; 17:0 Δ 9,10; C10:0:2-OH, C12:0:3-OH, C14:0:2-OH, C14:0:3-OH; C16:1:0:cis, C16:0:2-OH), actinomycetes (10Me16:0 and 10 Me18:0), arbuscular mycorrhizal fungi (AMF) (C16:1:11), and fungi (C18:2:9,12) (White and Rice, 2009). The abundance of phospholipid fatty acids was reported as nmol of PLFA per gram of dry soil. Total microbial biomass was estimated by the sum of all PLFA biomarkers in microbes with FAME for C11:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, and C20:0. Fungi to bacteria ratio was calculated by dividing the sum of AMF and saprophytic fungi by the sum of Gram(+), Gram(-), and actinomycetes.

Soil potential enzyme activities were measured using the colorimetric method described by Tabatabai (2018), however, without the use of toluene (Acosta-Martínez & Tabatabai, 2015). Moreover, the amount of soil and the volume of solution were reduced by half while maintaining the proportions of soil:solution ratio in the original assay methodology (Acosta-Martinez and

Cotton, 2017; Acosta-Martinez et al., 2019). Individual assays included a carbon-acquiring enzyme (β -glucosidase [β G]), a phosphorus-acquiring enzyme (acid phosphatase [AP]), a sulfur-acquiring enzyme (arylsulfatase [AS]), and a nitrogen-acquiring enzyme (N-acetyl- β -D-glucosaminidase [NAG]). For each respective assay, 0.5g of air-dried soil (sieved at <5mm) was incubated at 37°C in solution with its appropriate buffer and 0.5 mL of its appropriate substrate at optimal pH for each enzyme for 1 h. Each assay was performed with two replicates in addition to one control, to which substrate was added after the incubation time. After incubation and addition of stop solutions, the soil suspension was filtered through a Whatman® No. 2v filter, and the filtrate absorbance was measured colorimetrically at 400nm on a Genesys 20 Visible spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Absorbance values from the control samples were subtracted from those obtained for the two repetition samples before the calculation of the amount of *p*-nitrophenol released during the incubation time using a standard calibration curve. Potential soil enzyme activities were reported as micromoles of *p*-nitrophenol per gram of dry soil per hour. A description of soil texture, pH, extractable P, macroaggregates, and microaggregates for the study sites at the depths of 5-15 and 15-30 cm are described in Appendix C on Tables C.2 and C.3.

Crop yield measurements

Crop yields were obtained from each on-farm research location using combine-mounted yield monitors during the harvest. At Beloit, a CaseIH® AFS Pro 700 yield monitor was used in a CaseIH® 7130 combine (Case International Harvest®, Racine WI). At Bucyrus, a GreenStar® active yield monitor system was used in a John Deere® S760 combine (John Deere®, Moline, IL). At Solomon, an AgLeader® yield monitor (Ag Leader®, Ames, IA) was used in a Gleaner®

S98 combine (AGCO ®, Duluth, GA). Raw yield monitor shapefiles were exported by the cooperating farmers and subsequently cleaned and processed in ArcGIS Pro (Esri, Redlands, CA) to extract georeferenced yield information for each georeferenced sampling point. Yield monitor data for each site and year were imported into ArcGIS Pro as point feature layers containing georeferenced yield values. For each on-farm research field, previously delineated polygon shapefiles representing the experimental strips were used to spatially constrain the yield dataset. A spatial mask was applied to retain only yield points located strictly within the strip boundaries, ensuring that yield values corresponded exclusively to the intended treatment areas. Furthermore, because each strip contained georeferenced sampling points, a 10-meter radius buffer was generated around each sampling point using the Buffer tool in ArcGIS Pro to associate yield data with each sampling point. The buffer distance was selected to capture an adequate number of yield monitor points in proximity to each sampling point while minimizing overlap between adjacent sampling areas.

After buffer creation, the masked yield point layer was intersected with each buffer to extract all yield monitor points within a 10-meter radius of each sampling point. For each buffer polygon, the mean yield of all included yield points was calculated. Additionally, raw yield values were corrected for the standard moisture values for each crop. Standard moisture values used for yield corrections were 15.5% for corn, 13% for soybean, and 13.5% for wheat. The average moisture-corrected crop yield was used to represent the crop yield at each sampling point for each site-year combination and was reported as Mg ha^{-1} or kg ha^{-1} .

Data analysis

The crop yield dataset was spatially transformed into an *sf* object using latitude and longitude coordinates from the GPS sampling points before statistical analysis (Pebesma & Bivand, 2023). Crop yield data were analyzed separately for each site and year to account for spatial and temporal variation across the on-farm network. For each crop, linear mixed models from the *nlme* R package (Pinheiro & Bates, 2000) were used to quantify the effects of cover cropping and year on crop yields. Treatment in each year-location combination was used as a fixed effect, while replications (blocks) were used as random effects to account for the non-independence among observations within sites. Model residuals were examined to verify assumptions of normality and homoscedasticity, and yield values were transformed when necessary to meet model assumptions. The normality of the model's residuals was tested using the Shapiro-Wilk test, and square root transformations were used when necessary. Exponential, spherical, Gaussian, and rational spatial correlation structures were tested in each model, and the spatial correlation structure was selected using the Akaike Information Criterion (AIC). Model means were extracted using the *emmeans* package in R Studio (version 2024.4.1.748) (R Core Team 2025; Lenth, 2025), and pairwise comparisons were employed to assess significant differences between treatments for the fixed effects. The significance levels for all tests reported in this study were evaluated using *p*-values level of 0.05.

To evaluate the relative performance of cover crop and no-cover treatments across environments, treatment yield ratios were calculated for each crop as the quotient of yield under cover crop treatment divided by yield under no-cover treatment. These ratios were plotted against the average observed annual precipitation to investigate the influence of water availability on treatment differences, to determine whether precipitation patterns modified the

magnitude and direction of yield responses to cover cropping, and to identify potential precipitation thresholds associated with yield benefits or penalties. Furthermore, yield adaptability and stability across producing environments were assessed using a Finlay-Wilkinson regression approach. An environmental index was calculated for each site-year using the mean annual crop yield for each treatment minus the overall grand crop yield mean across all treatment-years (Eberhart & Russell, 1966; Simão et al., 2023). While initially created to determine the interaction between crop genotypes and producing environments, environmental indices can also be applied to assess different agronomic management strategies (Reckling et al., 2021a; Simão et al., 2023; St. Luce et al., 2020; Xu et al., 2019). Treatment-specific yields were normalized within each site-year to remove scale effects and enable direct comparisons of cover crop and no-cover responses across the producing environments. For each crop, yield responses were regressed against the environmental index, and the resulting regression coefficients were interpreted as measures of yield adaptability and stability. The adaptability of each treatment and treatment-crop combinations was described by the slope (α) of the regression, where $\alpha > 1$ reflects adaptability to high-yielding environments, $\alpha = 1$ reflects broad adaptability, and $\alpha < 1$ reflects adaptability to low-yielding environments. Crop yield stability for each treatment and treatment-crop combination was described by the coefficient of determination (r^2) for each regression, where r^2 closer to 1 indicates more stable crop yields. Differences in slopes between treatments and treatment-crop combinations were used to characterize how cover crop treatments influenced the stability and adaptability of crop yields across the different producing environments presented in this study. All the statistical analyses were performed in R (R Core Team, 2025), and all the figures were generated using the *ggplot2* package in R (Wickham, 2016).

Results

Chemical and biological soil health indicators

Soil health indicators responded positively to cover crop adoption across the three study locations, with the magnitude of responses varying by site and indicator (Table 4.3). At Beloit, total microbial biomass increased by approximately 3% under the cover crop treatment relative to the no cover crop treatment, while soil total fungal biomass increased by approximately 21%. Extracellular enzyme activities exhibited apparent treatment differentiation, with soil β g, AS, AP, and NAG activities exhibiting 35%, 38%, 9%, and 32% of increase in the cover crop treatment relative to the no cover crop treatment, respectively. At Bucyrus, cover crop effects were substantially larger. The cover crop treatment increased soil organic carbon in 11.5%, soil total microbial biomass in 1161%, soil total bacteria in 2160%, and soil total fungi in 1770%. Extracellular enzyme activities, on the other hand, declined after 7 years of cover crops implementation. However, the cover crop treatment consistently yielded higher enzyme activities than the no cover crop treatment in 2024. Soil β g, AS, AP, and NAG activities in Bucyrus were 3-21% higher in the cover crop treatment, despite the overall decline from the baseline levels. At Solomon, the cover crop treatment also increased overall chemical and biological soil health indicators. Soil organic carbon (SOC) increased by approximately 19%, total microbial biomass by 33%, total bacteria by 32%, and total fungi by 35% in the cover crop treatment. Potential extracellular enzyme activities also increased in the cover crop treatment, where β g increased by 49%, AS by 50%, AP by 23%, and NAG by 50%. Results from soil health indicators across treatments and years in Beloit, Bucyrus, and Solomon for the depths of 5-15 and 15-30 cm are described in the supplementary Tables C.4 and C.5 in Appendix C.

Table 4.4. Characterization of chemical and biological soil health indicators at Beloit, Bucyrus, and Solomon at the baseline soil health assessment year and the last soil health assessment across treatments at 0-5 cm depth. SOC: soil organic carbon; TMB: soil total microbial biomass; TB: soil total bacteria biomass; TF: soil total fungi biomass; β g: soil potential β -glucosidase activity; AS: soil potential arylsulfatase activity; AP: soil potential acid phosphatase activity; NAG: soil potential N-acetyl- β -D-glucosaminidase activity.

Treatment	Year	SOC	TMB	TB	TF	βg	AS	AP	NAG
		g. kg ⁻¹	nmol PLFA g soil ⁻¹			μg PNP g soil ⁻¹ h ⁻¹			
		0-5 cm							
		Beloit							
NC	2019	1.7	52.1	30.4	4.3	300	67.2	842	77.4
CC		1.8	52.3	31.5	4.1	284	61.8	803	71.9
NC	2025	2.0	46.5	32.3	2.4	173	84.5	955	84.3
CC		2.2	48.0	31.2	2.9	233	116.6	1037	111.5
Bucyrus									
NC	2018	2.6	43.4	21.4	3.4	380	213.5	582	58.2
CC		2.5	43.2	21.7	3.2	363	199.1	529	58.0
NC	2024	2.8	470	416	54.5	230	217.3	724	68.3
CC		2.9	547	484	63.6	237	240.2	813	82.9
Solomon									
NC	2019	1.8	42	18.5	7.8	294	55.9	1135	101.8
CC		1.7	44.1	19.5	7.9	330	58.3	1112	117.4
NC	2025	2.1	203	182	20.4	165	65.5	993	164.7
CC		2.5	269	242	27.5	245	97.9	1220	244.7

Crop yield responses

At Beloit, crop yield responses to the cover crop treatment did not significantly differ from the no-cover treatment (Table 4.4). Average soybean yields in the cover crop treatment in 2021 were comparable between treatments, indicating that cover crop implementation did not influence soybean productivity under the conditions of that year (Fig. 4.1). Wheat yields

displayed a consistent pattern across the years evaluated, with no significant differences between treatments across the years. In 2019, wheat yields were low for both treatments, reflecting generally unfavorable growing conditions, yet no treatment differences were observed. Furthermore, wheat yields improved markedly in 2022 and again in 2025; however, despite the observed yield increases, no significant differences were observed between the cover crop and no-cover treatments.

Table 4.5. Summary of *p*-values for the effects of treatment (TRT) on corn and soybean yields in Beloit, KS.

Location	Crop	Year	TRT <i>p</i> -value
Beloit	Wheat	2019	0.820
Beloit	Soybean	2021	0.411
Beloit	Wheat	2022	0.512
Beloit	Wheat	2025	0.518

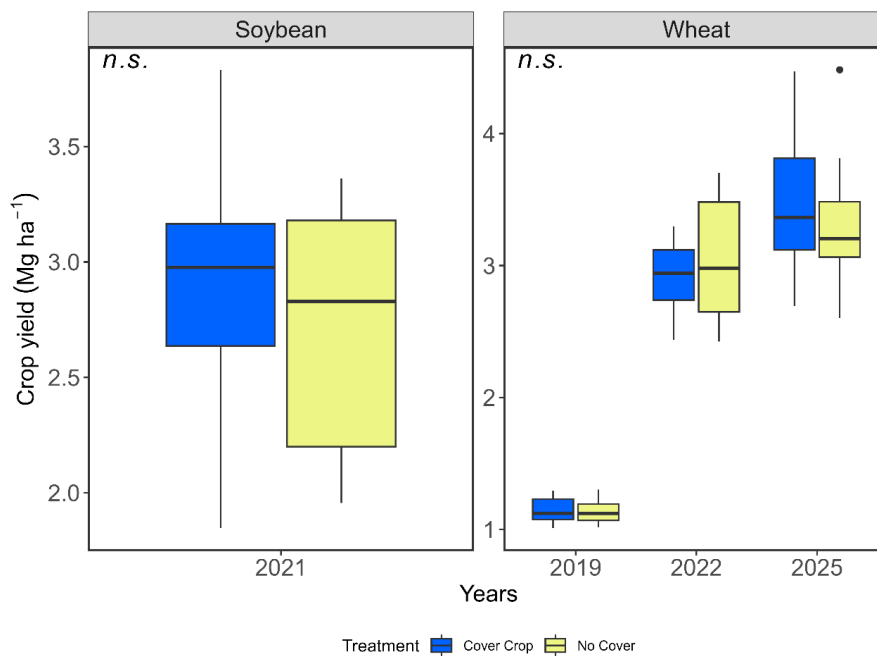


Figure 4.1. Crop yield differences for soybean and wheat at Beloit, KS, for 2019, 2021, 2022, and 2025 crop seasons under the cover crop and no-cover treatments. Means followed by the different letters indicate statistical difference according to the Tukey HSD test at $p < 0.05$. *n.s.*: no significant differences.

At Bucyrus, crop yields under the cover crop treatment did not significantly differ from those under no-cover treatment for either corn or soybeans across all the evaluated years (Table 4.5). For corn, yields exhibited a substantial year-to-year variability, ranging from approximately 10 Mg ha⁻¹ in 2020 to over 17 Mg ha⁻¹ in 2024 (Fig. 4.2), yet yields remained statistically similar between treatments within each year. Soybean yields in Bucyrus displayed a similar pattern, with no significant crop yield response to cover crop treatment relative to the no-cover treatment. Similar to corn yields, a substantial year-to-year variation in soybean yields was observed in Bucyrus, with yields ranging from 3 Mg ha⁻¹ in 2023 to nearly 6 Mg ha⁻¹ in 2025.

Table 4.6. Summary of p -values for the effects of treatment (TRT) on corn and soybean yields in Bucyrus, KS.

Location	Crop	Year	TRT p-value
Bucyrus	Corn	2018	0.890
Bucyrus	Soybean	2019	0.926
Bucyrus	Corn	2020	0.051
Bucyrus	Soybean	2021	0.247
Bucyrus	Corn	2022	0.377
Bucyrus	Soybean	2023	0.444
Bucyrus	Corn	2024	0.738
Bucyrus	Soybean	2025	0.717

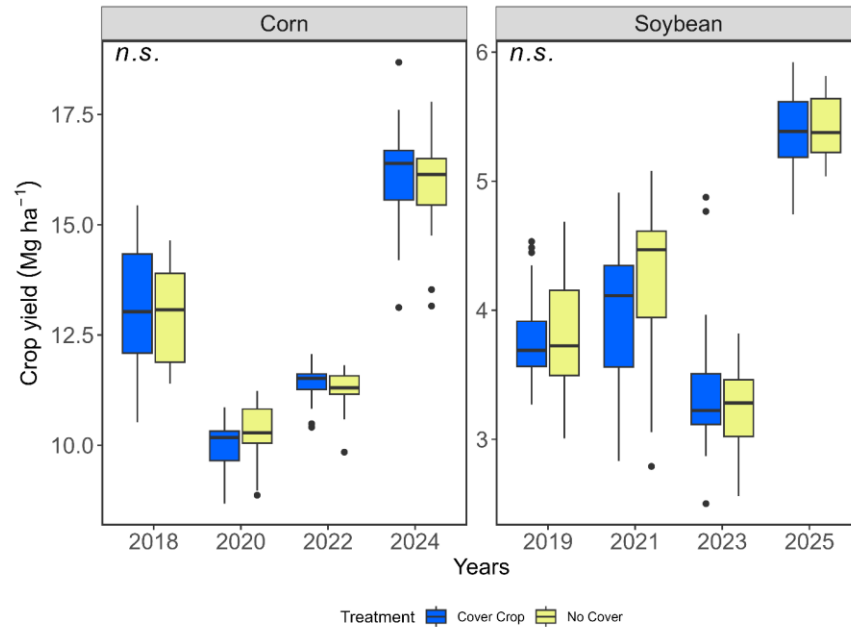


Figure 4.2. Crop yield differences for corn and soybean at Bucyrus, KS, for 2018, 2019, 2020, 2021, 2022, 2023, 2024, and 2025 crop seasons under the cover crop and no-cover treatments. Means followed by different letters indicate statistical difference according to the Tukey HSD test at $p < 0.05$. *n.s.*: no significant differences.

At Solomon, wheat yields in 2025 were significantly influenced by treatment (Table 4.6), with the cover crop treatment producing significantly higher yields than the no-cover treatment. In contrast, no significant wheat yield differences between treatments were observed in 2020 and 2022. Furthermore, wheat yields also varied considerably across the years, with markedly lower yields in 2022 compared to 2020 and 2025 (Fig. 4.3). Soybean yields at Solomon were not significantly different between the cover crop and no-cover treatments (Figure 4.4). Although crop yields in Solomon did not statistically differ between treatments in most of the years for wheat and soybean, the cover crop treatment tended to exhibit consistent reduction in in-field variability (Fig. 4.3; 4.4), which may indicate greater yield stability under the cover crop treatment.

Table 4.7. Summary of p -values for the effects of treatment (TRT) on corn and soybean yields in Solomon, KS.

Location	Crop	Year	TRT p -value
Solomon	Wheat	2020	0.608
Solomon	Soybean	2021	0.339
Solomon	Wheat	2022	0.113
Solomon	Wheat	2025	0.003***

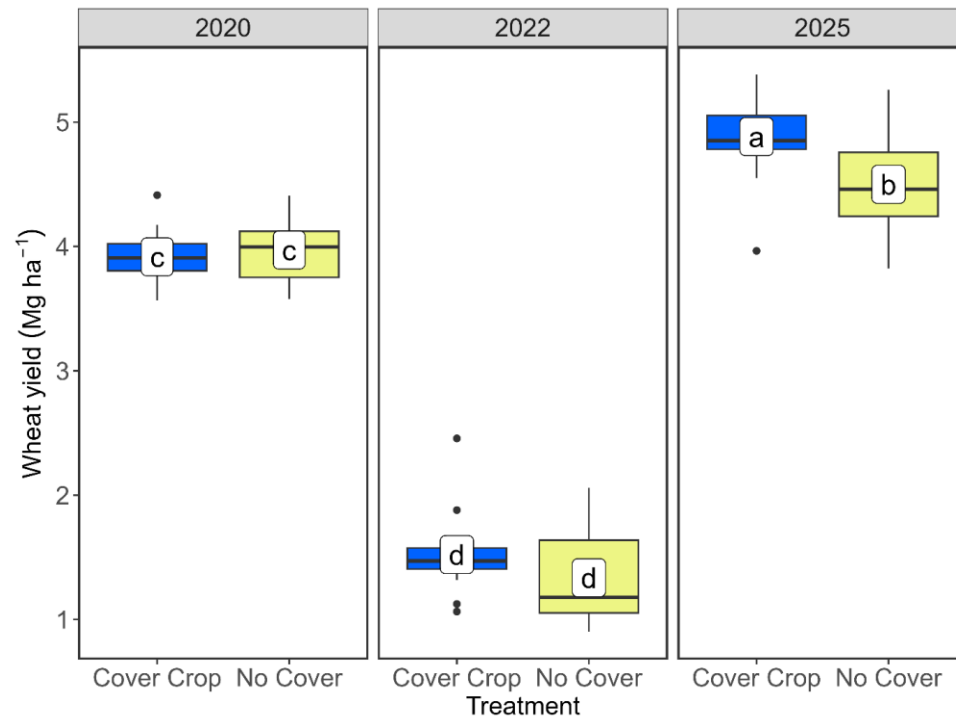


Figure 4.3. Crop yield differences for wheat at Solomon, KS, for 2020, 2022, and 2025 crop seasons under the cover crop and no-cover treatments. Means followed by different letters indicate statistical difference according to the Tukey HSD test at $p < 0.05$. *n.s.*: no significant differences.

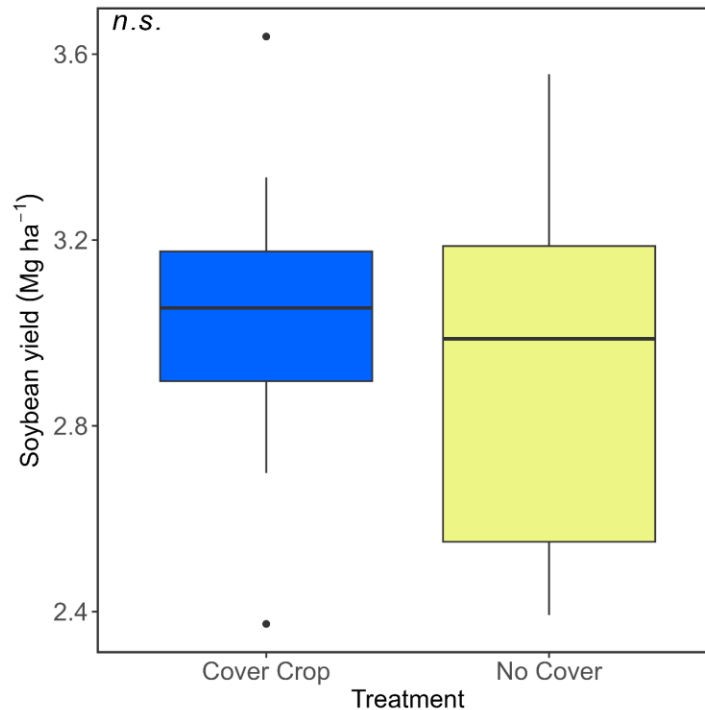


Figure 4.4. Crop yield differences for soybean at Solomon, KS, for the 2021 crop season under the cover crop and no-cover treatments. Means followed by different letters indicate statistical difference according to the Tukey HSD test at $p < 0.05$. *n.s.*: no significant differences.

Yield stability and adaptability

Finlay-Wilkinson regressions had consistent and largely linear yield responses to producing environmental quality across the different crops, treatments, and locations (Fig. 4.5a). For corn, relative yield responses had strong sensitivity to the environmental index with both cover crop and no-cover treatments, with slope coefficients (α) of 1 and 0.97 for the cover crop and no-cover treatments, respectively, and adjusted coefficients of determination (Adj.R^2) over 0.9 between the two treatments. Corn yields responded proportionally to environmental favorability, and cover crops did not alter the stability of the responsiveness of corn yields in this environment.

Soybean yields were more variable in responses, especially with the cover crop treatments. Slope coefficients (α) differed between treatments (0.82 and 1.2 for the cover crop and no-cover treatments, respectively), and adjusted coefficients of determination were higher for the no-cover treatment when compared to the cover crop treatment (Adj.R² 0.86 and 0.71, respectively) (Fig. 4.5a). Soybean yield stability and adaptability were more dependent on site-specific conditions. Moreover, the α value from soybean responses in the cover crop treatment was lower than 1 and lower than the no-cover treatment (Figure 4.5a). This indicates greater adaptability of soybean yields to low-yielding environments in the cover crop treatment, though with lower stability (Adj.R² 0.86 for the no-cover and Adj.R² 0.71 for the cover crop treatment) compared to the no-cover treatment. Wheat yields had consistent responses across locations and treatments (Fig. 4.5a), with α values near 1 and Adj.R² values greater than 0.9 for both cover crop and no-cover treatments (Fig. 4.5). These findings indicate a strong coupling between wheat yields and environmental conditions, regardless of management practices. Furthermore, when relative yield responses for all crops were averaged and similarly plotted against a relative environmental index, no apparent differences between treatments were observed (Fig. 4.5b). Both cover crop and no-cover treatments had slope (α) values close to 1 (1 for the cover-crop and 0.99 for the no-cover treatment), and Adj.R² values identical to each other (0.99 for both treatment responses) (Fig. 4.5b). The overall crop responses thus indicate that yield responses across crops in both treatments were proportional to the producing environments, without a clear distinction between the use or absence of cover crops.

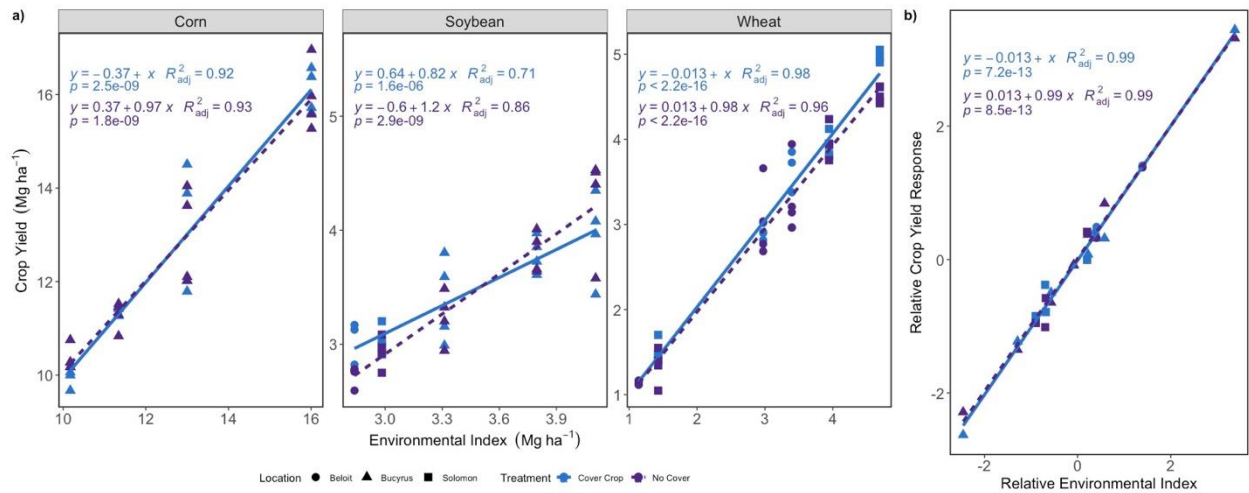


Figure 4.5. Finlay-Wilkinson regression of crop yields against environmental indices for corn, soybean, and wheat under cover crop and no-cover treatments at Beloit, Bucyrus, and Solomon, KS (a). Finlay-Wilkinson regression for the relative yield response across corn, soybean, and wheat crop yields against the relative environmental index for corn, soybean, and wheat crops (b). The inset equations report fitted slope, intercept, adjusted coefficient of determination (Adj. R^2), and significance level. Shapes correspond to the different locations. Lines correspond to treatment-specific linear regressions.

Across all site-year combinations, yield responses to the cover crop treatment were evaluated by the precipitation received during the whole growing season and phenological periods at the three locations. Soybean yield responses to cover crops had no clear or consistent relationship observed between precipitation and yield response across the different phenological windows (Fig. 4.6). Data points, in this situation, were scattered, with both positive and negative yield responses to the cover crop treatments occurring across the full range of precipitation conditions. Although slight tendencies toward reduced yield benefits in the cover crop treatment under higher precipitation were visually apparent in some cases, these patterns were inconsistent.

For both corn and wheat, yield responses to cover cropping also exhibited no consistent response patterns to precipitation across the growing season, and within specific phenological phases (Fig. 4.7; Fig. 4.8). Similar to soybean, no clear or consistent relationship was observed between precipitation and yield responses to the cover crop treatment for either crop. Data points were also scattered across the precipitation range, with both positive and negative yield responses occurring under both drier and wetter conditions.

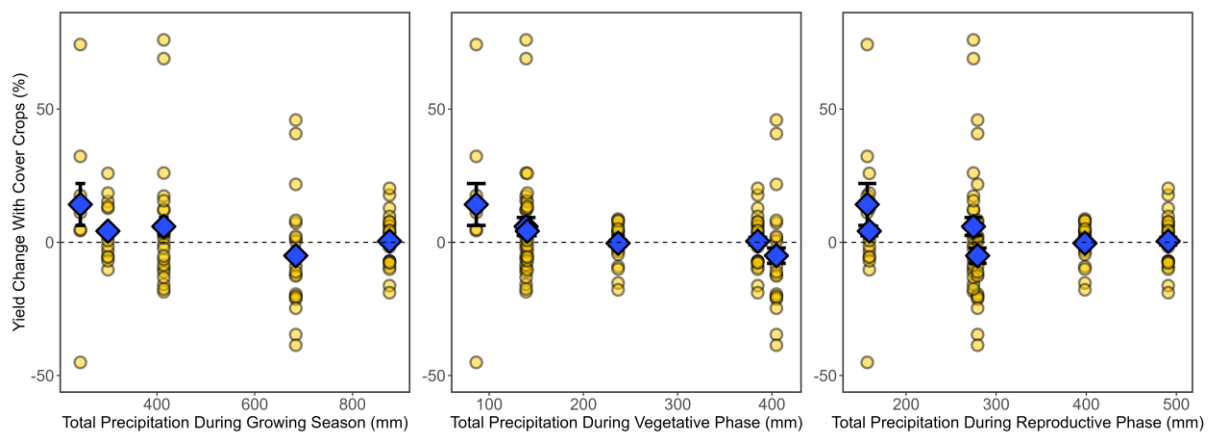


Figure 4.6. Relationship between soybean yield changes under the cover crop treatment and precipitation accumulated during the whole growing season (left), the soybean vegetative phase (center), and the soybean reproductive phase (right). Yield change (%) represents the relative difference between cover crop and no-cover treatments. Each yellow point represents a site-year combination observation. Blue symbols represent the main yield change across precipitation patterns, with error bars indicating the standard error of the mean. Horizontal dashed lines indicate no yield difference between the treatments (0% yield change).

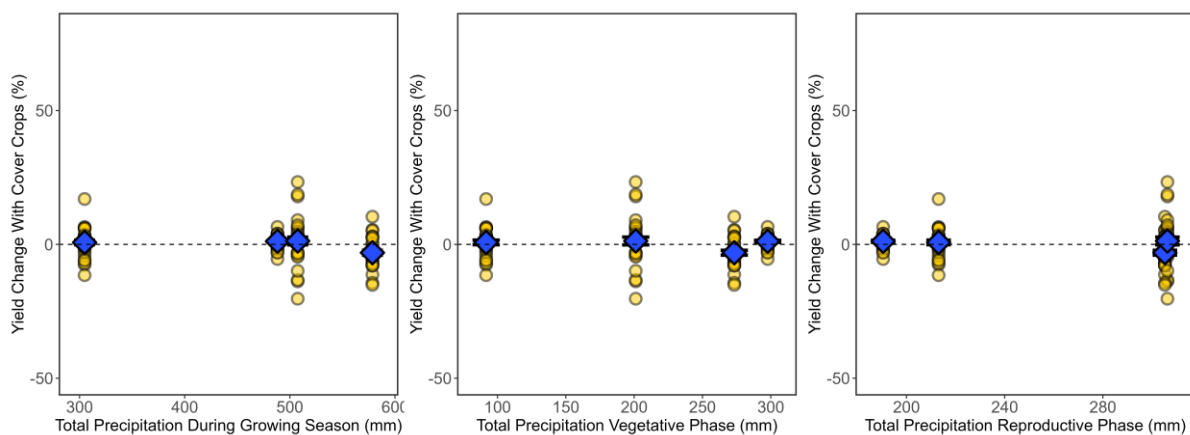


Figure 4.7. Relationship between corn yield changes under the cover crop treatment and precipitation accumulated during the whole growing season (left), the corn vegetative phase (center), and the corn reproductive phase (right). Yield change (%) represents the relative difference between cover crop and no-cover treatments. Each yellow point represents a site-year combination observation. Blue symbols represent the main yield change across precipitation patterns, with error bars indicating the standard error of the mean. Horizontal dashed lines indicate no yield difference between the treatments (0% yield change).

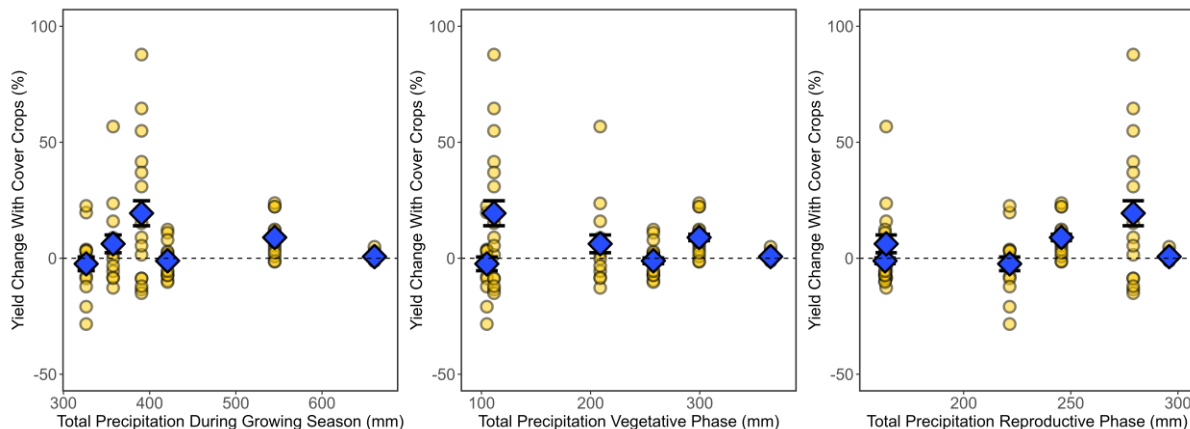


Figure 4.8. Relationship between wheat yield changes under the cover crop treatment and precipitation accumulated during the whole growing season (left), the wheat vegetative phase

(center), and the wheat reproductive phase (right). Yield change (%) represents the relative difference between cover crop and no-cover treatments. Each yellow point represents a site-year combination observation. Blue symbols represent the main yield change across precipitation patterns, with error bars indicating the standard error of the mean. Horizontal dashed lines indicate no yield difference between the treatments (0% yield change).

Discussion

The effect of cover crops adoption, replacing fallow periods in between cash crops in crop rotations, on overall soybean, corn, and wheat yield performance was assessed in this study. In general, the use of cover crops as a regenerative agricultural practice primarily aims to improve soil chemical, physical, and biological characteristics that are important to biogeochemical cycles in the soil ecosystem (Acosta-Martínez et al., 2003; Chavarría et al., 2016; Hsiao et al., 2018). Across the three study locations, cover crop adoption was associated with increases in soil health indicators at 0-5 cm depth, including SOC, TMB, TB, TF, and extracellular enzyme activities. These results were consistent with the previously reported meta-analysis by Muhammad et al. (2021), in which cover crop use increased soil microbial community abundance metrics and microbial biomass carbon compared with no-cover crop treatments. Moreover, increasing the diversity of cropping systems through the use of cover crops has been widely reported as beneficial to overall soil health (Lin et al., 2023; Liu et al., 2023; Simão et al., 2023), through the incorporation of increased levels of plant biomass, plant-derived carbon, and root exudates (Chavarría et al., 2016) that positively interplay with the soil microbiome (Kim et al., 2020). However, the magnitude and consistency of these improvements were strongly location-specific, reflecting differences in cropping systems, cover crop species

composition, and cumulative annual precipitation. These results indicate that while cover crops consistently enhanced soil health indicators, their influence on crop productivity was conditional and mediated by crop-specific and environment-specific constraints.

While improvements in soil health indicators in the cover crop treatments across the three study locations were observed, crop yield responses to cover crop adoption were generally neutral. This observed trend of yield neutrality suggests that replacing fallow periods between cash crops with cover crops resulted in neither a yield gain nor a yield penalty under the conditions evaluated in this on-farm research network across the years. This overall pattern is consistent with previous studies reporting small yield responses in crop yield outcomes under cover cropping systems (Peng et al., 2024; Qin et al., 2021; Tonitto et al., 2006). Likewise, the lack of significant yield responses aligns with the context-dependent nature of cover crop effects on subsequent grain crop yields, where outcomes are influenced by crop type, cover crop species, management practices, and environmental conditions (Blanco-Canqui et al., 2015; Chahal et al., 2023; Deines et al., 2023).

Specifically, by crop, in higher annual precipitation environments, such as Bucyrus, KS, corn yields showed neutral responses to cover cropping, especially when nitrogen availability, residue quality, and cover crop termination timing were well managed. Furthermore, improved soil health parameters, such as soil aggregation, water infiltration rates, and well-balanced water dynamics, can potentially offset early-season competition for nitrogen or soil moisture, thus resulting in neutral net yield effects (Basche et al., 2016; Finney et al., 2016; Marcillo & Miguez, 2017; White et al., 2017). Conversely, some studies emphasize that this balance is particularly sensitive to cover crop termination timing, which can shift these responses to the negative side when competition for resources persists into early crop growth stages (Muniz et al., 2025; Rosa

et al., 2021). Moreover, corn yields frequently exhibited neutral responses to cover cropping, particularly in systems using diverse cover crop mixtures or where no water or nutrient limitations are present (Finney et al., 2016; Hunter et al., 2019; Marcillo & Miguez, 2017; White et al., 2017).

For winter wheat, studies in the U.S. Great Plains have shown yield reductions following cover crops in years with below-average water availability, while no yield differences were observed under favorable rainfall conditions (Hunter et al., 2019; Nielsen et al., 2016). In our study, besides the 2024-2025 winter wheat season in Solomon, where the cover crop treatment yielded significantly higher winter wheat yields, there were no overall significant differences in wheat crop yields in both dry and wet years. Furthermore, previous studies suggested that cover crops may reduce stored soil water before winter wheat establishment in dry years, thus resulting in yield penalties (Nielsen et al., 2015; Nielsen, 2016; Holman et al., 2018). Other studies implied that potential neutral to positive responses would be expected when precipitation is sufficient, and soil water recharge occurs before winter wheat planting (Nielsen et al., 2016; Robinson & Nielsen, 2015). Therefore, a wide range of responses depending on cover crop biomass production, termination timing, and moisture availability have been reported (Eash et al., 2021; Holman et al., 2018; Obour, Simon, Holman, Carr, Schipanski, Fonte, Ghimire, Nleya, & Blanco-Canqui, 2021).

For soybean, a similar trend was observed with no significant differences in yield between treatments. Similar to corn, soybean yields have often been reported as non-responsive to cover crop adoption, with previous studies documenting no significant differences following either single-species or mixed cover crops (Hunter et al., 2019; Qin et al., 2021). Additionally, this lack of response may reflect that soybean are often less responsive to changes in soil

nitrogen dynamics induced by cover crops, given their potential for biological nitrogen fixation. Despite this trend, soybean yield responses still varied according to planting conditions and cover crop termination timing (Qin et al., 2021). Regional studies across Kansas similarly reported no soybean yield responses to cover crops, with variability mostly driven by cover crop species selection and year-to-year weather conditions rather than by systematic yield penalties or gains (Hunter et al., 2019; Shoup et al., 2017). In locations with lower cumulative annual rainfall (700 mm in Beloit and 848 mm in Solomon, compared to 998 mm in Bucyrus), yield responses to cover cropping tended to be more constrained by soil water dynamics and precipitation variability. More broadly, Rodriguez et al. (2023) implied that the magnitude of yield responses to cover cropping varies widely across environments, reinforcing that no yield responses are expected when cover crops are adopted in precipitation-limited regions. Taken together, these crop- and location-specific mechanisms suggest that the yield responses observed across the three on-farm research sites may reflect a balance between soil functional improvements and environmental constraints that vary by crop, rotation, and year.

While no significant effects of cover crop use on crop yields were observed, Finlay-Wilkinson regressions revealed crop-specific differences in yield stability and environmental responsiveness. Corn and wheat responses, with and without cover crops, were proportional, suggesting that yield variation was driven primarily by environmental quality rather than by cover crop use. The yield-environment relationships observed for corn and wheat in this study align with multi-environment studies demonstrating that cereal crop yields are largely governed by environmental quality, with water availability acting as a dominant driver of yield variation across different environments (Piepho & Blancon, 2023; Reckling et al., 2021b; van Eeuwijk et al., 2019). Conversely, in this study, more diversified rotations and soil health practices

displayed potential to shift soybean yields toward lower environmental responsiveness, buffering potential yield decreases under non-optimal conditions. This effect on soybean yields can be linked to practices that improve soil biological activity and enhance soil structure, including no-tillage and cover cropping (Li et al., 2019; Reed et al., 2019; Rosa et al., 2021). These observed patterns support the assumption that cover crops may act as a stabilizing mechanism, reducing yield losses under resource-limited conditions, rather than as a yield-enhancing practice in favorable environments (Kaye & Quemada, 2017). Moreover, recent studies have suggested interpreting these outputs in terms of environmental variables, such as soil water and precipitation, to better explain observed deviations (Piepho & Blancon, 2023; Reckling et al., 2021b).

Additionally, the inconsistent relationship between yield gains under the cover crop treatment and the precipitation during the crop growing season indicated that the yield responses to cover cropping were not directly driven by total precipitation alone. These findings are consistent with previous studies showing that the amount of rainfall received explains only a limited portion of yield variability in cover cropping systems, especially when soil water availability, infiltration rates, and distribution of rainfall events are more important than cumulative precipitation totals (Basche & DeLonge, 2017; Blanco-Canqui et al., 2015). For soybean, the lack of a stronger relationship between precipitation and yield gains in the cover crop treatment suggested that cover crops' benefits may operate through buffering mechanisms that reduce sensitivity to short-term moisture stress rather than through enhanced yield potential in wetter environments. Similar conclusions have been reported in long-term field studies where cover cropping improved yield stability without producing consistent yield gains under high precipitation conditions (Ruis et al., 2020). In corn and wheat, the lack of systematic

precipitation effects on relative yield changes further supports the interpretation that yield outcomes were primarily controlled by a broader environmental quality, which may also include soil water storage and temperature interactions, rather than precipitation solely (Hatfield & Dold, 2019). Together, these results reinforce the trade-offs observed in the Finlay-Wilkinson regression, suggesting that the use of cover crops influenced crop yields mainly by modifying crop-environment interactions and buffering yield variability, rather than amplifying yield responses under favorable moisture conditions.

Overall, results from this study indicated that cover crop adoption influenced crop performance by altering yield stability and crop-environment interactions rather than by consistent changes in absolute yield values. Across crops, locations, and years, yield responses were generally neutral, while Finlay-Wilkinson regression and precipitation-based analyses revealed that environmental conditions, and especially water availability, remained the main drivers of observed yield variability. In this sense, cover crops appeared to function mainly as a buffering management practice, moderating yield responses under less favorable conditions without enhancing yield potential in high-productivity environments. These findings highlight an important distinction between yield maximization and yield resilience, reinforcing the idea that the agronomic value of cover crops may be better captured by stability-oriented metrics rather than absolute yield comparisons. Importantly, the lack of yield responses with cover crops should not be interpreted as a lack of agronomic benefit. Yield neutrality, when accompanied by improvements in soil health and yield stability, may allow producers to reduce reliance on certain inputs, such as synthetic fertilizers and herbicides, thereby improving overall system efficiency even in the absence of apparent yield gains. Some limitations of this study should be acknowledged. The observational nature of on-farm research introduces variability in

management practices, cropping systems, cover crop species composition, and termination timing that may have obscured detectable treatment effects. In addition, this study did not explicitly quantify input costs or economic return for the three farming operations assessed, thus limiting the ability to directly assess the profitability of cover crops adoption in each situation. As such, future research should integrate agronomic, environmental, and economic analyses to evaluate whether yield-neutral responses under cover cropping translate into reduced input requirements or improved net returns over time. Incorporating detailed cost-benefit assessments alongside long-term stability metrics would provide a more complete evaluation of the role of cover crops in resilient and economically sustainable cropping systems.

Conclusion

This study evaluated the effect of cover crop adoption on crop yields and yield stability across a multi-year, three-location on-farm network in Kansas, encompassing contrasting crop rotations, precipitation regimes, and soil conditions. Across all sites, cover crops consistently improved soil health indicators, including soil organic carbon, microbial biomass, and microbial activity, reinforcing their capacity to enhance soil biological and chemical functioning under no-tillage management. However, these improvements in soil health did not translate into consistent yield gains for corn, soybean, or winter wheat. Instead, yield responses were generally neutral, indicating that cover crops adoption neither enhanced nor penalized crop productivity under the range of environmental conditions evaluated. Analyses of crop yield adaptability and stability revealed that environmental quality, especially water availability, remained the dominant driver of yield variability across crops and locations. Finlay-Wilkinson regressions demonstrated

largely proportional yield responses to environmental favorability for corn and wheat, regardless of cover crop use, while soybean exhibited evidence of different responsiveness under cover cropping, suggesting greater buffering under less favorable conditions. Complementary precipitation-based analyses further indicated that total precipitation alone explained only a small portion of yield variation, reinforcing that yield outcomes in cover-cropped systems are governed by complex interactions among soil water storage, rainfall timing, crop phenology, and management practices. Taken together, these findings indicate that the primary agronomic value of cover crops in these systems lies in their ability to enhance soil health and modulate crop-environment interactions rather than increase short-term yield potential.

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Chapter 5 - Conclusion

The overall goal of this dissertation was to evaluate how regenerative agriculture management in cropping systems influences soil health and agronomic performance in rainfed systems of Kansas. Specifically, this research examined seasonal dynamics of biological soil health indicators, the effects of cropping systems intensity and diversity under no-tillage management, and the impact of cover crop adoption on soil function, yield outcomes, and stability across contrasting environments. Collectively, these chapters contribute to a broader understanding of how biological, chemical, and physical soil health indicators respond to management transitions and how those responses shape agronomic outcomes under environmental variability.

Chapter two examined how biological soil health indicators fluctuate across seasons and crop growth stages. Results demonstrated that soil microbial biomass and extracellular enzyme activities are highly dynamic, responding to crop phenology, soil moisture, and temperature. This chapter highlighted that soil biological activity is not static, and the interpretation of soil health assessments must consider sampling timing. Identifying a consistent and representative sampling window provided important methodological guidance for long-term soil health monitoring efforts in winter wheat-based cropping systems. These findings reinforce, therefore, that biological soil health indicators are sensitive early signals of management and environmental changes.

Chapter three evaluated whether increasing the intensity and diversity of cropping systems under a no-tillage management system would enhance soil health over a five-year period. The transition from a conventional tillage system to a no-tillage system at the beginning

of the experiment produced clear improvements in surface soil organic carbon, microbial biomass, and aggregate stability. However, differences between more intense and diverse cropping systems relative to the winter wheat-summer fallow baseline treatment were less pronounced within the timeframe studied. These results suggest that reducing soil disturbance is a critical first step in rebuilding soil health, while the additional effects of crop intensification and diversification may take longer to become evident. Likewise, these findings indicate that changes in soil carbon accrual and aggregation stability develop progressively and depend upon sustained organic inputs and management continuity.

Results from chapter four extended the analysis to the on-farm scale by evaluating the effects of cover crop adoption on soil health indicators and crop yield performance across multiple locations and cropping systems. The integration of cover crops increased biological soil health indicators, including soil organic carbon, microbial biomass, extracellular enzyme activity, and aggregate stability. Despite improvements in soil health indicators, crop yields of corn, soybean, and winter wheat were generally similar between treatments across locations and years. However, analysis of yield stability revealed that cover crops improved system resilience by buffering crop yields under drier conditions, especially for soybean. These findings suggest that the agronomic benefits of regenerative agriculture practices may be expressed more strongly through enhanced yield stability and resilience rather than through immediate increases in average absolute crop yields.

Taken together, the results of this dissertation demonstrate that soil biological processes play a central role in mediating the development of soil health under regenerative management systems. Biological soil health indicators responded rapidly to seasonal environmental conditions and crop growth stages, reflecting the dynamic interactions between plant inputs, microbial

activity, and climatic drivers. Over longer time scales, practices such as no-tillage and continuous soil cover improved soil structure, organic matter accumulation, and microbial activity, contributing to the gradual enhancement of soil health. Notably, these results indicate that improvements in soil health do not necessarily translate into immediate increases in crop yield, but instead contribute to the stabilization and resilience of crop production systems under variable environmental conditions.

This research provides important implications for soil health assessment and the management of regenerative cropping systems in the U.S. Great Plains. The results highlight the importance of accounting for seasonal dynamics when evaluating biological soil health indicators, as sampling timing can strongly influence the interpretation of soil health status. In particular, the findings of this dissertation demonstrate that regenerative management practices can generate measurable improvements in soil biological functioning within relatively short timeframes, even though system-level benefits may require longer periods to become fully expressed. Additionally, the observed relationships between soil health and crop yield responses emphasize that regenerative agriculture should be evaluated not only based on absolute yield outcomes but also on its potential to enhance long-term stability, resilience, and sustainability of cropping systems. Overall, this dissertation advances the understanding of how regenerative agricultural practices influence soil health and agronomic performance in rainfed cropping systems of the U.S. Great Plains, contributing to a more integrated understanding of soil health in agricultural systems. Also, it provides an understanding of regenerative agriculture practices as a pathway for improving soil function, strengthening system resilience, and supporting the long-term sustainability of agricultural production in climate variability scenarios.

Despite the comprehensive scope of this research, some opportunities remain for future investigation. The temporal duration of the cropping system experiment (Chapter 3) may not yet be sufficient to fully capture the long-term effects of cropping intensification and diversification on soil health indicators, thus suggesting that soil health assessments at longer durations will be essential to better understand the cumulative impacts of more intense and diverse winter wheat-based crop rotations. In addition, while this study evaluated several key biological soil health indicators, further investigation of the underlying mechanisms regulating microbial activity and soil carbon dynamics would strengthen the interpretation of these responses (Chapter 2). In particular, future research integrating measurements of labile organic matter pools, such as particulate organic matter and dissolved organic carbon, would provide insight into the seasonal variability of substrates that drive microbial biomass and extracellular enzyme activity responses. Likewise, the quantification and characterization of root exudates and other rhizosphere carbon inputs could improve the understanding of how plant-soil interactions regulate microbial activity across crop growth stages and environmental conditions. At a broader scale, future studies should encompass the integration between soil health and agronomic performance, integrating the economic sphere (Chapter 4). Including economic analyses of input costs, profitability, and risk reduction would help to clarify whether improvements in soil health and system resilience translate into meaningful benefits for producers. Together, these research directions would contribute to a more mechanistic and system-level understanding of how regenerative cropping systems influence soil processes, farm performance, system resilience, and overall sustainability of the U.S. Great Plains cropping systems.

Appendix A - Chapter 2

Table A.1. Chronological summary of sampling times conducted from November 2021 to July 2023 across winter and summer crops planting, flowering, and harvest. Each sampling moment corresponds to a specific crop growth stage, planting (P), flowering (F), and harvest (H) for either winter (W) or summer (S) crops. Days after the first sampling indicates the elapsed time (in days) relative to the initial sampling conducted at the winter wheat planting in 2021.

Sampling Moment	Date	Year	Crop Growth Stage	Crop season	Days after the first sampling
WP	11/21	2021	Planting	Winter	0
WF	04/22	2022	Flowering	Winter	152
WH	07/06	2022	Harvest	Winter	227
SP	07/22	2022	Planting	Summer	243
SF	9/22	2022	Flowering	Summer	305
SH	10/22	2022	Harvest	Summer	335
WP	11/22	2022	Planting	Winter	366
WF	04/23	2023	Flowering	Winter	518
WH	07/23	2023	Harvest	Winter	609

Table A.2. Description of soil physiochemical characteristics across the sampling moments.

GWC: gravimetric water content; SOC: soil organic carbon; K: plant-available potassium; P: plant-available phosphorus; TN: soil total nitrogen; In. N: plant available nitrogen. Sampling moments are divided into: P-W: winter crops planting in the first season; F-W: winter crops flowering in the first season; H-W: winter crops harvest in the first season; P-S: summer crops planting; F-S: summer crops flowering; H-S: summer crops harvest; P-W2: winter crops planting in the second season; F-W2: winter crops flowering in the second season; and H-W2: winter crops harvest in the second season. Errors are reported as standard errors.

	GWC	pH	SOC	K	P	TN	In. N
	g kg ⁻¹		g kg ⁻¹	mg kg ⁻¹			
	0-5 cm						
P-W	19.1 ± 0.4	6.6 ± 0.09	16.6 ± 0.2	438.9 ± 10.4	47.1 ± 2.7	10.8 ± 0.4	7.9 ± 0.8
F-W	20.6 ± 0.9	6.6 ± 0.07	15.1 ± 0.2	383.1 ± 10.6	36.2 ± 2.2	14.7 ± 0.2	6.3 ± 0.4
H-W	12.6 ± 0.9	6.6 ± 0.08	15.7 ± 0.2	376.3 ± 5.6	41 ± 2.5	14.5 ± 0.3	2.5 ± 0.1
P-S	18.6 ± 0.7	6.5 ± 0.1	15.5 ± 0.3	399.9 ± 7.7	47.4 ± 3	13 ± 0.3	7.8 ± 1.5
F-S	10.6 ± 0.6	6.4 ± 0.1	16.3 ± 0.3	390.4 ± 8	38.8 ± 3.2	16.1 ± 0.2	6.5 ± 1.5
H-S	23.8 ± 0.5	6.4 ± 0.1	16.5 ± 0.3	366.6 ± 6	41.3 ± 3.5	15 ± 0.2	5.1 ± 0.4
P-W2	22.1 ± 6.1	6.4 ± 0.1	15.8 ± 0.3	357.3 ± 8.5	40.7 ± 3.2	13.9 ± 0.2	8.9 ± 0.5
F-W2	9.6 ± 12.1	6.9 ± 0.1	16.2 ± 0.3	306.8 ± 9.5	37.6 ± 2.1	14.6 ± 0.2	6.1 ± 0.9
H-W2	12.5 ± 6.8	6.7 ± 0.1	15.9 ± 0.3	369.4 ± 8.9	47 ± 2.3	15.6 ± 0.2	4.9 ± 0.2
	5-15 cm						
P-W	20.2 ± 0.2	6.3 ± 0.09	14 ± 0.2	305.3 ± 9.6	27.8 ± 2.9	12.9 ± 0.1	3.75 ± 0.2
F-W	23.1 ± 0.8	6.3 ± 0.1	13.5 ± 0.2	251.7 ± 8.4	11.5 ± 1.1	13.4 ± 0.2	1.95 ± 0.1
H-W	15.6 ± 0.6	6.4 ± 0.1	13.6 ± 0.1	249.5 ± 5.6	11.3 ± 0.8	13.6 ± 0.1	0.9 ± 0.05
P-S	19.2 ± 0.5	6.3 ± 0.1	12.7 ± 0.2	285.5 ± 8.2	18 ± 1.7	11.6 ± 0.2	1.56 ± 0.1
F-S	14.3 ± 0.7	6.2 ± 0.1	13.8 ± 0.1	275.9 ± 6.3	13.5 ± 1.5	14.8 ± 0.1	2.8 ± 0.5
H-S	19.9 ± 0.1	6.2 ± 0.1	12.9 ± 0.1	218.8 ± 4.4	8.9 ± 1.2	12.4 ± 0.1	1.3 ± 0.2
P-W2	18.7 ± 4.5	6.1 ± 0.1	12.8 ± 0.1	196.5 ± 4.4	7.6 ± 0.8	10.9 ± 0.1	2.6 ± 0.2
F-W2	11.8 ± 3.2	6.3 ± 0.1	12.8 ± 0.1	202.3 ± 8.2	16.5 ± 2	11.6 ± 0.1	1.4 ± 0.2

H- W2	14 ± 4.3	6.4 ± 0.1	12.8 ± 0.1	182.2 ± 5.3	9.1 ± 0.8	12.6 ± 0.1	1.1 ± 0.1
15-30 cm							
P-W	26.1 ± 0.8	6.4 ± 0.06	11.4 ± 0.2	289.6 ± 8	3.4 ± 0.5	10.6 ± 0.1	1.1 ± 0.05
F-W	26.9 ± 1.2	6.3 ± 0.06	11.6 ± 0.2	261.1 ± 9.1	2.2 ± 0.3	12.2 ± 0.2	0.5 ± 0.04
H-W	18.9 ± 0.3	6.4 ± 0.05	12.2 ± 0.2	304.2 ± 8.4	2.5 ± 0.3	12.2 ± 0.1	0.4 ± 0.02
P-S	24.2 ± 0.7	6.3 ± 0.08	11 ± 0.3	316.8 ± 9.9	3.1 ± 0.5	11.4 ± 0.1	0.6 ± 0.05
F-S	20.5 ± 0.7	6.3 ± 0.05	10.3 ± 0.3	320.8 ± 9.3	2.2 ± 0.3	12.3 ± 0.1	0.7 ± 0.06
H-S	25 ± 0.3	6.3 ± 0.07	10.9 ± 0.3	295.1 ± 8.9	2.4 ± 0.3	11.4 ± 0.2	0.7 ± 0.09
P-W2	20.8 ± 2.1	6.2 ± 0.05	10.7 ± 0.2	233.7 ± 6.4	2.5 ± 0.8	9.9 ± 0.1	1.1 ± 0.1
F-W2	12.5 ± 2	6.4 ± 0.1	10.7 ± 0.2	237.9 ± 6.8	5.3 ± 0.4	10.1 ± 0.2	0.7 ± 0.1
H- W2	14.1 ± 2.1	6.2 ± 0.08	11.3 ± 0.2	202.4 ± 5.3	3.2 ± 0.3	11.5 ± 0.2	0.7 ± 0.04

Table A.3. Summary of candidate generalized additive mixed model (GAMM) configurations tested for biological soil health indicators. Each model incorporated combinations of climatic covariates (air temperature, soil temperature, precipitation), temporal aggregation windows (Window, 7, 14, 30, or 60 days prior to sampling), lagged temporal dependencies, the inclusion or exclusion of random effects, and correlation structures (continuous autoregressive [CAR1] and autoregressive [AR1]). The Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) were used to identify the most appropriate model for each variable. TMB: total microbial biomass; TF: total fungal biomass; TB: total bacterial biomass; β G: potential β -glucosidase activity; AP: potential acid phosphatase activity; AS: potential arylsulfatase activity; NAG: potential N-acetyl- β -D-glucosaminidase activity; B: blocks (repetitions); ID: sample identification number; D; soil depth. T: true; F: false.

Variable	Model	Window	Climate	Lag	D. Effect	Random	Correlation	AIC	BIC
TMB	GAMM	14 days	T	T	Interaction	B, ID	CAR1	1283.1	1452.6
TMB	GAMM	30 days	T	T	Interaction	B, ID	CAR1	1184.8	1354.4
TMB	GAMM	60 days	T	T	Interaction	B, ID	CAR1	1213.2	1382.8
TMB	GAMM	30 days	F	T	Interaction	B, ID	CAR1	1438.7	1594.9
TMB	GAMM	30 days	T	F	Interaction	B, ID	CAR1	1228.4	1393.6
TMB	GAMM	30 days	T	T	Additive	B, ID	CAR1	1212.2	1337.2
TMB	GAMM	30 days	T	T	Random	B, ID, D	CAR1	1225.3	1345.8
TMB	GAMM	30 days	T	T	Interaction	B, ID	none	1183.5	1348.6
TMB	GAMM	30 days	T	T	Interaction	B, ID	AR1	1184.8	1354.4
TB	GAMM	7 days	T	T	Interaction	B, ID	CAR1	1537.6	1707.2
TB	GAMM	14 days	T	T	Interaction	B, ID	CAR1	1611.4	1781.0
TB	GAMM	30 days	T	T	Interaction	B, ID	CAR1	1528.3	1697.9
TB	GAMM	60 days	T	T	Interaction	B, ID	CAR1	1564.6	1734.2
TB	GAMM	30 days	F	T	Interaction	B, ID	CAR1	1481.2	1637.3
TB	GAMM	30 days	T	F	Interaction	B, ID	CAR1	1570.5	1735.7
TB	GAMM	30 days	T	T	Additive	B, ID	CAR1	1577.2	1702.2
TB	GAMM	30 days	T	T	Random	B, ID, D	CAR1	1582.8	1703.3

TB	GAMM	30 days	T	T	Interaction	B, ID	none	1526.2	1691.3
TB	GAMM	30 days	T	T	Interaction	B, ID	AR1	1528.3	1697.9
TF	GAMM	14 days	T	T	Interaction	B, ID	CAR1	946.5	1116.1
TF	GAMM	30 days	T	T	Interaction	B, ID	CAR1	908.0	1077.6
TF	GAMM	60 days	T	T	Interaction	B, ID	CAR1	973.0	1142.6
TF	GAMM	30 days	F	T	Interaction	B, ID	CAR1	1359.4	1515.5
TF	GAMM	30 days	T	F	Interaction	B, ID	CAR1	918.7	1083.8
TF	GAMM	30 days	T	T	Additive	B, ID	CAR1	907.4	1032.3
TF	GAMM	30 days	T	T	Random	B, ID, D	CAR1	927.4	1047.8
TF	GAMM	30 days	T	T	Interaction	B, ID	none	906.0	1071.1
TF	GAMM	30 days	T	T	Interaction	B, ID	AR1	908.0	1077.6
β G	GAMM	7 days	T	T	Interaction	B, ID	CAR1	6500.3	6668.3
β G	GAMM	14 days	T	T	Interaction	B, ID	CAR1	6606.7	6774.7
β G	GAMM	60 days	T	T	Interaction	B, ID	CAR1	6529.9	6698.0
β G	GAMM	7 days	T	F	Interaction	B, ID	CAR1	6528.9	6692.5
β G	GAMM	7 days	T	T	Additive	B, ID, D	CAR1	6573.5	6697.7
β G	GAMM	7 days	T	T	Random	B, ID, D	CAR1	6595.9	6715.8
β G	GAMM	7 days	T	T	Interaction	B, ID	none	6498.3	6661.8
β G	GAMM	7 days	T	T	Interaction	B, ID	AR1	6500.3	6668.3
AP	GAMM	7 days	T	T	Interaction	B, ID	CAR1	288.9	458.6
AP	GAMM	14 days	T	T	Interaction	B, ID	CAR1	326.9	496.6
AP	GAMM	30 days	T	T	Interaction	B, ID	CAR1	243.9	413.7
AP	GAMM	60 days	T	T	Interaction	B, ID	CAR1	396.7	566.4
AP	GAMM	30 days	F	T	Interaction	B, ID	CAR1	408.2	564.5
AP	GAMM	30 days	T	F	Interaction	B, ID	CAR1	265.5	430.7
AP	GAMM	30 days	T	T	Additive	B, ID	CAR1	231.3	356.3
AP	GAMM	30 days	T	T	Random	B, ID, D	CAR1	232.7	353.3
AP	GAMM	30 days	T	T	Interaction	B, ID	none	241.9	407.2
AP	GAMM	30 days	T	T	Interaction	B, ID	AR1	243.9	413.7
AS	GAMM	7 days	T	T	Interaction	B, ID	CAR1	99.8	269.5
AS	GAMM	30 days	T	T	Interaction	B, ID	CAR1	110.7	280.4
AS	GAMM	60 days	T	T	Interaction	B, ID	CAR1	47.6	217.3
AS	GAMM	60 days	F	T	Interaction	B, ID	CAR1	103.5	259.8
AS	GAMM	60 days	T	F	Interaction	B, ID	CAR1	45.2	210.4
AS	GAMM	60 days	T	T	Additive	B, ID	CAR1	43.8	168.9

AS	GAMM	60 days	T	T	Interaction	B, ID	none	45.6	210.8
AS	GAMM	60 days	T	T	Interaction	B, ID	AR1	47.6	217.3
NAG	GAMM	7 days	T	T	Interaction	B, ID	CAR1	712.2	881.8
NAG	GAMM	14 days	T	T	Interaction	B, ID	CAR1	704.6	874.3
NAG	GAMM	30 days	T	T	Interaction	B, ID	CAR1	710.5	880.2
NAG	GAMM	60 days	T	T	Interaction	B, ID	CAR1	638.0	807.8
NAG	GAMM	60 days	F	T	Interaction	B, ID	CAR1	781.8	938.1
NAG	GAMM	60 days	T	F	Interaction	B, ID	CAR1	635.9	801.1
NAG	GAMM	60 days	T	T	Additive	B, ID	CAR1	638.1	763.1
NAG	GAMM	60 days	T	T	Random	B, ID, D	CAR1	654.2	774.8
NAG	GAMM	60 days	T	T	Interaction	B, ID	none	636.0	801.3
NAG	GAMM	60 days	T	T	Interaction	B, ID	AR1	638.0	807.8

Table A.4. Summary of the best-performing generalized additive mixed models (GAMMs) fitted to biological soil health indicators across treatments, soil depths, and sampling times. Each model incorporated combinations of climatic covariates (air temperature, soil temperature, precipitation) aggregated over temporal windows (7, 14, 30, or 60 days prior to sampling), the inclusion of temporal lags, and depth effects modeled either as additive or interactive terms with treatments. Random effects were specified for blocks (B) and plot identity (ID) to account for the nested experimental design, and correlation structures included CAR(1) for irregularly spaced observations when required. Model performance was evaluated using the Akaike Information Criterion (AIC), coefficient of determination (R^2), root mean squared error (RMSE), and deviance explained (Dev.ex.). The models presented correspond to those minimizing AIC while maintaining high deviance explained and interpretability for each soil health indicator. TMB: total microbial biomass; TF: total fungal biomass; TB: total bacterial biomass; β G: potential β -glucosidase activity; AP: potential acid phosphatase activity; AS: potential arylsulfatase activity; NAG: potential N-acetyl- β -D-glucosaminidase activity; B: blocks (repetitions); ID: sample identification number; D; soil depth. T: true; F: False.

Variable	Window	Climate	Lag	D. Effect	Random	Correlation	AIC	R^2	RMSE	Dev.ex.
TMB	30 days	T	T	Interaction	B, ID	none	1183.5	0.51	26.1	0.50
TB	30 days	F	T	Interaction	B, ID	CAR1	1481.2	0.32	21.2	0.33
TF	30 days	T	T	Interaction	B, ID	none	906.0	0.75	1.6	0.74
β G	7 days	T	T	Interaction	B, ID	none	6498.3	0.72	42.9	0.72
AP	30 days	T	T	Additive	B, ID	CAR1	231.3	0.72	139.8	0.70
AS	60 days	T	T	Additive	B, ID	CAR1	43.8	0.67	14.4	0.66
NAG	60 days	T	F	Interaction	B, ID	CAR1	635.9	0.47	21.9	0.49

Equation A.1.

In our statistical analysis, we used the following model structure:

$$Y_{ijkl}(t) = \beta_0 + \mathbf{f}_{(k)}(t) + \beta_1 T_k + \beta_2 D_l + \beta_3 (T_k \times D_l) + \sum_{m=1}^M \gamma_m X_m(t) + \delta Y_{ijkl}(t-1) + b_i + \mu_{i(j)} + \epsilon_{ijkl}(t)$$

Where $Y_{ijkl}(t)$ is the observed soil health indicator for the block i^{th} , plot j^{th} , treatment k^{th} depth l^{th} , and time t^{th} . β_0 denotes the overall intercept. $\mathbf{f}_{(k)}(t)$ denotes the smooth penalized spline function of time (numeric day since study start) fitted by treatment k^{th} using a base dimension k . $\beta_1 T_k + \beta_2 D_l$ denotes the fixed categorical effects of treatment k^{th} and depth l^{th} . $\beta_3 (T_k \times D_l)$ denotes the interaction effects of treatment k^{th} and depth l^{th} . $X_m(t)$ denotes the climatic covariates (air temperature, soil temperature, precipitation) aggregated over time windows (7, 14, 30, and 60 days), with corresponding regression coefficients γ_m . $Y_{ijkl}(t-1)$ corresponds to the lagged response term accounting for temporal dependency between consecutive sampling times, with an autoregressive parameter δ . b_i denotes the random intercept for the block i^{th} , and $\mu_{i(j)}$ denotes the random intercept of plot identification nested within block i^{th} . Finally, $\epsilon_{ijkl}(t)$ denotes the residual error term modeled using either an AR(1) or CAR(1) correlation structure to account for temporal autocorrelation among repeated measures.

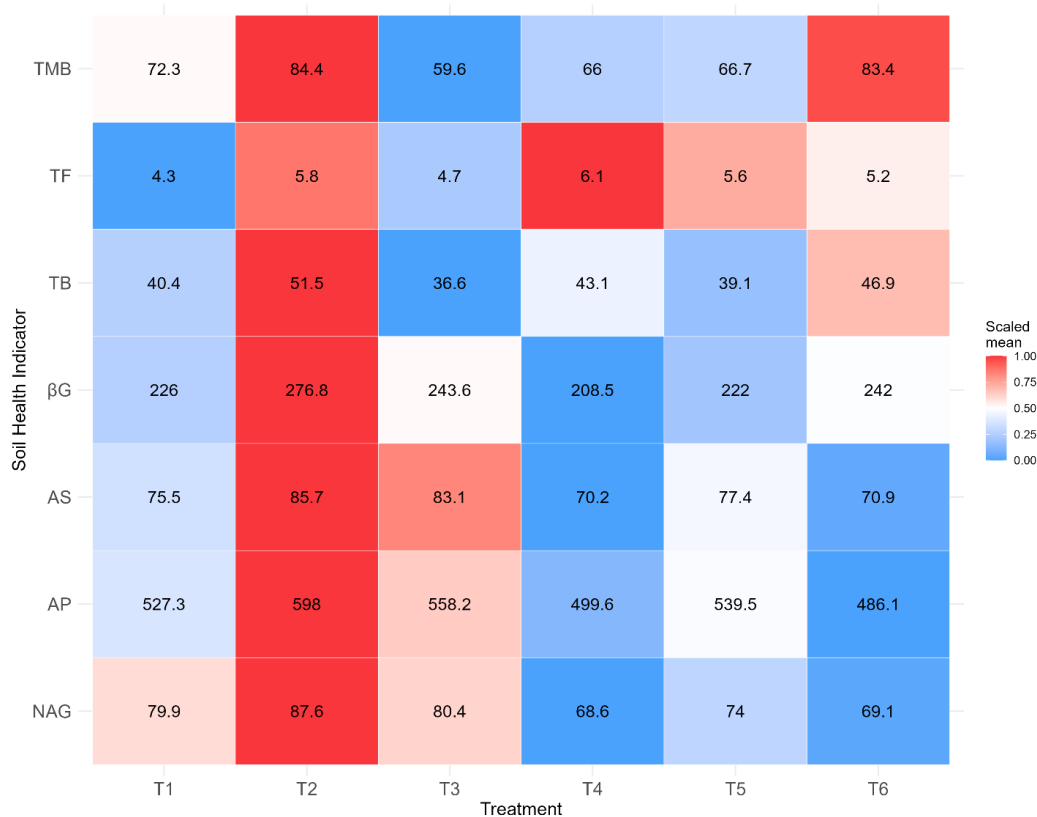


Figure A.1. Heatmap displaying estimated marginal means of soil health indicators at 0-5 cm depth across six treatments. Values represent the scaled means derived from the mixed-model analysis. Red indicates higher relative values, and blue indicates lower relative values. TMB: soil total microbial biomass abundance; TF: soil total fungal biomass abundance; TB: soil total bacterial biomass abundance; βG: soil potential β-glucosidase activity; AS: soil potential Arylsulfatase activity; AP: soil potential acid phosphatase activity; NAG: soil potential N-acetyl-β-D-glucosaminidase activity.

Appendix B - Chapter 3

Table B.1. Crop management information from the study site across the six treatments from 2019 to 2024. Crops, planting dates, and harvest/mow dates. T1: Control treatment; T2: Adaptive Forage treatment; T3: Semi-intensive treatment; T4: Intensive treatment; T5: Dual-purpose Semi-intensive treatment; T6: Dual-purpose Intensive treatment.

Season	Treatment	Crop	Management	
			Planting Date	Harvest/Mow Date
19/20	T1	Wheat	10/24/19	7/7/20
19/20	T2	Wheat	10/24/19	7/7/20
19/20	T3	Wheat	10/24/19	7/7/20
19/20	T4	Wheat	10/24/19	7/7/20
19/20	T5	Wheat	10/24/19	7/7/20
19/20	T6	Wheat	10/24/19	7/7/20
20	T1	Fallow	-	-
20	T2	Forage Soybeans	5/22/20	multiple
20	T3	Soybeans	7/8/20	10/20/20
20	T4	Sorghum	5/22/20	11/3/20
20	T5	Fallow	-	-
20	T6	Moth beans	5/22/20	10/20/20
20/21	T1	Wheat	10/15/20	6/21/21
20/21	T2	Wheat	9/24/20	6/21/21
20/21	T3	Wheat	11/7/20	6/21/21
20/21	T4	Fallow	-	-
20/21	T5	Triticale	9/24/20	6/21/21
20/21	T6	Wheat	9/24/20	6/21/21
21	T1	Fallow	-	-
21	T2	Fallow	-	-
21	T3	Fallow	-	-

21	T4	Soybeans	7/6/21	10/25/21
21	T5	Soybeans	7/6/21	10/25/21
21	T6	Alfalfa		multiple
21/22	T1	Wheat	10/22/21	6/30/22
21/22	T2	Wheat	10/5/21	6/30/22
21/22	T3	Wheat	10/22/21	6/30/22
21/22	T4	Wheat	11/8/21	6/30/22
21/22	T5	Wheat	10/22/21	6/30/22
21/22	T6	Alf+Trit		
22	T1	Fallow	-	-
22	T2	Forage Soybeans	5/17/22	multiple
22	T3	Soybeans	7/12/22	11/7/22
22	T4	Corn	7/12/22	11/7/22
22	T5	Fallow	-	-
22	T6	Alfalfa		multiple
22/23	T1	Wheat	10/13/22	6/23/23
22/23	T2	Wheat	9/19/22	6/23/23
22/23	T3	Wheat	11/21/22	6/23/23
22/23	T4	Triticale	11/21/22	6/23/23
22/23	T5	Triticale	9/19/22	6/23/23
22/23	T6	Wheat	9/19/22	6/23/23
23	T1	Fallow	-	-
23	T2	Forage Soybeans	5/22/23	multiple
23	T3	Fallow	-	-
23	T4	Soybeans	5/22/23	10/20/23
23	T5	Soybeans	5/22/23	10/20/23
23	T6	Alfalfa		multiple
23/24	T1	Wheat	10/9/23	6/30/24
23/24	T2	Wheat	9/21/23	6/30/24
23/24	T3	Wheat	11/7/23	6/30/24
23/24	T4	Wheat	11/7/23	6/30/24
23/24	T5	Wheat	9/21/23	6/30/24

23/24	T6	Triticale	9/21/23	6/30/24
24	T1	Fallow	-	-
24	T2	Forage Soybeans	5/14/24	multiple
24	T3	Soybeans	7/4/24	10/29/24
24	T4	Corn	7/4/24	10/14/24
24	T5	Fallow	-	-
24	T6	Forage Soybeans	5/14/24	multiple

Table B.2. Monthly mean temperature (T) and accumulated precipitation (PP) recorded from October 2019 to October 2024 in the study site. Values between brackets indicate the absolute deviation from the historical value (30 years). Source: Kansas Mesonet Station in Manhattan (2025).

Month	2019		2020		2021	
	T (°C)	PP (mm)	T (°C)	PP (mm)	T (°C)	PP (mm)
Jan	-	-	-0.1 (+1.2)	17 (+3.1)	1.3 (+2.4)	22.9 (+9.8)
Feb	-	-	1.4 (+0.2)	20.1 (-1.1)	-5.1 (-6.3)	2.5 (-18.7)
Mar	-	-	8.7 (+1.4)	64.2 (+19)	9.4 (+2.1)	91.7 (+46.5)
Apr	-	-	11.7 (-1.1)	49.3 (-29.3)	12.1 (-0.7)	52.3 (-26.3)
May	-	-	16.5 (-2.1)	141.2 (+19.3)	16.7 (-1.9)	133.9 (+12)
Jun	-	-	25.6 (+1.5)	88.9 (-35.1)	25.1 (+1)	66.8 (-57.2)
Jul	-	-	26.1 (-0.3)	168.4 (+74.3)	25.4 (-1)	161.8 (+67.7)
Aug	-	-	24.6 (-0.9)	45.5 (-59.9)	26.4 (+0.9)	71.6 (-33.8)
Sep	-	-	19.3 (-1.5)	54.6 (-21.8)	23.1 (+2.3)	69.1 (-7.3)
Oct	10.5 (-3.3)	60.9 (+5)	11.4 (-2)	16.8 (-39.1)	15.5 (+1.7)	82.8 (+26.9)
Nov	4.9 (-1.8)	7.4 (-25)	8.6 (+1.9)	60.2 (+27.8)	8.6 (+1.9)	28.9 (-3.5)
Dec	2.4 (+1.7)	17.8 (-4)	2.2 (+1.5)	24.4 (+26)	5.5 (+4.8)	8.1 (-13.7)
Month	2022		2023		2024	
	T (°C)	PP (mm)	T (°C)	PP (mm)	T (°C)	PP (mm)
Jan	-1.1 (0)	6.9 (-7)	0.3 (+1.4)	56.4 (+42.5)	-3.8 (-2.8)	50.8 (+36.9)
Feb	0.1 (-1.1)	2 (-19.2)	2.6 (+1.4)	26.7 (+5.5)	6.7 (+5.5)	32.2 (+11)
Mar	6.6 (-0.6)	56.6 (+11.4)	5.8 (-1.4)	23.6 (-21.6)	8.7 (+1.5)	37.6 (-7.6)
Apr	12.4 (-0.4)	25.6 (-53)	13.3 (+0.5)	51.3 (-27.3)	14.7 (+1.9)	101.6 (+26)
May	19 (+0.4)	231 (+109)	19.4 (+0.8)	131.8 (+9.9)	18.7 (+0.1)	197.6 (+75.7)
Jun	24.6 (+0.5)	154 (+30.4)	23.8 (-0.3)	82.8 (-41.2)	25.2 (+1.1)	106.7 (-17.3)
Jul	26.1 (-0.3)	128 (+33.9)	25.8 (-0.6)	81.5 (-12.6)	25.5 (-0.9)	122.2 (+28.1)
Aug	25.6 (+0.1)	33.3 (-72.1)	26.5 (+1)	49.8 (-55.6)	24.9 (-0.6)	70.9 (-34.5)
Sep	22 (+1.2)	58.2 (-18.2)	22.8 (+2)	17.5 (-58.9)	21.6 (+0.8)	14.9 (-61.5)
Oct	14.5 (+0.7)	29.9 (-26.1)	14.4 (+0.6)	53.6 (-2.4)	17.2 (+3.4)	26.7 (-29.3)
Nov	5.6 (-1.1)	90.7 (+58.3)	7.3 (+0.6)	51.5 (+19.1)	-	-
Dec	-0.9 (-1.6)	29.7 (+7.9)	3.7 (+3)	68.8 (+47.4)	-	-

Table B.3. Contrast estimates of treatment effects on changes (2019-2024) in water-stable aggregates (WSA) fractions at 5-15 and 15-30 cm depth, relative to the control. Positive values indicate greater increases in WSA mass relative to the control from 2019 to 2024, while negative values indicate smaller increases or decreases.

Contrast	WSA > 2.0 mm	WSA 2 - 0.25 mm	WSA 0.25 – 0.053 mm	WSA 0.053 – 0.02 mm
g kg soil ⁻¹				
5-15 cm				
T2 – T1	-1.3	10.4	-9.2	0.3
T3 – T1	-9.2	-8.7	21.6	-2.5
T4 – T1	-8.6	3.1	6.3	-0.3
T5 – T1	-2.1	12.4	29.6	0.1
T6 – T1	-6.5	7.1	-4.5	2.3
15-30 cm				
T2 – T1	-3.1	-0.1	7.7	-1.2
T3 – T1	-6.7	-6.4	7.9	0.9
T4 – T1	-3.4	-5.3	3.1	1.6
T5 – T1	5.5	3.0	-10.8	-0.6
T6 – T1	-0.5	-1.4	-3.3	0.1

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

Table B.4. Contrast estimates of treatment effects on changes (2019-2024) in soil organic carbon and soil microbial community composition at 5-15 cm and 15-30 cm depth. relative to the control. Positive values indicate greater increases in SOC and microbial community composition metrics relative to the control from 2019 to 2024, while negative values indicate smaller increases or decreases. SOC: soil organic carbon; TMB: soil total microbial biomass; TB: soil total bacterial biomass; TF: soil total fungal biomass.

Contrast	SOC	TMB	TB	TF
	g kg ⁻¹	nmol PLFA g soil ⁻¹		
		5-15 cm		
T2 – T1	-1.6	12.9	8.3	-0.8
T3 – T1	-0.5	10.3	7.7	-0.1
T4 – T1	1.7	23.1	26.4	-0.8
T5 – T1	-1.5	19.9	20.3	-0.9
T6 – T1	-2.6	2.0	-7.9	1.9
		15-30 cm		
T2 – T1	-0.4	16.1	12.9	0.9
T3 – T1	0.3	6.4	7.8	-0.2
T4 – T1	0.1	30.0	27.2	0.7
T5 – T1	1.4	19.5	18.8	0.5
T6 – T1	-0.3	10.2	4.8	1.9

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

Table B.5. Contrast estimates of treatment effects on changes (2019-2024) in soil organic carbon and soil microbial community composition at 5-15 cm and 15-30 cm depth. relative to the control. Positive values indicate greater increases in greater Gram +, Gram -, Actinomycetes, and AMF biomass from 2019 to 2024, while negative values indicate lower Gram +, Gram -, Actinomycetes, and AMF biomass from 2019 to 2024. Gram +: gram-positive bacteria; Gram -: gram-negative bacteria; AMF: arbuscular mycorrhizal fungi.

Contrast	Gram +	Gram -	Actinomycetes	AMF
nmol PLFA g soil ⁻¹				
5-15 cm				
T2 – T1	7.3	-0.3	1.3	0.1
T3 – T1	6.7	-1.1	2.1	0.5
T4 – T1	-5.4	32.3	-0.5	0.2
T4 – T1	3.0	17.3	-0.1	-0.2
T6 – T1	11.1	-21.2	2.1	0.5
15-30 cm				
T2 – T1	2.8	9.6	0.5	0.8
T3 – T1	-1.7	9.9	-0.5	-0.2
T4 – T1	1.4	25.9	-0.1	0.6
T4 – T1	1.3	17.6	-0.1	0.4
T6 – T1	1.4	3.2	0.1	1.3

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

Table B.6. Contrast estimates of treatment effects on changes (2019-2024) in potential soil extracellular enzyme activities at 5-15 cm and 15-30 cm depth. relative to the control. Positive values indicate greater increases in β G, AP, AS, and NAG activities from 2019 to 2024, while negative values indicate lower β G, AP, AS, and NAG activities from 2019 to 2024.

Contrast	β G	AP	AS	NAG
	$\mu\text{g PNP g soil}^{-1} \text{ hour}^{-1}$			
	5-15 cm			
T2 – T1	16.7	142.6	20.9*	12.3
T3 – T1	10.4	-323.3	19.7*	14.1
T4 – T1	8.3	101.1	5.9	3.5
T4 – T1	41.4	-69.2	8.7	15.4
T6 – T1	23.0	-110.9	13.9	1.9
	15-30 cm			
T2 – T1	-3.8	25.4	6.9	29.4
T3 – T1	-2.8	9.0	4.5	-50.6
T4 – T1	-6.4	-6.5	-2.4	27.6
T4 – T1	-14.2	-5.3	8.4	17.8
T6 – T1	0.1	-9.1	4.5	7.3

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

Appendix C - Chapter 4

Table C.1. Description of crop rotation sequences for the on-farm research experiments in Beloit, Bucyrus, and Solomon, KS. W. Wheat: winter wheat; Mix: multi-species mix of cover crops; C: corn; SB: soybeans; WW: winter wheat; GS: grain sorghum; TT: triticale; CR: cereal rye; Mix: cover crop species mixture (grasses, legumes, brassicas).

Site	2018		2019		2020		2021		2022		2023		2024		2025	
Beloit	NC		WW		-		GS		-		SB		WW		GS	
	CC		WW		Mix		GS		TT		SB		WW		GS	
Bucyrus	NC	C	-	SB	-	C	-	SB	-	C	-	SB	-	C	-	SB
	CC	C	CR	SB	Mix	C	CR	SB	Mix	C	CR	SB	Mix	C	C.R	SB
Solomon	NC		-		WW		-		SB		WW		-		GS	
	CC		-		WW		Mix		SB		WW		Mix		GS	

Table C.2. Characterization of pH, texture, extractable P, macroaggregates, and microaggregates at Beloit, Bucyrus, and Solomon at the baseline soil health assessment year and the last soil health assessment across treatments at 5-15 cm depth. P: extractable phosphorus; Macro: water-stable macroaggregates; Micro: water-stable microaggregates.

Treatment	Year	pH	Sand	Silt	Clay	P	Macro	Micro
			%			g kg ⁻¹	g 100 g ⁻¹	
			5-15 cm					
			Beloit					
NC	2019	4.9	25.6	47.8	26.6	39.7	19.9	58.8
CC		5	21.2	50.4	27.9	38.2	30.4	47.3
NC	2025	4.9	-	-	-	45.6	28.6	60
CC		5.2	-	-	-	38.6	31.6	56.9
			Bucyrus					
NC	2018	5.6	12.4	59.4	28.2	17.3	53.6	36.6
CC		5.7	12	59.1	29	15.3	61.9	27.8
NC	2024	7	-	-	-	23	66.4	27.7
CC		7.1	-	-	-	17.1	71.2	22.4
			Solomon					
NC	2019	5.1	13.1	58.9	28	19.7	18.8	64
CC		5.3	13.1	56.9	30.1	16.7	20.6	61.5
NC	2025	5.2	-	-	-	25.9	30.4	60
CC		5.4	-	-	-	17	29.2	60.4

Table C.3. Characterization of pH, texture, extractable P, macroaggregates, and microaggregates at Beloit, Bucyrus, and Solomon at the baseline soil health assessment year and the last soil health assessment across treatments at 15-30 cm depth. P: extractable phosphorus; Macro: water-stable macroaggregates; Micro: water-stable microaggregates.

Treatment	Year	pH	Sand	Silt	Clay	P	Macro	Micro
			%			g kg ⁻¹	g 100 g ⁻¹	
			15-30 cm					
			Beloit					
NC	2019	6.3	19.2	49.4	31.3	3.7	30.4	50.9
CC		6.4	19.3	46.5	34.2	4.8	37.1	45.2
NC	2025	5.5	-	-	-	5.5	32.6	56.6
CC		5.7	-	-	-	5.8	29.4	58.5
			Bucyrus					
NC	2018	5.7	11.7	58.2	30.1	7.3	61	28.4
CC		5.8	11.6	57.6	30.8	4.1	60.7	26.6
NC	2024	6.8	-	-	-	6.8	59.3	34.1
CC		6.9	-	-	-	6.9	63.5	30.1
			Solomon					
NC	2019	5.4	12.7	58.5	28.7	11	36.7	58.9
CC		5.5	12.3	57	30.7	9.2	40.2	55.7
NC	2025	5.4	-	-	-	6.6	22.5	66.5
CC		5.6	-	-	-	5.5	26.3	62.3

Table C.4. Characterization of chemical and biological soil health indicators in Beloit, Bucyrus, and Solomon at the baseline soil health assessment year and last soil health assessment across treatments at 5-15 cm depth. SOC: soil organic carbon; TMB: soil total microbial biomass; TB: soil total bacteria biomass; TF: soil total fungi biomass; β g: soil potential β -glucosidase activity; AS: soil potential arylsulfatase activity; AP: soil potential acid phosphatase activity; NAG: soil potential N-acetyl- β -D-glucosaminidase activity.

Treatment	Year	SOC	TMB	TB	TF	β g	AS	AP	NAG
		g. kg ⁻¹	nmol PLFA g soil ⁻¹			μ g PNP g soil ⁻¹ h ⁻¹			
			5-15 cm						
			Beloit						
NC	2019	1.1	23.8	15.5	1.0	63.8	22.5	355.8	21.9
CC		1.1	24.1	15.6	1.1	65.3	26.5	356.8	22.9
NC	2025	1.2	23.5	18.2	0.6	62.1	33.0	442.5	27.9
CC		1.2	22.9	17.2	0.6	61.5	37.8	444.0	29.7
			Bucyrus						
NC	2018	1.7	20.9	10.9	1.0	56.2	54.7	285.4	24.6
CC		1.7	19.8	10.9	0.9	60.3	52.7	258.8	24.7
NC	2024	1.8	193.7	180.8	12.9	88.0	98.9	309.3	24.8
CC		1.9	239.8	220.8	18.9	107.9	133.1	361.3	31.6
			Solomon						
NC	2019	1.3	22.7	11.6	3.7	93.5	43.4	593.0	33.1
CC		1.3	24.1	12.3	3.9	96.6	48.1	589.2	34.2
NC	2025	1.3	168.8	117.8	5.9	216.4	35.9	443.2	20.9
CC		1.3	276.3	210.7	8.3	227.8	42.9	443.8	24.7

Table C.5. Characterization of chemical and biological soil health indicators in Beloit, Bucyrus, and Solomon at the baseline soil health assessment year and last soil health assessment across treatments at 15-30 cm depth. SOC: soil organic carbon; TMB: soil total microbial biomass; TB: soil total bacteria biomass; TF: soil total fungi biomass; β g: soil potential β -glucosidase activity; AS: soil potential arylsulfatase activity; AP: soil potential acid phosphatase activity; NAG: soil potential N-acetyl- β -D-glucosaminidase activity.

Treatment	Year	SOC	TMB	TB	TF	βg	AS	AP	NAG
		g. kg ⁻¹	nmol PLFA g soil ⁻¹			μg PNP g soil ⁻¹ h ⁻¹			
		15-30 cm							
		Beloit							
NC	2019	0.9	23.8	15.5	1.0	63.8	22.5	355.8	21.9
CC		0.9	24.1	15.6	1.1	65.3	26.5	356.8	22.9
NC	2025	1.1	23.5	18.2	0.6	62.1	33.0	442.5	27.9
CC		1.1	22.9	17.2	0.6	61.5	37.8	444.0	29.7
Bucyrus									
NC	2018	1.7	20.9	10.9	1.0	56.2	54.7	285.4	24.6
CC		1.7	19.8	10.9	0.9	60.3	52.7	258.8	24.7
NC	2024	1.8	193.7	180.8	12.9	88.0	98.9	309.3	24.8
CC		1.9	239.8	220.8	18.9	107.9	133.1	361.3	31.6
Solomon									
NC	2019	1.3	21.3	10.9	3.3	76.5	41.6	444.7	39.1
CC		1.3	20.5	10.4	3.2	89.5	42.8	461.5	36.4
NC	2025	1.2	149.9	111.0	4.2	157.1	32.8	317.1	14.4
CC		1.2	192.9	141.8	6.0	175.1	42.1	357.3	17.4