The interaction of precipitation and land use effect on biological and chemical soil health indicators across a Kansas precipitation gradient

by

Tiffany Poydras

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Approved by:

Major Professor
Charles W. Rice

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Abstract

As a concept and principal, soil health is intricately entwined with climate change. The health and stability of the soil is vital to reducing agricultural CO2 emissions and increasing sequestered carbon to combat climate change. To improve the effectiveness of soil health practices, it is essential that thorough investigations be made on the interactions between soil health principles in climate change as well as how responses can change with different land uses and increasing depth. Analysis of the effect of temperature, elevation, water content, and seasonal variability have been thoroughly explored, but the legacy that precipitation has on soil health in different land uses has been widely looked over. Conveniently, Kansas is the perfect place for this investigation because it has a rather consistent soil-type, land formation and a grassland ecosystem with a linear precipitation gradient. To test this relationship, three different land uses were sampled, representing three different levels of disturbance (Native Prairie, Restored Prairie, and AG) across 12 different locations, creating a linear gradient increasing from west to east from 400 mm to 1100 mm of mean annual precipitation. These sites were sampled to a depth of 120 cm and divided into five depth increments of 0-5 cm, 5-15 cm, 15-30cm, 30-75cm, and 75-120cm. Analysis of soil was conducted using soil health indicators, including soil organic carbon, total nitrogen, enzyme activity, PLFA, and CO2 respiration. Statistical analysis was performed in R using both univariate and multivariate statistical models to show the complex interaction between factors. Results show that disturbance impacted the relationship between soil health even after restoration, and sites with active agricultural use were decoupled from the effects of precipitation.

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Dedication

I would like to dedicate this to my mother and sister. Thank you for supporting me and always being patient with me.

Chapter 1 - Introduction and Literature Review

Dust Bowl

During the 1930s, Kansas, Colorado, Texas, and Oklahoma experienced an environmental disaster unlike anyone in the US had ever seen before. About a century before, westward expansion had led to the colonizing of the Great Plains, with the main appeal of this region being farming the fertile Mollisols, rich in key essential nutrients and easy to till (Baumhardt et al., 2015; Chen et al., 2020). The farms in the Dust Bowl region, from the southwestern tip of Kansas to the northernmost region of Texas, used primarily fallow cropping due to a lack of precipitation that made fallow water storing period necessary (Cook et al., 2009; Baumhardt et al., 2015). However, the excessive plowing practices, the loss of native grasses, and prolonged drought and high temperatures brought by the El Nino Southern Oscillation were enough to cause complete devastation (Cook et al., 2008; 2009). The Dust Bowl was a social, environmental, and economic disaster. In some regions, upwards of 25 percent of topsoil was lost, and due to the dust storms blocking the sun for a good portion of the growing season, yields were suppressed (Caprettini and Voth, 2022).

The stress and unlivable living conditions in this region lead to a mass exodus, farm abandonment, business loss, and a tremendous increase in unemployment (Long and Siu, 2018). This man-made environmental disaster was the wake of call for the US government regarding the importance of farming and good farming practices (Hobbs et al., 2007). Policies and programs such as The Soil Conservation Service were established, which focused on soil conservation and land rehabilitation. Efforts were made to provide farmers with the financial support needed to implement more sustainable farming practices (Hobbs et al., 2007; Hyde and Mahalov, 2022).

The catastrophe of the Dust Bowl highlighted the intricate relationship between human activity, climate, and soil health.

History of Soil Health

The concept of Soil Health was derived from an earlier phrase, “soil quality” (Karlen et al., 1997). Soil quality was used for decades before soil health was introduced. It was first described as “the ability to yield corn, soybeans, and wheat under conditions of high-level management,” considering that crops differed in other regions (Mausel, 1971). Essentially, this meant that soil quality was first defined as the ability of the soil to support agricultural production, be it forage or crop (Wienhold et al., 2004). As the term “soil quality” became more popular, the definition was redefined often to encompass more of the ecosystem services provided by soil (Parr et al., 1992).

New definitions of soil quality reflected the original focus of the soil science community, the support of crop and animal production as well as minimizing soil erosion, but more environmentally focused researchers expressed distaste at the quality of soil being based on its ability to benefit agricultural production (Larson and Pierce, 1991). Additionally, research showing the effect that biological activity had on protecting plants from diseases and creating resistance to erosion showed the importance of the biological components of the soil (Fierer et al., 2021). Earlier research and assessments on soil quality comprised physical and chemical indicators, mainly organic matter, pH, available P, and water storage (Bünemann et al., 2018). While the biological components of soil were researched early on, the novelty of this research delayed its movement into the analysis of soil quality.

The official definition of soil quality was defined by the Soil Science Society of America (Karlen et al. 1997), stating that it is “the capacity of a specific kind of soil to function, within

natural or managed boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation." Soil quality resides in environmental quality control with air and water quality. However, the research on soil quality did not reflect changing views and environmental control (Andrews et al., 2002). Much debate came with this redefining due to its over-emphasis on crop production and the redefining of soil quality to fit the growing emphasis on soil's ecosystem service. Additionally, the adoption of newer practices targeted to improve soil quality was minimal due to the lack of visibility of the importance of the soil in comparison to air and water, as well as the complexity of the soil ecosystem (Andrews et al., 2002; Nortcliff, 2002). The redefining of soil quality not being fully accepted led to the newer definitions used to define soil health and for these two terms to be used interchangeably. Thus, the critical difference between these two concepts is shown by Cardoso et al. (2013), who used soil quality in terms of referring to "a soil's fitness for a specific use" and defined soil health as being "the capacity of the soil to function as a vital living system, within ecosystem and land-use boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and promote plant and animal health."

Soil Health

Over the past decade, due to population growth, industrialization, manmade disasters, and a growing emphasis on One Health, the importance of the field and concept of Soil Health has significantly expanded (Zinsstag et al., 2011; Baum et al., 2017; Yang et al., 2022). Understanding that all forms of life are intimately linked together brings us one step closer to creating integrated health systems focused on decreasing problems instead of treating problems as they come (Zinsstag et al., 2011). Soil health principles use this way of thinking and apply it to the soil. Many common management practices are detrimental to the stability, functioning, and

"health" of the soil, including over-fertilizing, tillage, excess pesticides, and excessive biomass removal (Diacono & Montemurro, 2010; Crovo et al., 2021). These practices are designed to create the best yield, and for a long time, soil management wholly disregarded the soils to which they were applied (Diacono & Montemurro, 2010). These practices have largely negative impacts on the physical and chemical aspects of the soil, but most notably, biological characteristics of the soil (Franzluebbers, 2016; Hermans et al., 2017). The biological aspect of the soil consists of micro- and meso-fauna that rely on stable soil systems and a living root to function best (Banerjee & Heijden, 2022; Kou et al., 2021). Microorganisms facilitate the cycling of nutrients in the soil ecosystem directly through exoenzymes used for decomposition and indirectly through their necromass (Schimel et al., 2007; Wagg et al., 2014). Microbial activity in the soil plays important roles in increasing nutrient uptake in plants, disease resistance, erosion resistance, aggregation, and carbon sequestration (Chen et al., 2020; Muramoto et al., 2022). However, under the presence of improper land management practices, microbial activity and diversity are greatly diminished, leaving soils and crops vulnerable to the environment (Smith et al., 2016; Wagg et al., 2014; Zeiss et al., 2022).

Soil health practices are directly designed to protect the biological and chemical aspects of the soil while maintaining physical balance (Karlen et al. 1997). This is done by incorporating management practices that protect the soil ecosystem, such as crop rotations, cover crops, and no-till/conservation tillage (Schmidt et al., 2018). Soil health practices have been shown to influence the soil beneficially, but when combined with climate limitations such as water content, there can be a negative influence on the yield of agricultural fields (Blanco-Canqui et al., 2015). Governmental subsidies and carbon credits have supported the application of soil health practices and have worked to cover the economic hurdle that may come with changing

management style as beneficial effects can sometimes take multiple years to develop (Randazzo et al., 2023; Qin et al., 2023). Soil health practices are sensitive to increasing depth, but as most research focuses on the most active portion of the soil, the top 30 cm, there is a lack of information about how soil health can change with depth (Costantini & Mocali, 2022). Soil health indicators are sensitive to ecosystem and land management changes, making them useful indicators for predicting future effects of climate change and the effectiveness of land management practices (Muramoto et al., 2022). As biological and chemical soil health indicators directly support physical soil health indicators, the focus shall remain on the former.

Chemical Soil Health Indicators

Carbon

The soil is the largest terrestrial stock of C and the main source of atmospheric carbon (Lal, 2004; Batjes, 1996). As the genesis of most atmospheric carbon postindustrial comes from terrestrial or subterranean portions of the earth, some strategies to recapture C include replenishing soil C stocks (Cheng et al., 2007; Legg, 2021). Agricultural soils contain much smaller pools of carbon due to anthropogenic activity but have more potential to increase carbon content in comparison to native soils (Senthilkumar et al., 2009; Bruun et al., 2013). However, more productive ecosystems may not be able to store additional soil carbon (Plaza et al., 2022). This effect might not be a concern for soils under management that have already degraded carbon stocks (Lal, 2004).

Soil organic matter (SOM) is the backbone of all ecosystem functions within the soil and is primarily comprised of soil organic carbon (SOC) (Schlesinger and Bernhardt, 2013; Soil Science Society of America, 1997). Soil OC exists in many forms and is constantly cycled through the ecosystem (Singh et al., 2018). Soil OC is comprised primarily of degraded material

and the amount is dependent on management, soil type, and climate (Bronick and Lal, 2005). Soil OC can indicate soil stability and resilience, as it contributes to soil aggregation that then creates resistance to erosion and leaching (Sahoo et al., 2019; Sadeghi, 2021). Additionally, changes in the amount of SOC can show the effectiveness of conservation practices, as ineffective conservation practices will disturb soil carbon stocks (Sahoo et al., 2019). Soil C pools are greater in colder ecosystems and are vulnerable to increased temperatures, which can increase microbial metabolism, leading to C degradation (Plaza et al., 2022).

As SOC is supplied by adding aboveground and belowground biomass, there is a natural decrease in amount and dynamics with depth (Henneron et al., 2022). In the subsoil, C age is older and can be considered relatively sequestered. The stability of subsurface C can be attributed to N and oxygen unavailability, nutrient deficiency, soil type, and aggregation. Fontaine et al. (2007) studied the stability of a previously buried A horizon by adding fresh C to the subsoil. Subsurface C decreased due to microbial mineralization of older C. Subsurface soils have minimal nutrient additions, as belowground nutrient inputs are minimal. Clay content can increase with the amount of subsurface C, but the stability of subsurface C was not found to be related to mineral fixation (Fontaine et al., 2007). Additionally, deep tillage has shown the ability to mix soil to the point of increasing diffusion of C and N throughout the soil profile (Veenstra and Burras, 2015). Soil carbon dynamics are slower at lower depths due to low energy supplied by carbon coupled with the high amount of energy required for decomposition (Henneron et al., 2022). The stability of subsurface C can be attributed to the constraints on microbial activity in the subsoil environment. (Fontaine et al., 2007).

The SOC fractions can be divided into particulate organic matter (POM) and mineral-associated organic matter (MAOM) (Cotrufo et al., 2015). In the soil, POM fraction primarily

consists of plant material, that is easily decomposed and sensitive to disturbance making it labile (Li et al., 2008). Depending on the soil and land use, POM can be associated with either stable or unstable carbon fractions and is seen to persist and even accumulate indefinitely (Cotrufo et al., 2019). The MAOM fraction consists of microbial derived carbon, resistant to disturbance due to being mineral bound (Jamala, 2013; Canarini et al., 2017; Cotrufo et al., 2019). This form of carbon is found abundantly in grasslands and has been found to significantly correlate with clay content, cation exchange capacity, and iron content in the soil (Yu et al., 2023).

Nitrogen

Nitrogen (N) is essential for ecosystem functioning and greatly limits biological activity when deficient. Nitrogen directly impacts plant productivity and physiology by facilitating photosynthesis and increasing biomass. In most ecosystems, nitrogen is limited due to minimal nitrogen inputs coming from wildlife and nitrogen deposition (Olin et al., 2015; Luo et al., 2004). Nitrogen dynamics are correlated to external factors such as climate and land use. In grassland ecosystems, increased N mineralization, nitrification rates, and total nitrogen content strongly correlate with increased annual precipitation (Feyissa et al., 2021; Wang et al., 2023).

Additionally, elevation, SOM, and silt correlate with the amount of soil nitrogen that exists in grassland ecosystems (Wang et al., 2023). In water and nutrient-limited agricultural systems, land use greatly influenced SOC and TN in the top 40 cm while soil type and texture played a greater influence from 100-300 cm (Cao et al., 2021). Thus, soil type and texture responded differently by depth.

For grassland ecosystems, limited nitrogen can disrupt the relationship between the ecosystem and precipitation, as there is enough moisture but not enough nitrogen, thus limiting growth and photosynthesis. Controlled burns contribute to increased nitrogen by releasing

nitrogen from organic matter, controlling invasive species, and encouraging grazing (Hobbs et al., 1991; Tilman et al, 2006; Pathak et al., 2017) For agricultural soils, an additional barrier is placed between that of the soil and nitrogen dynamic through tillage. N-rich fertilizers applied to agricultural soils ensure productivity and yield. Fertilizing for yield and not for optimization has contributed to environmental degradation coupled with climate change as excess N is released during rain events as N_2O or leached to water sources (Carpenter et al., 1998). For this reason, it is important to continue to analyze the dynamic that N has with precipitation, land use, and depth.

Carbon: Nitrogen Ratio

The ratio of carbon to nitrogen directly controls ecosystem function, as it influences the availability of nutrients and decomposition rates (Garnier et al., 2004). An imbalance in the C:N ratio can lead to adverse effects on the soil ecosystem, from microbial activity limiting immobilization to accelerated decomposition, especially of previously "sequestered" or stored C stocks. In prairie ecosystems, it is more common to find higher C:N ratios in comparison to agricultural sites due to the lack of N inputs to the system. This leads to protected carbon that, if never disturbed, could be considered sequestered. For agricultural soils derived from grasslands, an initial increase in the C:N ratio is seen in the first years, but as cultivation continues, a decline in C:N (Pan et al., 2022). This simulates the initial uptake of N stored in the soil and the destruction of stored carbon in aggregates by tillage.

CO_2 Respiration

Most carbon applied to the soil is lost to the atmosphere as CO_2, caused by microbial respiration (Lloyd & Taylor, 1994). As a result of climate change, atmospheric CO_2 levels have risen by 50% in comparison to preindustrial levels (Legg, 2021). As C is the primary source of

food for plants, the implication that elevated CO_2 levels have been widely studied, showing mixed effects on both aboveground and belowground respiration. For aboveground and belowground ecosystems, responses to elevated CO_2 levels are directly controlled by climate as well as nutrient and water availability (Zou et al., 2023). This has primarily been shown to be due to C content and energy requirements. As temperatures rise, CO_2 respiration increases as the energy requirements for decomposition have increased (Lloyd & Taylor, 1994; Melillo et al., 2011). This effect is limited by the availability of nutrients and water, that are necessary to facilitate the increased metabolic activity (Melillo et al., 2011). Under controlled simulations where nutrients are not limited, the effects of elevated CO_2 levels are positive and can even facilitate an increase in soil water content (Liu et al., 2015; Zou et al., 2023). Elevated CO_2 causes increased biomass in plants, as there is less effort exerted by the plant to capture CO_2, while soil microbial activity relies on having a stable C:N ratio to facilitate degradation, and excess C can cause N immobilization (Niklaus et al., 1998; Zou et al., 2023). However, elevated CO_2 levels may not correlate with increased microbial biomass as much as it does increase microbial activity (Liu et al., 2015; Mann et al., 2019).

Biological Soil Health Indicators

Microbial Biomass

Microbial biomass abundance and composition can be measured by phospholipid fatty acid analysis (PLFA), which uses the fatty acids found in the cell membrane of living organisms to specify what type of microorganism is being analyzed (Olsson, 1999; Bååth et al. 2004). This technique does not require culturing, creating a better representation of the living community (Hill et al., 2000). The use of phospholipids as a biomarker is similar to using 16S rRNA gene sequence analysis as it pioneered culture independent microbial analysis, but PLFA is more

functional for characterizing the community composition (Pace, 1997). Because cell membranes and their fatty acids are one of the first things to degrade after the death of microorganisms, PLFA identifies the living or recently living organisms, creating a clear picture of the living community composition (Kirk et al., 2004; Kaur et al., 2005). The composition of PLFAs has been shown to shift with soil health indicators, such as pH (Rousk et al., 2010). This technique has been shown to sufficiently replicate the community composition and is useful for assessing how the microbial community can change under different conditions (Duncan et al., 2016; Mann et al., 2019).

Fungi

The main two groups of fungi found in the soil of grassland ecosystems are saprophytic and mycorrhizal fungi. Saprophytic fungi are fungi with no symbiosis and obtain nutrients from SOM decomposition (Lindahl et al., 2006). Like other microorganisms, they play a role in the cycling soil nutrients and mineralization, and their activity can be limited by substrate and pH (Francioli et al., 2020) In general, fungal diversity is greater in native prairie soils in comparison to restored prairie sites, but both increase with Mean Annual Precipitation (MAP) (Francioli et al., 2020; Dea et al., 2022). For saprophytic fungi specifically, plant composition is the main driving force of soil fungal community (Francioli et al., 2020).

Arbuscular Mycorrhizal Fungi

Arbuscular mycorrhizal fungi (AMF) are soil fungi that form a symbiotic relationship with over 80% of plants where it exchanges nutrients acquired in areas the roots cannot reach in exchange for carbohydrates and lipids (Smith & Read, 2008; Bravo et al., 2017). AMF creates a spiderweb-like hyphal network to lengthen the area that roots can reach, thus increasing nutrient acquisition (Johansson et al., 2004). The consistency of hyphal networks is threadlike, allowing

it to be able to access nutrients (Jansa et al., 2013). A byproduct of this fungal network is its ability to help form macroaggregates, as the hyphal network acts as a glue and increases the overall stability of the soil (Tisdall and Oades, 1979). Nutrient acquisition by the fungal hyphae creates their own sphere of influence, called the hyphosphere, where bacterial communities associate and may assist in accessing recalcitrant forms of soil nutrients (Johansson et al., 2004; Jansa et al., 2013; Zhang et al., 2018; Wang et al., 2022).

Under natural or non- intensive land management practices, the function and association of the AMF hyphal network can work at maximum capacity, only limited by external limiting factors, such as plant availability, nutrient availability, climate, pH, and plant type. Intensive agricultural practices break apart the hyphal network, destabilizing and degrading it (Jansa et al., 2013; Köhl et al., 2014). When the AMF hyphal network is maintained, it becomes a beneficial asset to agricultural crops and soils, by enhanced nutrient acquisition and increasing soil structure (Kumawat et al., 2022). Less intensive agricultural practices, specifically no-till, benefits AMF in comparison to tilled practices by not breaking the fungal network (Mann et al., 2019). AMF is fundamental to the acquisition of diffusion limited nutrients such as Zn and P. AMF can act as a direct indicator of the health and stability of the soil (Mann et al., 2019; Saboor et al., 2021).

Bacteria

Soil bacteria play an essential role in the microbial decomposition of organic matter. As with other microorganisms, soil bacteria amount and diversity are strongly impacted by land use and crop presence (Duncan et al., 2016). In restored prairies, after the initial restoration, community differences can be seen and microbial functional capacity enhanced but increased diversification may not be measured even years later (Duncan et al., 2016). This is likely due to

native bacterial groups being exterminated at the time of land use change and a lack of reintroduction of new bacterial groups. The importance of soil bacteria in agriculture is highlighted by the way that soil bacterial groups change nutrient dynamics (Dennis et al., 2010). Bacterial community structure is influenced by soil pH (Rousk et al., 2010; Wu et al., 2017). This relationship has been found in several situations, including land use, crop rotation, nitrogen fertilization, liming, and the addition of biochar (Rousk et al., 2010; Dai et al., 2018; Qiu et al., 2019; Zhang et al., 2019).

Gram Negative Bacteria

Bacterial groups exist as two main types defined by their cell wall structure: Gram-negative (G-) and Gram-positive bacteria(G+). G- bacteria, in comparison to G+ bacteria consists of more complex cell wall (Beveridge, 1999). The outer membrane consists of an inner membrane of cytoplasm as well as a peptidoglycan layer, that creates resistance to antibiotics (Beveridge, 1999; Tietze et al., 2006) In studies, G- bacteria correlate with fungi and have similar response to land management practices and land use (Herzberger et al., 2014; Duncan et al., 2016). Both fungi and G- bacteria are more active when the soil is covered such as a perennial grassland (Butler et al., 2003; Treonis et al., 2004). G- abundance has been found to increase in forest soils as well, in comparison to cultivated soils (Zhong et al, 2009).

Gram Positive Bacteria

G+ bacteria differ from that of G- due to the lack of an additional extracellular membrane, and have a much thicker peptidoglycan layer (Beveridge, 1999). This creates lower resistance towards human derived stressors, potentially making it a reliable indicator of the soil health. This can be seen in response to the presence of heavy metals, disturbance, temperatures, and water availability. G+ bacteria have been found to be uniquely capable of manipulating the

community structure and even biology of soil fauna, to make the soil ecosystem more in their favor (Dhinaut et al., 2017).

Actinomycetes play an important role in soil health and the rhizosphere. Like other microbial groups, actinomycetes are fundamental to the decomposition of organic matter (Ardhi et al., 2019; Helfrich et al., 2015). Nutrient availability is further increased through the activity of actinomycetes, which can solubilize insoluble phosphates (Karthikeyan et al., 2018; Alfikri et al., 2020; Crawford et al., 1993) as well as N fixation (AbdElgawad et al., 2020). Actinomycetes contribution to the soil ecosystem includes antimicrobial properties and other secondary metabolites such enzymes that contribute to nutrient cycling in the soil.

Enzymes

Soil enzymes facilitate nutrient cycling in the soil (Dick, 1994). For this reason, soil extracellular enzymes are often used in soil health assessments. Extracellular enzymes degrade substrates not presently bioavailable (Dick and Kandeler, 2005). The enzyme groups primarily used for soil health analysis are oxidoreductases, transferases, and hydrolases, but the most well-known and widely used enzymes for soil health indicators are the hydrolases (Dick and Kandeler, 2005; Mori et al., 2023). Enzymes such as acid phosphatase, β-glucosidase (BG), and N-acetyl-β-glucosaminidase (NAG) perform hydric depolymerization that is otherwise rate limiting for SOM turnover (Dick and Kandeler, 2005). Extracellular enzymes are a significant energy investment, so it can be assumed that soil enzyme production reflects nutrient limitations as well as the nutrient composition of the soil (Burns et al., 2013). Soil enzymes are influenced by several soil factors, including temperature, depth, moisture, pH, substrate availability, substrate type, depth, soil type, and land management practices, but the way that different enzymes correlate to these factors are not consistent (Acosta-Martínez et al., 2011; Nakayama et

al., 2023; Mori et al., 2023; Margenot and Wade, 2023). For example, pH can affect enzyme activity, but it may not be the only factor (Mori et al., 2023; Margenot and Wade, 2023).

Despite the popularity of enzyme activity measurement, there are many contradictions and oversimplifications for assessing enzyme activity as a soil health indicator (Sainju et al., 2022; Margenot and Wade, 2023). It is important to emphasize that soil enzymes are not only made by microorganism but are also a product of roots. Additionally, it has been found that enzymes such as NAG and BG are multifaceted, in that they can assist in the degradation of multiple nutrients (Burns et al., 2013; Margenot and Wade, 2023).

β-1,4-glucosidase

The enzyme, β-1,4-glucosidase (BG), is the most widely available soil enzyme, that directly correlates with carbon cycling. BG is released by both plants and microbes into the soil in order to hydrolyze polysaccharides, which assists in the degradation of plant litter (Turner et al., 2002; Dick and Kandeler, 2005; Adetunji et al., 2017). BG is the final phase of the degradation of many carbon compounds and directly produces glucose for microbial consumption. As an enzyme most directly related to the immediate acquisition of energy, it is natural that BG activity typically correlates with SOC and microbial biomass (Acosta-Martínez et al., 2003). This can explain the reason for the sharp decrease of BG activity with depth, mirroring that of total microbial biomass (Acosta-Martínez et al., 2003). BG activity is often substrate-induced, correlating to an increase in the preferred nutrient supply (Zeng et al., 2021). In agricultural soils, BG activity decreases with increased disturbance and lack of litter, making it a suitable indicator for assessing the effect of land management practices on soil (Pandey et al., 2014). General assumptions made about BG over emphasize its role in carbon cycling and further research on the hydrolysis and structural dynamics in comparison to other soil enzymes,

not only hydraulic ones, is necessary to increase enzyme understanding (Margenot and Wade, 2023).

N-acetyl-β-D-glycosaminidase

N-acetyl-β-D-glycosaminidase (NAG) is a hydrolyzing enzyme used in the degradation of chitin (Nannipieri et al., 2002). Chitin is found in the exoskeleton of invertebrates as well as in plants and is difficult to degrade due to its original role in structural support/protection (Fu et al., 2014). In the degradation of chitin results in low molecular weight monomers for both C and N cycling (Cohen-Kupiec and Chet, 1998; Keyhani and Roseman, 1999; Mori, 2020). The multifaceted nature of NAG, while associated with N, can indicate shifts in C:N availability. Additionally, NAG has adverse reactions to abiotic stressors, such as water availability. Under drought conditions that limit root exudates, BG and NAG activity can be reduced by 50%, showing that the activity of both can be climate limited and that there is a strong association with water availability (Staszel et al., 2022).

Acid Phosphatase

Acid Phosphatase (AP) is a phosphomonoesterase enzyme that degrades organic phosphoric compounds at slightly acidic pH (Eivazi & Tabatabai, 1977; Acosta-Martínez et al., 2011) Acid Phosphatase is more appropriate for analyzing the soil's relationship with phosphorous (P) under the given pH index, liming, and crop production (Juma & Tabatabai, 1988; Wright & Reddy, 2001; Adetunji et al., 2017). Phosphorus is a key nutrient for biological activity, as it is necessary for efficient conversion of energy as well as cellular functions (Algborey & Hassan, 2022). This makes the analysis of soil enzymes responsible for the cycling of P in the soil essential as a soil health indicator. Increased phosphatase activity is typically induced by P limitation (Olander and Vitousek, 2000; Turner and Wright, 2013; Fleischer et al.,

2019; Nakayama et al., 2023), which has been found to be related to substrate availability, climate, and soil type (Sardans & Peñuelas, 2004; Jing et al., 2016; Margalef et al., 2017; Widdig et al., 2019). While phosphatase is primarily related to the cycling of P, under demand for C, AP can contribute to the liberation of C, showing a multifaceted nature to acid phosphatase as well (Spohn and Kuzyakov, 2013; Turner and Wright, 2013; Margenot and Wade, 2023).

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Chapter 2 - Materials and Methods

Site Descriptions

A precipitation gradient spanning 12 locations across the state of Kansas ranged in precipitation from an annual mean of 400 to 1100 mm (Fig. 2.1 and 2.2). For each location, three land uses were sampled representing three levels of disturbance: native prairie, restored prairie, and agricultural land under tillage. All sites had the same or similar soil mapping units, primarily silt loam soils that increased in clay content with depth. Sampling time varied due to distance, availability of equipment and people, as well as rain and field conditions. The first 8 sites were sampled in 2019 with four more sites sampled in 2021. Each site was sampled 4 times, representing the four quadrants on the site and samples were then mixed. Soil cores were collected using a Giddings probe (Giddings Machine Company, Windsor, CO, USA) to a depth of 150 cm for sites sampled in 2019 and 120 cm for sites sampled in 2021. Soil samples were separated by 0-5, 5-10, 10-15, 15-40, 40-60, 60-90 cm. Site meta information can be found in Table 2.1 and 2.2.

Land Use

Native prairie sites were documented as having never been cultivated. The agricultural sites were tilled systems with different cropping systems due changes in precipitation. Restored prairie were sites that had previously been under cultivation but were later restored to native vegetation conditions. The time of establishment of restored prairie sites varied greatly, with the oldest known restoration date being the HAY site in the 1940s and newest being the HNC site in 2013. Due to the degree of variation as well as unspecified years of restoration for some restored prairie sites, restored prairie was excluded from the data analysis.

Chemical Soil Health Indicators

Soil organic carbon and total nitrogen

Soil organic carbon (SOC) and total nitrogen (TN) was determined by a LECO TruSpec Carbon/Nitrogen combustion analyzer (LECO Corporation, St. Joseph, MI, 2005). Total C was considered soil organic carbon for soils with pH <7.2, while soils with pH >7.2 were pretreated for carbonates with dilute Phosphoric Acid. Carbonates are released as CO_2 from calcium and magnesium carbonates in calcareous soils, leaving only the soil organic carbon present.

Soil pH

Soil pH was determined using $CaCl_2$ (Thomas, 2018). A 1:1 slurry of 10g <2 mm sieved air-dry soil with a 0.01 M $CaCl_2$ solution was shaken for 1h. Measurements of pH were then taken using a Skalar SP50 Robotic Analyzer (Skalar Inc., Buford, Georgia).

Biological Soil Health Indicators

Microbial phospholipid analysis

Phospholipid fatty acids (PLFA) were used to characterize the total microbial biomass and the microbial community composition. Total lipids were extracted from freeze-dried soil utilizing a modified version of the Bligh and Dyer lipid extraction method (Bligh and Dyer, 1959; White and Ringelberg, 1998; White and Rice, 2009). Phosphate buffer was added to the soil solution to prevent cell rupturing before adding methanol and chloroform to precipitate lipids from the bulk of the soil analyzed. PLFAs were separated from the total lipid extract using silicic acid column chromatography to separate the fraction of lipids that represent the living microbial biomass from the total microbial biomass in the soil (Axelsson & Gentili, 2014). Once separated, the fatty acids were converted via methylation, forming fatty acid methyl esters (FAME). The FAMEs were analyzed using a Thermo Scientific Trace GC-ISQ gas

chromatographer mass spectrometer from Thermo Fisher Scientific, equipped with a DB5 MS column. The FAMEs were analyzed using a Thermo Scientific Trace GC-ISQ gas chromatographer mass spectrometer from Thermo Fisher Scientific, equipped with a DB5 MS column with a 30m x 250 μm i.d. x 0.25 μm film thickness from Agilent Technologies. Identification of the produced FAME markers was made using 32 biomarkers, that were then grouped together based to form groups consisting of Gram-positive (+) bacteria (i15:0, a15:0, i16:0, i17:0, and a17:0), Gram-negative (-) bacteria (19:0:delta9,10; C18:1:11:cis; 17:0:delta9,10; C10:0:2-OH, C12:0:2-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), AMF (C16:1:11), and fungi (C18:2:9,12) (White and Rice, 2009). To form the total microbial biomass, FAMEs representing the sum of the most common biomarkers (C11:0; C12:0; C13:0; C14:0; C15:0; C16:0; C17:0; C18:0; and C20:0) were added together. Actinomycetes, Gram-positive, and Gram-negative bacteria were added together to represent the total bacterial biomass, and AMF and fungi were added together to represent the total fungal biomass. Additionally, "other" microbial biomass was created by subtracting the sum of total bacteria and fungi from total microbial biomass. Phospholipid fatty acid abundance was reported as nmol per gram of dry soil. Lastly, the fungal to bacterial ratio was calculated by dividing total fungal biomass by total bacteria biomass.

Soil respiration

Soil respiration was Lin (2021), a modified version of Schindelbeck et al. (2016) Lin (2021). Air dried soil (20g) was sieved, weighed into aluminum weigh boats, pierced throughout the bottom with a needle, to allow for water infiltration. Wide-mouth Ball Mason jar (1000 mL) were prepared by adding a Whatman filter paper placed on the bottom cut to size and 7.5 mL of DI water added before the soil weigh boats to ensure that water infiltrated slowly into the soil.

Soil filled weigh boats were gently placed in Mason jars, sealed and incubated for 4 days at 25 °C. To collect gas from jars, jar lids were modified by drilling a hole in the center and inserting a rubber septum in the center that was then fastened airtight. To ensure an air-tight seal of the lid to the mason jar, the perimeter of the lid was covered in vacuum seal grease before then further securing the jar lid with a ring. After incubation, 20 mL gas samples were taken per incubation jar, and 5 mL of the collected gas was analyzed for CO_2 using a Shimadzu Gas Chromatograph-8A (Shimadzu Scientific Instruments Inc., Columbia, MD). A control containing no soil was used to correct for ambient CO_2.

Fluorometric Enzyme assay

To assess the enzyme functioning of the soil ecosystems, three enzymes were assayed using a fluorometric enzyme assay (Wallenstein & Weintraub, 2008; German et al., 2011). These enzymes were β-1,4-glucosidase (BG), N-acetyl-β-D-glycosaminidase (NAG), and Acid Phosphatase (PHOS). This was done by taking 1 g of dry soil and 100 mL of 50 mM pH 5 acetate buffer and adding them to a deep Pyrex glass dish. A stir rod was added to the soil and acetate buffer to facilitate mixing, and the dish was placed on a stir plate, and covered with a lid to prevent splashing. The soil slurry was homogenized for 5 min. then pipetted using a multi-channel pipettor into all but the first 3 columns of a pre-prepared incubation plate. The remaining soil slurry was then re-pipetted into two more incubation plates, all prelabeled with what soil samples would go into the plate, one sample per row, and what enzymes the plate would be used for. Once all soil slurries were collected, reagents were then loaded into the plates using the table layout seen in Table 2.3. For the hydrolytic enzyme assay, BG used 4-MUB-b-D-glucoside, NAG used 4-MUB-N-acetyl-b-glucosaminide, and PHOS used 4-MUB-phosphate for substrate

solutions (Subs). Plates were then incubated in a dark drawer, 2 h for PG and PHOS and 3 h for NAG.

After incubation, further activity was stopped using 10 μL of 0.5 N NaOH as a stop solution. The time of stop solution addition was then recorded for later use. Fluorescence was measured using a Thermos scientific Varioskan Lux scanning multimode reader set to 365/445 to excite the enzyme-specific MUB buffer and Subs. Data was then saved and exported for further analysis.

To calculate the hydrolytic activity of the enzyme the formula bellow was used:

$$\frac{((\text{mean of assay wells} - \text{mean of negative control wells} - \text{mean of blank wells})(100\text{ mL}))}{((\text{emission coefficient})(\text{quench coefficient})(0.2\text{ mL})(\text{incubation, hr})(\text{g soil used in slurry}))}$$

In this formula, the emission coefficient was found by dividing the mean fluorescence of the reference standard by 0.5 Nmole, and the quench coefficient was found by dividing the mean fluorescence of the quench standard by the mean fluorescence of the reference standard.

Statistical Analysis

R scripts were custom written for the statistical analysis, using R 4.2.2 (R Core Team, 2022). To begin the statistical analysis, data was first cleared of outliers using the Dixon's Q test (Dean and Dixon, 1951). For further analysis, the dataset was divided by depth and land use using the "dplyr" package (Wickham et al., 2023). Precipitation accounted for the location effect. To examine if there is significance in the relationship between soil health indicators and environmental influences, individual type 3 two-way ANOVAs were done for each soil health indicator at each depth. This in turn further compared the slopes of the native prairie and ag sites

found in the multivariate linear models. This was also done regarding whole profile and its relationship with pH. While pH and precipitation can be covariate factors, both were kept and analyzed separately as well as together to find degrees of relation.

MANOVA

To create a summary of the interaction that land use, and precipitation have in total with soil health indicators, multivariate analysis of variance was used (MANOVA). To create this interaction, summary soil health indicators (MB, TBACT, TFUNG) were separated, to reduce repetition and covariance. The "MASS" package was used to perform the analysis (Venables, 2002). A linear model was created out of the dependent variables or soil health indicators, before being transformed using the MANOVA function utilizing a type 3 test to account of interactions.

Multivariate Linear Models

To visualize the different factors influencing the soil ecosystem, "ggplot2" was used to create linear mixed effect model, where land use and depth were kept as fixed, while the soil health indicator and precipitation remained fluid (Wickham, 2016). R^2 and p-values were added to determine the change in relationship between soil health indicator and precipitation as land use and depths change. P-values were created using type 3 one-way analysis of variance (ANOVA) with Satterthwaite's method to account for the interactions.

Correlation Matrixes and PCA Biplot

To create the base of these models, data set was cleared of characters, NAs, and scaled to address different measurement ranges of the variables analyzed. Correlations were found using the Pearson method, and correlation plots were visualized using the package "ggcorrplot" (Kassambara, 2022). Scores were made with a significance level of $p<0.05$ using Spearman correlation coefficient. The correlation matrix was converted to a principal components analysis

(PCA) that was then visualized through biplots using packages “factoextra” and “FactoMineR” (Venables et al., 2002; Sebastien et al., 2008). To examine the proportions and importance of PCA components, scree plots, loadings, and dimensions were analyzed. Dimensions 1 and 2 were used to create a PCA biplot, accounting for most of the findings associated with the soil microbial ecosystem. Depth and Precipitation were left in as neither caused skew in the dimensions of the PCA biplots. Vectors were grouped directionally, based on similarities and inverse vectors showed inverse relationships between variables.

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Tables

SITE INFORMATION FOR 2019 SAMPLES

LOC	Location	Land use	Lat.	Long.	MAP	MAT	Soil Name	Soil Texture (A horizon)	Sampling
EKS	Anderson	Native	38.181	-95.272	1040	13.23	Kenoma-Olpe (8780)	Silt loam	6-11-19
		Restored	38.181	-95.274	1040	13.23	Kenoma (8875)	Silt loam	6-11-19
	Franklin	Agriculture	38.538	**-95.248**	1009	13.01	Woodson(8961)	Silt loam	6-11-19
HAY	Ellis	Native	38.835	-99.303	604	12.28	Harney (2612)	Silt loam	6-24-19
		Restored	38.844	-99.316	602	12.23	Harney (2612)	Silt loam	6-24-19
		Agriculture	38.843	-99.315	602	12.23	Harney (2612)	Silt loam	6-24-19
KNZ	Riley	Native	39.105	**-96.604**	850	12.74	Reading (7174)	Silt loam	6-17-19
		Restored	39.103	-96.605	860	12.53	Reading (7170)	Silt loam	6-17-19
		Agriculture	39.103	**-96.604**	870	12.41	Reading (7170)	Silt loam	6-17-19
LVN	Leavenworth	Native	39.259	-94.979	1003	12.55	Shelby-Sharpsburg (s2389)	Silty/clay loam	6-25-19
		Restored	39.261	-95.016	997	12.58	Pawnee-Grundy (s2386)	Silty/clay loam	6-25-19
		Agriculture	N/A	N/A	N/A	N/A	N/A	N/A	N/A
SVR	Logan	Native	38.874	-100.983	477	11.68	Ulysses (1857)	Silt loam	6-25-19
		Restored	38.874	-100.984	477	11.68	Ulysses (1857)	Silt loam	6-25-19

		Agriculture	38.872	-100.983	477	11.68	Ulysses (1856)	Silt loam	6-25-19
TLI	Salina	Native	38.970	-97.469	760	13.30	Hord (3755)	Silt loam	6-25-19
		Restored	38.766	-97.574	781	13.30	McCook (2347)	Silt loam	6-25-19
		Agriculture	38.694	-97.591	780	13.19	Hord (3755)	Silt loam	6-25-19
TRB	Tribune	Native	38.469	-101.782	455	11.31	Richfield (1761)	Silt loam	6-25-19
		Restored	38.472	-101.785	455	11.31	Richfield (1761)	Silt loam	6-25-19
		Agriculture	38.469	-101.782	455	11.31	Richfield (1761)	Silt loam	6-25-19
RKS	Rooks	Native	39.175	-99.162	634	11.90	Heizer-Harney-Brownell-Bogue-Armo (s2536)	Sandy/silt loam	6-25-19
		Restored	39.164	-99.150	634	11.90	Heizer-Harney-Brownell-Bogue-Armo (s2536)	Sandy/silt loam	6-25-19
		Agriculture	39.242	-99.215	626	11.84	Uly-Holdredge-Harney (s2549)	Silt loam	6-25-19

Table 2.1 Site information for 2019 soil samples. LOC: abbreviation used for soil samples; Location: city/county in which soil cores were taken; Land use; Lat: Latitudinal coordinates; Long: longitudinal coordinates; MAP: Mean annual precipitation (mm yr-1); MAT: mean annual temperature (C); Soil Name: Map unit name and symbol; Soil Texture (A horizon); Sampling date (MM-DY-YR

SITE INFORMATION FOR 2021 SAMPLES

LOC	Location	Land use	Lat.	Long.	MAP	MAT	Soil Name	Soil Texture (A horizon)	Sampling date
HNC	Ellis	Native	38.892	-97.988	751	12.8	Lancaster-Hedville (3396)	Loam	8-06-21
		Restored	38.702	-98.231	723	12.8	Wells (3492)	Loam	8-06-21
		Agriculture	38.888	-97.991	751	12.8	Wells-Edalgo (3495)	Loam	8-06-21
JEF	Jefferson	Native	39.045	-95.205	981	12.61	Pawnee (7501)	Clay loam	6-23-21
		Restored	39.047	-95.209	981	12.55	Pawnee (7501)	Clay loam	6-23-21
		Agriculture	39.334	-95.508	959	12.66	Pawnee (7501)	Clay loam	6-23-21
LGN	Logan	Native	39.789	-101.167	533	10.94	Colby (1581)	Silt loam	7-07-21
		Restored	39.788	-101.167	533	10.94	Colby (1581)	Silt loam	7-07-21
		Agriculture	N/A	N/A	N/A	N/A	N/A	N/A	7-07-21
TRG	Trego	Native	39.015	-99.745	598	12.06	Holdrege (2674)	Silt loam	7-08-21
		Restored	N/A	N/A	N/A	N/A	N/A	N/A	7-08-21
		Agriculture	39.013	-99.760	597	12.11	Penden (2748)	Clay loam	7-08-21

Table 2.2 Site information for 2021 soil samples. LOC: abbreviation used for soil samples; Location: county in which soil cores were taken; Land use; Lat: Latitudinal coordinates; Long: longitudinal coordinates; MAP: Mean annual precipitation (mm yr-1); MAT: mean annual temperature (C); Soil Name: Map unit name and symbol; Soil Texture (A horizon); Sampling date (MM-DY-YR)

Plate Cell layout for Fluorometric Enzyme Analysis												
	1	2	3	4	5	6	7	8	9	10	11	12
A	x	x	x	x	x	x	x	x	x	x	x	x
B	x	x	x	x	x	x	x	x	x	x	x	x
C	x	x	x	x	x	x	x	x	x	x	x	x
D	x	x	x	x	x	x	x	x	x	x	x	x
E	x	x	x	x	x	x	x	x	x	x	x	x
F	x	x	x	x	x	x	x	x	x	x	x	x
G	x	x	x	x	x	x	x	x	x	x	x	x
H	x	x	x	x	x	x	x	x	x	x	x	x
	BLANK	NEG CTRL	REF STD	QUENCH STANDARDS			ASSAY WELLS					
	250uL Buff	**200uL Buff + 50uL Subs**	**200uL Buff + 50uL Std**	**200 uL Soil + 50 uL Std**			**200 uL Soil + 50 uL Subs**					
	**** for Soil Blank plates : add 50 uL Buffer to all wells**											

Table 2.3 Plate cell layout for Fluorometric enzyme reading. Buff: 50 mM Acetate buffer; Subs: substrate solution for desired enzyme; Std: 10 µM standard solutions made from 4-methylumbelliferone (MUB).

Figures

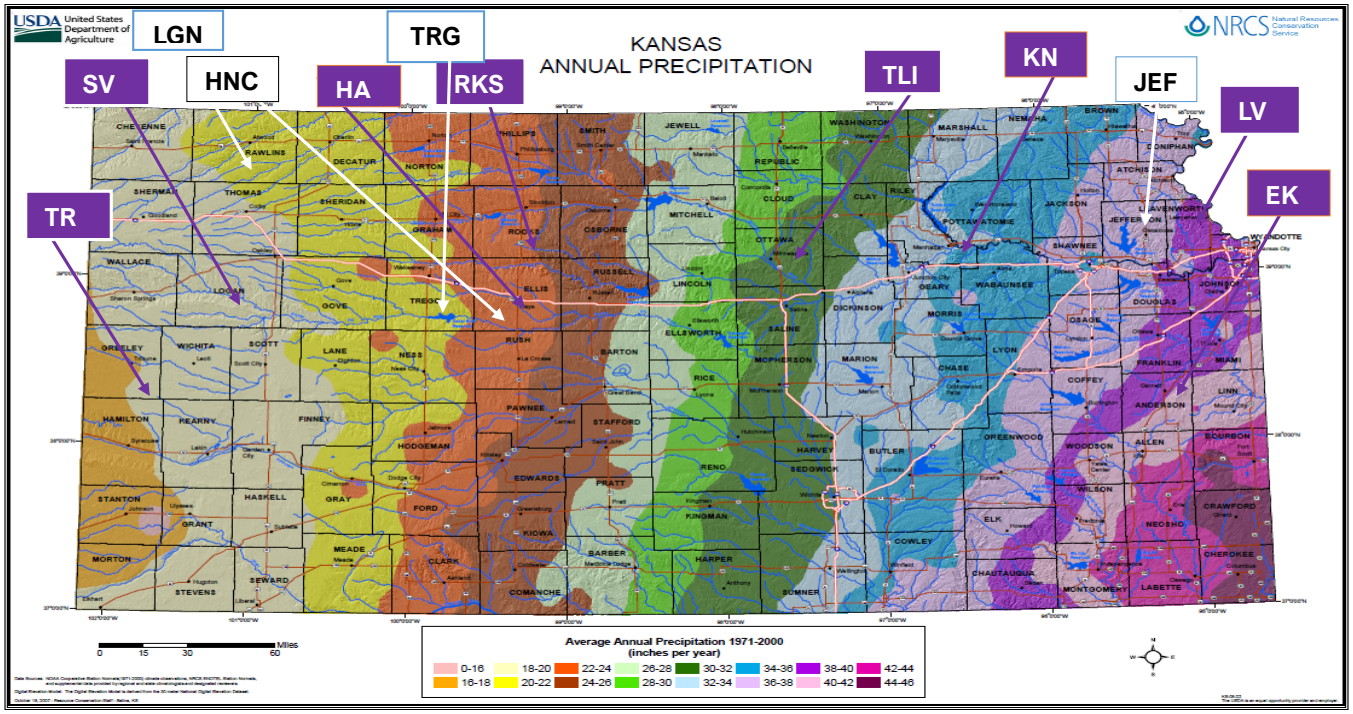

Figure 2.1 Map of Kansas's annual precipitation gradient procured from Kansas State University's Kansas Climate page. Average annual precipitation reflects the years 1971-2000 in inches and site location are marked with their corresponding site LOC. 2019 sites are in purple boxes and 2021 sites are in white.

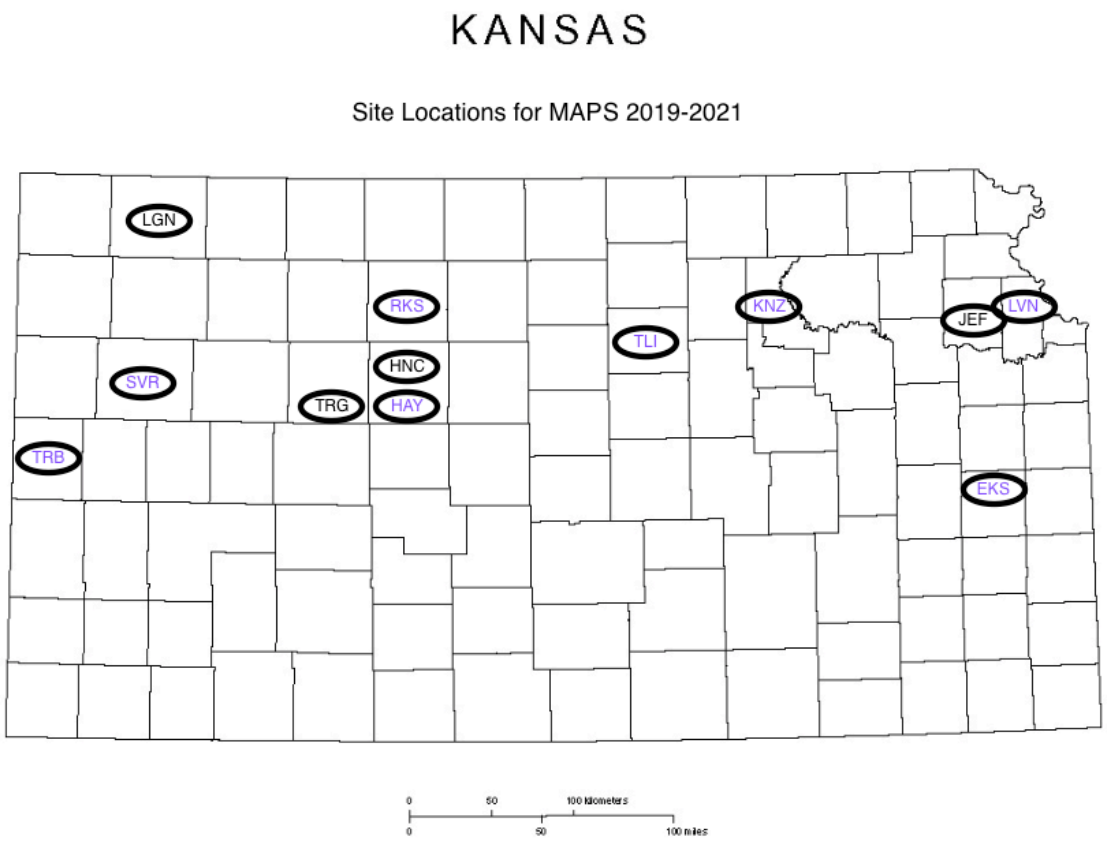

Figure 2.2 Map of site location for MAPS project 2019-2021. 2019 sites are in purple, and 2021 sites are in blac

Chapter 3 - Results

Biological Aspect of Soil Health

Microbial Biomass Distribution across Kansas

Microbial biomass (MB) and many of its components were significantly affected by land use, precipitation, and the interaction (Table 3.3) to a depth of 15 or 30 cm. Below 30 cm, only land use affected microbial biomass and its composition to 75 cm with no effects below 75 cm. Therefore, we examined the surface 30 cm separately from the subsoil (>30 cm). In the native prairie, MB was correlated with precipitation in the top 2 depths (0-5 and 5-15 cm) ($R^2 = 0.56$, $p<0.05$) and a weak correlation at the at 15-30 cm ($R^2 = 0.28$, $p<0.1$) (Fig. 3.1). For restored prairie, only a weak correlation was found at 0-5 cm ($R^2 = 0.28$, $p<0.1$). For the agricultural sites, there was no linear relationship with precipitation. In the subsoil (> 30 cm) only restored prairie held a weak correlation ($R^2 = 0.28$, $p<0.1$) (Fig 3.2). For the native prairie and the ag sites there were no relationships with precipitation.

For total arbuscular mycorrhizal fungi (AMF) in the topsoil (Fig. 3.3), correlations were not as abundant as MB between specific depth and precipitation. In the prairies, a weak correlation was found in native at 22.5 cm ($R^2 = 0.30$, $p<0.1$) and 10 cm ($R^2 = 0.27$, $p<0.1$) in restored. For Ag, there was no linear relationship for the top depths. In the subsoil (Fig 3.4), no correlations were found, but restored prairie and ag sites had similar amounts of AMF biomass which were lower than native prairie sites.

Both prairies had higher amounts of fungal biomass than the ag sites, with the native prairie having the greatest fungal biomass. Fungal biomass in the ag sites did change with precipitation. In the subsoil, there were no significant interactions with precipitation for any land use, but abundance was highest in native prairie while restored prairie and ag sites were similar (Fig 3.6). For total fungal biomass the native prairie was weakly correlated with precipitation at 2.5 cm ($R^2 = 0.33$, $p<0.1$) and 10 cm ($R^2 = 0.30$, $p<0.1$), and a stronger correlation at 22.5 cm ($R^2 = 0.36$, $p<0.05$) (Fig 5). For the restored prairie, there was no correlation between fungal biomass and precipitation at the topsoil top depth, but weak correlations were found at both 10 cm (R2 = 0.34, $p<0.1$) and 22.5 cm ($R^2 = 0.28$, $p<0.1$).

ACTINO abundance was still higher for the native prairie sites than the restored prairie sites, but the relationship with precipitation were similar. The Ag sites however, had much lower ACTINO biomass and no association with precipitation at any depth. Actinomycetes (ACTINO) had the greatest significant relationship with precipitation of all the microbial groups (Fig 3.7). For the native and restored prairies, all three topsoil depths were correlated with precipitation. For native prairie, 2.5 cm ($R^2 = 0.46$, $p<0.05$) and 22.5 cm ($R^2 = 0.40$, $p<0.05$) were correlated with precipitation, and a strong correlation at 10 cm ($R^2 = 0.65$, $p<0.01$). For the restored prairie, 2.5 cm ($R^2 = 0.40$, $p<0.05$), 10 cm ($R^2 = 0.36$, $p<0.05$). and 22.5 cm ($R^2 = 0.42$, $p<0.05$) were correlated with precipitation. In the subsoil, there were no significant relationships with precipitation The native prairie sites had higher biomass, while the restored prairie and ag sites were similar (Fig 3.8).

For total bacteria (TBACT), significant relationships with precipitation were much lower than ACTINO (Fig 3.9). In the native prairie, 10 cm was strongly correlation with precipitation ($R^2 = 0.66$, $p< .01$), and restored prairies at 2.5 cm was weakly correlated with precipitation (p<

.1). TBACT biomass decreased with the degree of disturbance (native>restored>ag) for all depths except at 97.5 cm.

Microbial biomass groups had similar reactions in response to precipitation and the relationship with disturbance and depth, indicating that the largest external influence for microbial biomass is the disturbance of its ecosystem. Linear relationships with precipitation were not found in ag sites and were most abundant in native prairie sites. Restored prairie sites were consistently similar to the native prairie sites in their relationship with precipitation, except with lower microbial biomass.

Disturbed vs non-disturbed sites

To test the relationship between that of no disturbance and disturbance, native prairie, and ag sites an ANOVA was performed for each soil health indicator, at each depth (Tab 3.3). Land use (Use) was compared to reflect differences between soil indicators and disturbance. To find significant difference in the relationship between precipitation and disturbance, precipitation was used (Precip). To find if the slopes found in the linear models of native and ag at each soil health indicator was significant (ex. Fig 3.1), the interaction of Use*Precip was measured to test the reliability of the visible differences. The interaction between these two could also be interpreted as the interaction of disturbance and precipitation.

MB was found to be significantly affected by Land use (Use) to a depth of 75 cm, with 0-5 cm ($p<.001$), 5-15 cm ($p<.001$), and 15-30 cm ($p<.001$) all having a high significance, and 30-75 cm having strong significance ($p<.01$), only a step lower. The relationship between MB and Precip has lower significance in comparison, with 0-5 cm ($p<.01$) and 5-15 cm ($p<.01$) still being strongly significant, but 15-30 cm ($p<.1$) only having weak significance. The interaction of

Use*Precip was significant at 0-5 cm ($p<.05$) and 5-15 cm ($p<.05$). Generally, significance diminished with increasing depth.

Bacteria was divided into gram negative bacteria (G-), gram positive bacteria (G+), ACTINO, and TBACT. First, G- was significantly affected by Land use (Use) to a depth of 75 cm, but 0-5 cm ($p<.1$), 5-15 cm ($p<.1$), 15-30 cm ($p<.1$), and 30-75 cm ($p<.1$) only were weakly significant (Table 3.1). There was no relationship with the Precip or Use*Precip. G+ was significantly affected by Land Use to a depth of 75 cm, with 0-5 cm ($p<.001$), 5-15 cm ($p<.001$), and 15-30 cm ($p<.001$) all having a high significance, and 30-75 cm having significance ($p<.05$) as well, like MB. In relation to Precip, 0-5 cm ($p<.001$) and 5-15 cm ($p<.001$) were highly significant in their relationship, and strong significance was found from 30-75 cm ($p<.01$). The interaction of Use*Precip was found to be significant at 0-5 cm ($p<.05$) and 5-15 cm ($p<.05$). Generally, significance diminished with increasing depth.

ACTINO was significantly affected by Land use to a depth of 75 cm, with 0-5 cm ($p<.001$), 5-15 cm ($p<.001$), and 15-30 cm ($p<.001$) all having a high significance (Table 3.3). At 30-75 cm ACTINO was significant ($p<.05$), similar to both MB and G+. In relation to Precip, ACTINO was significant to 30 cm. ACTINO has the highest interaction between Land Use*Precip but did not decrease with depth. The interaction between Land use and Precip, was significant to 30 cm. This supports the linear relationship found in native but not ag (Fig. 3.7).

TBACT was significantly affected by Land Use to a depth of 75 cm, with 0-5 cm ($p<.001$) and 5-15 cm ($p<.001$) having the highest significance, and 15-30 cm and 30-75 cm significant at the $p<.05$ level. Precip was strongly significant at 0-5 cm ($p<0.01$) and 5-15 cm ($p<.01$), and the interaction of Land Use*Precip was significant at 0-5 cm ($p<0.05$) and at 5-15

cm ($p<.01$). Bacterial groups had different relationships with precipitation and land use, but G+ and ACTINO were similar.

Fungal biomass was divided into three groups: general fungi (FUNGI), AMF, and TFUNG. Of the three, FUNGI had the least significant relationships. For FUNGI, Land use had a weak significance ($p< 0.1$) at 0-5 cm ($p<0.1$) and 5-15 cm ($p<0.1$). AMF was significantly affected by Land Use at 0-5 cm ($p<.05$) and 15-30 cm and 5-15 cm ($p<.01$). For Precip ($p<.1$) and Precip*Use ($p<.1$), only weak significance was at 15-30 cm.

The ratio of fungal biomass to bacterial biomass was compared at each depth was not significant for Precip and Land Use. Only at 75-120 cm, was the interaction of Land Use*Precip significant ($p<.01$).

Enzyme Activity Distribution across Kansas

Each enzyme activity responded differently to land use, precipitation, and depth (Table 3.4). For β- glucosidase (BG), there was no effect of treatment at 0-5 cm. Land use significantly affected BG at 5-15 and 15-30 cm. Precip was significant at 5-15, 30-75 and 75-120 cm.

Precip did not affect BG at each depth and land use. In the surface 30 cm, BG activity decreased with increased disturbance. Native prairie BG activity was significant at 30-75 cm ($R^2 = 0.32$, $p<0.1$) and 75-120 cm ($R^2 = 0.61$, $p<0.01$). In the restored prairie sites as well as the ag sites, there was no significant relationship between precipitation at each depth.

For NAG, Precip was significant from 0-120cm, A significant interaction between Land Use*Precip was found in the subsoil from 30-120 cm but ranged considerably between depths (Table 3.4). Regarding Land Use, 5-15 cm ($p<.05$), 15-30 cm ($p<.05$), and 30-75 cm ($p<.05$) were all significantly impacted, but 0-5 cm ($p<.1$) showed a weaker relationship. Precip was

significant below 5 cm throughout the profile Interactions between Land Use*Precip occurred only at 30-75 cm ($p<.1$), and 75-120 cm ($p<.1$).

The lack of interaction between NAG with Land Use and Precip was due to more variability negating significance (Fig 3.13). Only prairie sites showed a significant relationship with precipitation, that were depth dependent. In native prairie topsoil, significance was seen at 0-5 cm ($R^2 = 0.32$, $p<0.2$) and 15-30 cm ($R^2 = 0.41$, $p<0.05$). In restored topsoil, significance was only seen at 15-30 cm ($R^2 = 0.39$, $p<0.05$). Precipitation effect increased in the native prairie as depth increased, 30-75 cm ($R^2 = 0.42$, $p<0.05$). and 75-120 cm ($R^2 = 0.52$, $p<0.05$) (Fig 3.13; Fig 3.14). In restored prairie, only 75-120 cm ($R^2 = 0.31$, $p<0.1$) was significant. For the Ag sites, there was no significant relationship with precipitation at any depth.

For PHOS, precipitation was significant for all depths other than 0-5 cm. Land Use was also significant from 5-75 cm (Table 3.4). The interaction of Land Use*Precip was significant at 0-5 cm ($p<.05$), 15-30 cm ($p<.1$), and 30-75 cm ($p<.01$).

The relationship between Precip and PHOS in Figure 3.13 was like that of NAG and Precip (Fig 3.15). Only the native prairie sites had a significant relationship with precipitation, that were depth dependent. In native topsoil, Precip was significant at 0-5 cm ($R^2 = 0.67$, $p<0.001$) and 15-30 cm ($R^2 = 0.40$, $p<0.05$). In restored prairie, Precip was only significant at 15-30 cm ($R^2 = 0.29$, $p<0.1$). In the subsoil of the native prairie, 30-75 cm ($R^2 = 0.77$, $p<0.001$) and 75-120 cm ($R^2 = 0.63$, $p<0.01$) (Fig 3.16). In the restored prairie subsoil, 30-75 cm ($R^2 = 0.56$, $p<0.01$) and 97.5 cm ($R^2 = 0.84$, $p<0.001$) had a strong relationship with precipitation. For the Ag sites only had a correlation with precipitation and PHOS at 97.5 cm ($R^2 = 0.35$, $p<0.1$). PHOS activity as was highest in native>restored>ag and variability increased with disturbance.

CO2 Activity

CO_2 activity was significantly affected by Land use between native prairie and ag. Land Use significantly affected CO_2 activity at 0-5 cm ($p<0.05$), 5-15 cm ($p<0.05$), and 15-30 cm ($p<0.001$) and at 30-75 cm ($p<0.1$). For precipitation, only 15-30 cm ($p<0.05$) significantly affected CO_2 activity. The interaction between Land Use and Precip was significant at 5-15 cm ($p<0.05$) and 15-30 cm ($p<0.05$). Similar to microbial biomass than carbon and nitrogen. Distribution of CO_2 activity was a positively related to precipitation in the topsoil of native prairie and undefined or negative slope in Ag, while restored prairie was in between (Fig 3.21). In the native prairie, 5-15 cm ($R^2 = 0.32$, $p<0.1$). and 15-30 cm ($R^2 = 0.41$, $p<0.05$) were significant, while in restored 15-30 cm ($R^2 = 0.36$, $p<0.1$) was the only significant depth. In the subsoil, only restored prairie was correlated with precipitation, at both 30-75 cm ($R^2 = 0.38$, $p<0.05$) and 75-120 cm ($R^2 = 0.42$, $p<0.05$) (Fig 3.22).

Chemical Aspects of Soil Health

The relationship between soil organic carbon and total nitrogen with precipitation mirrors each other. Both soil C and N had a highly significant association with Land Use (Table 3.4). Soil C and N were significantly affected by Land Use at all three surface depths ($p<0.001$). The relationship changed with Precip and Precip*Use. For nitrogen, Precip highly significant in the topsoil, 0-5 cm ($p<.01$), 5-15 cm ($p<.001$) and 15-30 cm ($p<.01$). For soil C, Precip was significant at 0-5 cm ($p<.01$), 5-15 cm ($p<.001$), 30-75 cm ($p<.01$) 30-75 cm ($p<.05$), and 75-30 cm ($p<.1$) Lastly, the interactions between land use and Precip on C and N, occurred only at 5-15 cm, with carbon ($p<.01$) and nitrogen ($p<.1$).

There was a linear relationship between Precip and soil C in the topsoil (Fig 3.17). In the native prairie soil C was related to Precip 0-5 ($R^2 = 0.51$, $p<0.01$), 5-15 ($R^2 = 0.62$, $p<0.01$), and

15-30 ($R^2 = 0.68$, $p<0.001$) with increased significance with increased depth. In the restored prairie, 0-5 cm ($R^2 = 0.29$, $p<0.1$), 5-15 cm ($R^2 = 0.51$, $p<0.05$). and 15-30 cm ($R^2 = 0.43$, $p<0.05$) also had a strong correlation. For ag, Precip was only significant at 0-5 cm ($R^2 = 0.38$, $p<0.1$) and 10-15 cm ($R^2 = 0.44$, $p<0.05$). Precipitation and carbon were not correlated in the subsoil.

For nitrogen in the topsoil, the precipitation effect was similar to soil carbon, but to a lesser degree (Fig. 3.19). First, in native 2.5 cm ($R^2 = 0.39$, $p<0.05$) and 10 cm ($R^2 = 0.54$, $p<0.01$), and were correlated, with 10 cm having the highest significant. For the restored prairie, soil N and precipitation were significant 5-15 cm ($R^2 = 0.36$, $p<0.1$). and 15-30 cm ($R^2 = 0.32$, $p<0.1$, but not as strong as the native prairie. For ag, soil N was affected by precipitation at 0-5 cm ($R^2 = 0.49$, $p<0.05$) and 5-10 cm ($R^2 = 0.39$, $p<0.1$). While soil N and Precip were correlated in the topsoil, there was no correlation between precipitation and N in the subsoil (Fig 3.20).

Correlations

Comparison of Native and Ag

Native

PCA biplots were created to understand the relationship between the variable and observations. For the whole profile of the native prairie, PC1 accounted for 85.4% of the variation in soil health indicators, across the x axis, that was based on the relationship with depth. (Fig 3.32). All but depth, pH, and the ratio of F:B resided on the right side of the axis. The "DEPTH" vector was added to show the importance of depth to the PC1 distinction (the inclusion did not cause skew or change to the biplot and only added support).

PC2 accounted for 6.7% of the soil health indicator variation along the y axis and created groupings, that reflected more of the difference between activity and microbial biomass.

Microbial biomass fell into the negative proportion of y, that correlated more with depth, and pH, while enzyme activity and CO_2 respiration fell in the positive portion. Carbon and nitrogen were grouped with BG and bacterial groups, that were mostly unaffected or unassociated with PC2. The length of the vector for NAG and precipitation were less as they are both represented by the 3rd PC, not represented in Figure 3.32.

Native Topsoil

For the native prairie topsoil, PC1 accounted for 66.6% of the variation while PC2 accounted for 16.4% of variation (Fig 3.35). PC1 represented the abundance of these groups, in relation to depth, with the groups most represented from greatest to smallest being N, C, MB, GRAMPOS, TFUNG, and Depth. PC2 represented the fungal components, with the groups most represented from greatest to smallest being GRAMNEG, FBRATIO, CO2, FUNGI, and AMF. PC3, not represented but considered, represented precipitation, with the groups most represented from greatest to smallest being NAG, pH, and PRECIP.

Native Subsoil

For the native prairie subsoil, PC1 accounted for 72.6% of the variation while PC2 accounted for 11.9% of variation (Fig 3.4). PC1 represented the abundance of these groups, in relation to depth, with the groups most represented from greatest to smallest GRAMPOS, MB, DEPTH, BG, and CO2. Enzyme activities and soil C were more related in the subsoil. PC2 represented the fungal components, with the groups most represented from greatest to smallest being GRAMNEG, FBRATIO, AMF, TFUNG, and FUNGI. PC3, not represented but considered, represented the precipitation effect, with the groups most represented from greatest to smallest being PRECIP, pH, N, and PHOS.

Ag

For the whole soil profile of the Ag land use, PC1 accounted for 81% of the variation in the soil health indicators across the x axis, based on the grouping of microbial groups decreasing with depth (Fig 3.36). Depth, pH, and the ratio of F:B resided on the left side of the axis, while microbial activity and biomass remained on the left. PC2 accounted for 11.9% of the variation soil health indicators along the y axis and created groupings, that reflected more of the difference between activity and microbial biomass. Indicators most strongly influenced by PC2 were related to fungal biomass, pH, PHOS, NAG and precipitation.

Ag Topsoil

For the ag topsoils, PC1 accounted for 66.7% of the variation while PC2 accounted for 20.4% of total variation (Fig 3.37). The most obvious difference between ag topsoils and native topsoils, was in land use flipping the axis, as soil health indicators were no longer linear with precipitation. PC1 represented the abundance of these groups, in relation to depth, with the groups most represented from greatest to smallest being MB, TBACT, DEPTH, TFUNG, and GRAMPOS. PC2 represented the fungal components, with the groups most represented from greatest to smallest being pH, PHOS, FBRATIO, AMF, FUNGI, and Carbon. PC3, not represented but considered, held 6.6% of variation and represented the precipitation effect, with the groups most represented from greatest to smallest being PRECIP, GRAMNEG, NAG, BG, CO2 and N.

Ag Subsoil

For the ag subsoils, PC1 accounted for 58.2% of the variation while PC2 accounted for 29.1% of variation (Fig 3.38). In the ag subsoil, there was a flip in the vectors across the x axis, that mirrored that of the native prairie subsoil. PC1 represented the abundance of these groups, in

relation to depth and an emphasis on G+ bacteria, with the groups most represented from greatest to smallest being TBACT, ACTINO, MB, GRAMPOS, DEPTH, and C. PC2 represented the fungal components, with the groups most represented from greatest to smallest being FBRATIO, AMF, TFUNG, and pH. PC3, not represented but considered, held 5.5% of variation and represented the precipitation effect, with the groups most represented from greatest to smallest being PRECIP, C, CO2, and FUNGI.

Summary MANOVAs

The native prairie and ag sites were used to find the cumulative interaction between Use, Precip, and Use*Precip at each depth (Table 3.1; Table 3.5).. Land Use was significant at 0-5 cm ($p<0.05$), 5-15 cm ($p<0.1$), 15-30 cm ($p<0.05$)., and 30-75 cm ($p<0.01$). Precip was only significant at 15-30 cm ($p<0.05$) and 30-75 cm ($p<0.01$). The interaction between Use*Precip was significant at 15-30 cm ($p<0.05$) and 30-75 cm ($p<0.01$).

Depth, pH and Precip significantly interacted with native prairie and ag. In Native Prairie, depth ($p<0.001$), Precip ($p<0.001$), and the interaction of Depth:Precip ($p<0.001$) were significant . Depth:pH ($p<0.01$) were strongly significant, and pH ($p<0.1$) and the interaction between pH:Precip ($p<0.1$) were slightly significance. For Ag, depth ($p<0.001$), pH ($p<0.001$), and Precip ($p<0.01$) were significant. The interaction with Depth:pH ($p<0.05$) and Depth:Precip ($p<0.05$) were also significant and Precip:pH was slightly significant.

For the topsoil in Native Prairie, depth ($p<0.001$), Precip ($p<0.001$), were significant. The importance of pH ($p<0.05$) was enhanced in the topsoil and the interaction between Precip:pH ($p<0.1$),. For Ag, depth ($p<0.001$) remained as the most significant influence on the soil, but Precip ($p<0.05$), and pH ($p<0.05$) were also significant.). In the native subsoil, Precip ($p<0.05$) and Precip:pH ($p<0.05$), had the strongest influence on soil properties. Depth ($p<0.1$) and pH

($p<0.1$) were significant. In the Ag subsoil, Precip:pH ($p<0.05$) and Depth:Precip:pH ($p<0.05$), were significant. Slight significance occurred with Precip ($p<0.1$) and the interaction of Depth:Precip ($p<0.1$).

Tables

MANOVA OF THE INTERACTION BETWEEN LAND USE AND PRECIPITATION

COLUMN1	**0-5 cm**	**5-15 cm**	**15-30 cm**	**30-75 cm**	**75-120 cm**
USE	**0.0350***	**0.0508***	**0.0157***	**0.00310****	0.433
PRECIP	0.421	0.271	**0.0278***	**0.00169****	0.255
USE*PRECIP	0.528	0.468	**0.0361***	**0.00113****	0.192

Table 3.1 Summary of the p-values using MANOVA for the effects and interaction between land use and precipitation on soil health indicators divided by depth. Includes: CARBON: soil organic carbon (mg/g); NITRO: total nitrogen (***μ***g/g); pH; BG: β- glucosidase (mg kg-1hr-1); NAG: N-acetyl-β-D-glucosaminidase (mg kg-1hr-1); PHOS: acid phosphatase (mg kg-1hr-1); CO2: soil respiration (mg CO2 g-1); MB: PLFA microbial biomass (nmol PLFA g-1); GRAMPOS: Gram-positive bacteria (nmol PLFA g-1); GRAMNEG: Gram-negative bacteria (nmol PLFA g-1); ACTINO: actinomycete (nmol PLFA g-1); AMF: arbuscular mycorrhizal fungi (nmol PLFA g-1); FUNGI: fungi (nmol PLFA g-1); FB: fungi to bacteria ratio; OTHER: unidentified microbial biomass (nmol PLFA g-1).

ANOVA table of effect of pH on Soil Health Indicators with Interactions

	BG	NAG	PHOS	CO2	G+	G-	Actino	T.Bact
pH	**<0.001*****	**<0.001*****	**<0.001*****	**<0.001*****	**<0.001*****	0.123	**<0.001*****	**<0.001*****
USE:pH	**0.0207***	**0.0234***	**0.00614****	**0.00283****	**<0.001*****	**0.0183***	**<0.001*****	**<0.001*****
DEPTH:pH	**<0.001*****	**0.0132***	**<0.001*****	**<0.001*****	**<0.001*****	**<0.001*****	**<0.001*****	**<0.001*****
PRECIP:pH	**0.00286****	**<0.001*****	**<0.001*****	0.274	**<0.001*****	0.839	**<0.001*****	**0.00404****
USE:DEPTH:pH	0.262	0.616	**0.0353***	**0.0167***	**<0.001*****	0.139	**<0.001*****	**<0.001*****
USE:PRECIP:pH	0.456	**0.00483****	**0.00563****	**0.0412***	**<0.001*****	0.928	**<0.001*****	**0.0200***
DEPTH:PRECIP:pH	0.539	0.106	0.187	0.492	**<0.001*****	0.992	**0.00976****	0.196
USE:DEPTH:PRECIP:pH	0.740	0.320	0.512	0.280	**0.00641****	0.954	**<0.001*****	0.215
	Fungi	**AMF**	**T. Fung**	**F:B**	**Other**	**C**	**N**	**MB**
pH	**0.0526.**	**0.00163****	**<0.001*****	**<0.001*****	**<0.001*****	**<0.001*****	**<0.001*****	**<0.001*****
USE:pH	**0.0335***	**0.00417***	**<0.001*****	0.391	**<0.001*****	**<0.001*****	**<0.001*****	**<0.001*****
DEPTH:pH	**<0.001*****	**<0.001*****	**<0.001*****	**0.00541****	**<0.001*****	**<0.001*****	**<0.001*****	**<0.001*****
PRECIP:pH	0.479	**0.00317****	**<0.001*****	0.847	**<0.001*****	**<0.001*****	**<0.001*****	**<0.001*****
USE:DEPTH:pH	0.229	**0.0132***	**<0.001*****	0.378	**<0.001*****	**<0.001*****	**<0.001*****	**<0.001*****
USE:PRECIP:pH	0.466	**0.0180***	**0.00631****	0.146	**<0.001*****	**0.00627****	**0.138**	**<0.001*****
DEPTH:PRECIP:pH	0.911	**0.0645.**	**0.0215***	0.815	**0.00330****	**<0.001*****	**0.00105****	**0.00418****
USE:DEPTH:PRECIP:pH	0.896	0.406	0.219	0.480	**0.0291***	0.491	0.300	**0.0280***

Table 3.2 Summary of the effect/interactions that pH has in conjunction with land use (Native and Ag) and precipitation on all main soil health indicators. C : soil organic carbon (mg/g); N: total nitrogen (***μ***g/g); BG: β- glucosidase (mg kg-1hr-1); NAG: N-acetyl-β-D-glucosaminidase (mg kg-1hr-1); PHOS: acid phosphatase (mg kg-1hr-1); CO2: soil respiration (mg CO2 g-1); MB: PLFA microbial biomass (nmol PLFA g-1); G+: Gram-positive bacteria (nmol PLFA g-1); G-: Gram-negative bacteria (nmol PLFA g-1); Actino: actinomycete (nmol PLFA g-1); AMF: arbuscular mycorrhizal fungi (nmol PLFA g-1); Fungi: fungi (nmol PLFA g-1); F:B: fungi to bacteria ratio; OTHER: unidentified microbial biomass (nmol PLFA g-1); T. Fung: total fungi (nmol PLFA g-1); T. Bact: total bacteria (nmol PLFA g-1).

ANOVA p-value table for each soil health indicator: Microbial Biomass										
0-5 cm	**MB**	**G+**	**G-**	**Actino**	**T. Bact**	**Fungi**	**AMF**	**T. Fung**	**F:B**	**Other**
Use	**<0.001*** **	**<0.001*** **	**0.052.**	**<0.001*** **	**<0.001*** **	0.247	**0.0237***	**0.00695****	0.678	**<0.001*** **
Precip	**0.00515****	**<0.001*** **	0.529	**0.0213***	**0.00663****	0.739	0.105	**0.0603.**	0.928	**0.0215***
Use*Precip	**0.0270***	**0.0116***	0.703	**0.0397***	**0.0437***	0.748	0.192	0.127	0.657	**0.0626.**
5-15 cm	**MB**	**G+**	**G-**	**Actino**	**T. Bact**	**Fungi**	**AMF**	**T. Fung**	**F:B**	**Other**
Use	**<0.001*** **	**<0.001*** **	**0.07.**	**<0.001*** **	**<0.001*** **	**0.0742.**	**0.004****	**<0.001*** **	0.433	**<0.001*** **
Precip	**0.00576****	**<0.001*** **	0.733	**0.00592****	**0.00211****	0.544	0.112	**0.0714.**	0.871	**0.0180***
Use*Precip	**0.0137***	**0.0100***	0.199	**0.00238****	**0.00335****	0.628	0.203	0.147	0.995	**0.0515.**
15-30 cm	**MB**	**G+**	**G-**	**Actino**	**T. Bact**	**Fungi**	**AMF**	**T. Fung**	**F:B**	**Other**
Use	**<0.001*** **	**<0.001*** **	**0.065.**	**<0.001*** **	**0.00193****	**0.076.**	**0.0243***	**0.00594****	0.858	**<0.001*** **
Precip	**0.0551.**	**0.00609****	0.852	**0.0555.**	0.126	0.997	**0.0644.**	**0.0396***	0.978	**0.0372***
Use*Precip	0.119	**0.0556.**	0.857	**0.0225***	0.263	0.976	**0.0963.**	**0.0631.**	0.27	**0.0637.**
30-75 cm	**MB**	**G+**	**G-**	**Actino**	**T. Bact**	**Fungi**	**AMF**	**T. Fung**	**F:B**	**Other**
Use	**0.00753****	**0.0201***	**0.069.**	**0.0314***	**0.00775****	0.161	0.377	0.23	**0.0694.**	**0.00647****
Precip	0.451	0.147	0.469	0.824	0.507	0.864	0.558	0.567	0.242	0.424
Use*Precip	0.934	0.727	0.294	0.301	0.639	0.71	0.379	0.403	**0.00379****	0.611
75-120 cm	**MB**	**G+**	**G-**	**Actino**	**T. Bact**	**Fungi**	**AMF**	**T. Fung**	**F:B**	**Other**
Use	0.148	0.291	0.354	0.177	0.22	0.354	0.909	0.986	0.211	0.236
Precip	0.28	**0.0565.**	0.969	0.981	0.65	0.551	0.591	0.624	0.562	**0.0538.**
Use*Precip	0.513	0.25	0.959	0.945	0.823	0.326	0.454	0.52	0.808	0.248

Table 3.3 Summary of the p-values from ANOVA for effects of land use and precipitation on each primary biological soil health indicator separated into each depth increment. MB: PLFA microbial biomass (nmol PLFA g^{-1}); G+: Gram-positive bacteria (nmol PLFA g^{-1}); G-: Gram-negative bacteria (nmol PLFA g^{-1}); Actino: actinomycete (nmol PLFA g^{-1}); AMF: arbuscular mycorrhizal fungi (nmol PLFA g^{-1}); Fungi: general fungi (nmol PLFA g^{-1}); F:B: fungi to bacteria ratio; Other: unidentified microbial biomass (nmol PLFA g^{-1}); T. Fung: total fungi (nmol PLFA g^{-1}); T. Bact: total bacteria (nmol PLFA g^{-1}).

ANOVA p-value table for each soil health indicator: Chemical and Microbial activity						
0-5 cm	**BG**	**NAG**	**PHOS**	**C**	**N**	**CO2**
Use	0.358	**0.0689.**	0.158	**<0.001*** **	**<0.001*** **	**0.0172 ***
Precip	0.359	**0.0338***	**0.0818.**	**0.00562 ****	**0.00609 ****	0.401
Use*Precip	0.608	0.395	**0.0146***	0.352	0.665	0.475
5-15 cm	**BG**	**NAG**	**PHOS**	**C**	**N**	**CO2**
Use	**0.0556.**	**0.031***	**0.0478***	**<0.001*** **	**<0.001*** **	**0.0108***
Precip	**0.0689.**	**0.041***	0.188	**<0.001*** **	**<0.001*** **	0.293
Use*Precip	0.906	0.371	0.221	**0.00863****	**0.0789.**	**0.0351***
15-30 cm	**BG**	**NAG**	**PHOS**	**C**	**N**	**CO2**
Use	**0.0165***	**0.0318***	**0.0186***	**<0.001*** **	**<0.001*** **	**<0.001*** **
Precip	0.239	**0.00649 ****	**0.0417***	**0.00831****	**0.00972****	**0.0179***
Use*Precip	0.386	0.187	**0.0614.**	0.14202	0.5536	**0.0492***
30-75 cm	**BG**	**NAG**	**PHOS**	**C**	**N**	**CO2**
Use	0.23	**0.101.**	**0.00740****	0.298	0.434	**0.0826.**
Precip	**0.106.**	**0.0137***	**<0.001*** **	**0.0165 ***	0.849	0.536
Use*Precip	0.283	**0.066.**	**0.00199****	0.571	0.998	0.576
75-120 cm	**BG**	**NAG**	**PHOS**	**C**	**N**	**CO2**
Use	0.237	0.208	0.299	0.4541	0.196	0.994
Precip	**0.0643.**	**0.0625.**	0.105	**0.0951.**	0.25	0.868
Use*Precip	**0.0956.**	**0.066.**	0.183	0.509	0.375	0.939

Table 3.4 Summary of the p-values from ANOVA for effects of land use and precipitation on chemical and microbial activity based soil health indicator separated into each depth increment. C: soil organic carbon (mg/g); N: total nitrogen (***μ***g/g); BG: β- glucosidase (mg $kg^{-1}hr^{-1}$); NAG: N-acetyl-β-D-glucosaminidase (mg $kg^{-1}hr^{-1}$); PHOS: acid phosphatase (mg $kg^{-1}hr^{-1}$); CO2: soil respiration (mg CO2 g-1 h-1).

	Native	Native (Top)	Native(Sub)
DEPTH	**<0.001*** **	**<0.001*** **	**0.0887.**
PRECIP	**<0.001*** **	**<0.001*** **	**0.0244***
PH	**0.0551.**	**0.0110***	**0.0893.**
DEPTH:PRECIP	**<0.001*** **	0.230	0.811
DEPTH:PH	**0.00373****	0.142	0.141
PRECIP:PH	**0.0600.**	**0.0784.**	**0.0177***
DEPTH:PRECIP:PH	0.454	0.234	0.241
	Ag	Ag (Top)	Ag (Sub)
DEPTH	**<0.001*** **	**<0.001*** **	0.102
PRECIP	**0.00156****	**0.0243***	**0.0548.**
PH	**<0.001*** **	**0.0122***	0.390
DEPTH:PRECIP	**0.0474***	0.166	**0.0838.**
DEPTH:PH	**0.0449***	0.807	0.539
PRECIP:PH	**0.0755.**	0.450	**0.0406***
DEPTH:PRECIP:PH	0.198	**0.0698.**	**0.04940***

Table 3.5 Summary of the p-values using MANOVA for the effects and interaction between depth, pH and precipitation on soil health indicators divided by land use and soil type. Includes: CARBON: soil organic carbon (mg/g); NITRO: total nitrogen (***μ***g/g); pH; BG: β- glucosidase (mg $kg^{-1}hr^{-1}$); NAG: N-acetyl-β-D-glucosaminidase (mg $kg^{-1}hr^{-1}$); PHOS: acid phosphatase (mg $kg^{-1}hr^{-1}$); CO2: soil respiration (mg CO2 g-1); MB: PLFA microbial biomass (nmol PLFA g^{-1}); GRAMPOS: Gram-positive bacteria (nmol PLFA g^{-1}); GRAMNEG: Gram-negative bacteria (nmol PLFA g^{-1}); ACTINO: actinomycete (nmol PLFA g^{-1}); AMF: arbuscular mycorrhizal fungi (nmol PLFA g^{-1}); FUNGI: fungi (nmol PLFA g^{-1}); FB: fungi to bacteria ratio; OTHER: unidentified microbial biomass (nmol PLFA g^{-1}).

Figures

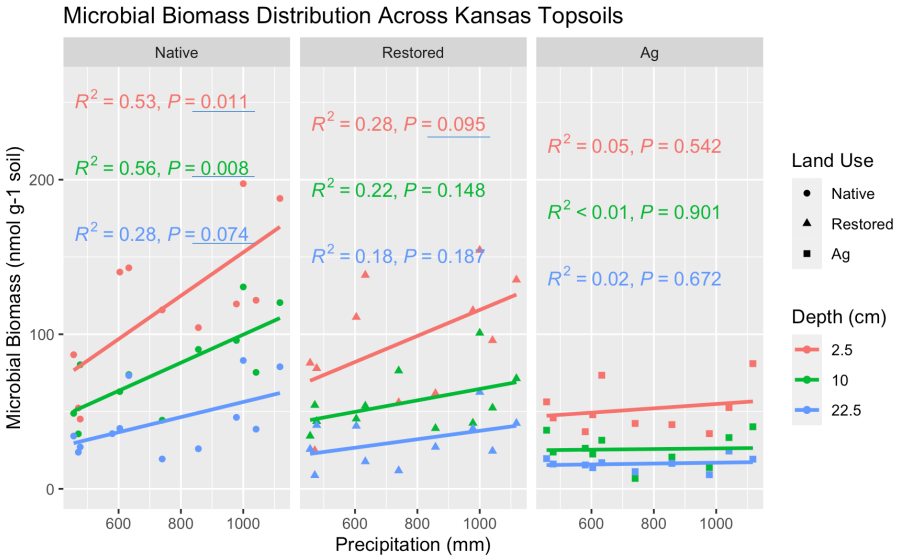

Figure 3.1 Microbial biomass in the topsoil of the Kansas precipitation gradient by land use and top 3 depths of 2.5 cm (0-5cm), 10 cm (5-15 cm), and 22.5 cm (15-30 cm). Proportional variance of linearity represented by R2, ANOVA with letters representing significant difference (p<0.1).

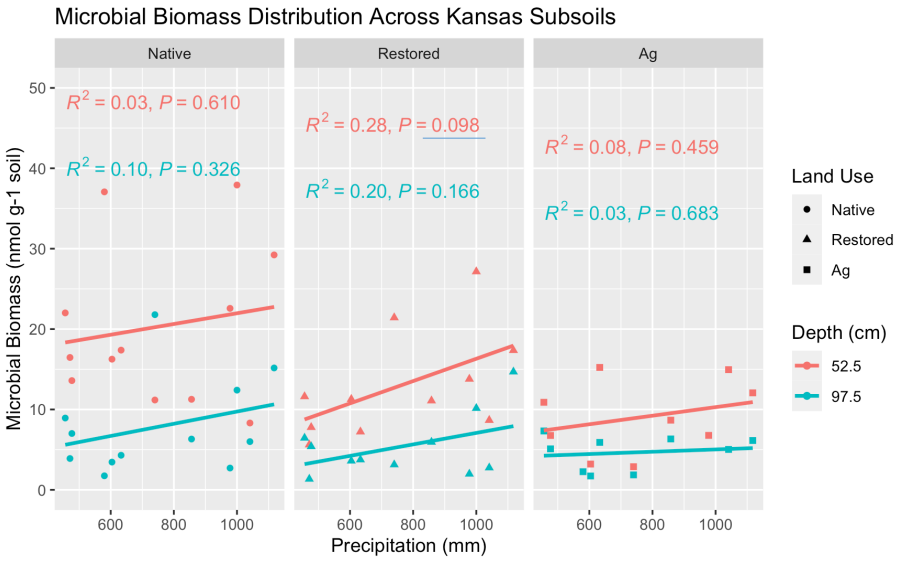

Figure 3.2 Microbial biomass in the subsoils of the Kansas precipitation gradient by land use and bottom 2 depths of 52.5 cm (30-75 cm), and 97.5 cm(75-120cm). Proportional variance of linearity represented by R2, ANOVA with letters representing significant difference ($p<0.1$).

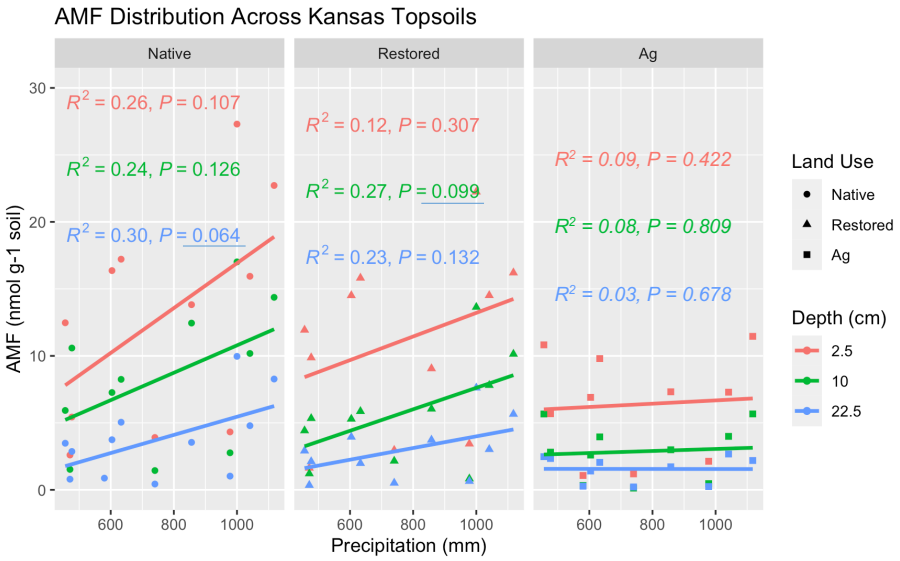

Figure 3.3 Arbuscular mycorrhizal fungi biomass in the topsoils of the Kansas precipitation gradient by land use and top 3 depths of 2.5 cm (0-5cm), 10 cm (5-15 cm), and 22.5 cm (15-30 cm). Proportional variance of linearity represented by R2, ANOVA with letters representing significant difference ($p<0.1$).

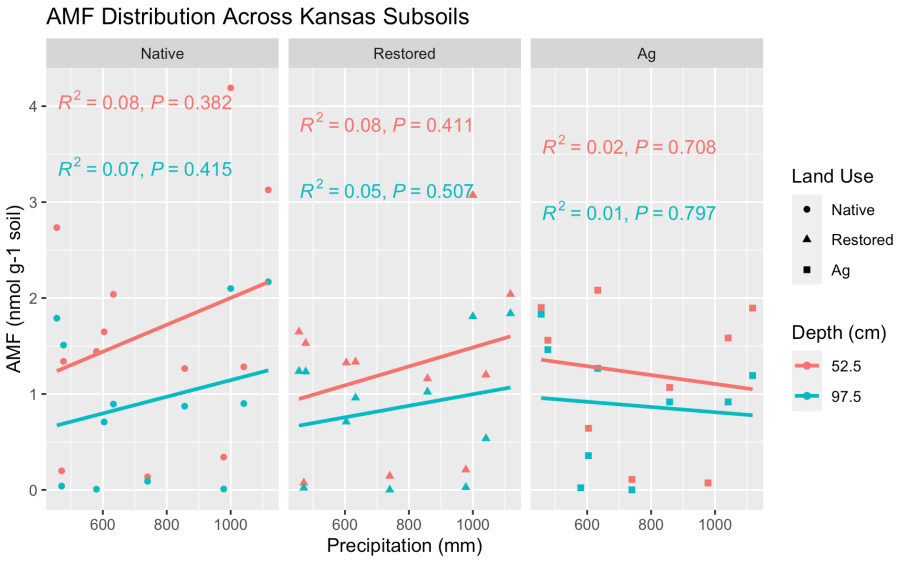

Figure 3.4 Arbuscular mycorrhizal fungi biomass in the subsoils of the Kansas precipitation gradient by land use and bottom 2 depths of 52.5 cm (30-75 cm), and 97.5 cm(75-120cm), Proportional variance of linearity represented by R^2, ANOVA with letters representing significant difference ($p<0.1$).

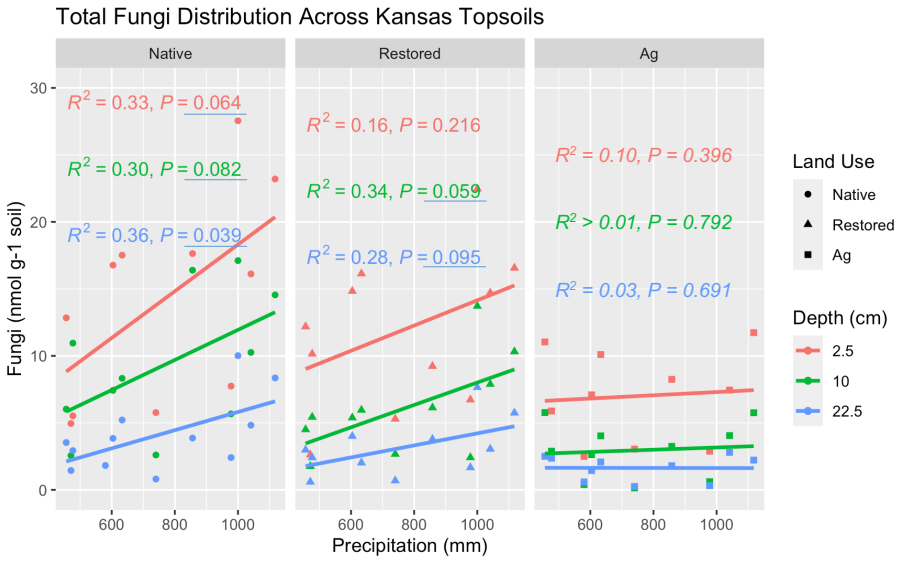

Figure 3.5 Total fungal biomass in the topsoils of the Kansas precipitation gradient by land use and top 3 depths of 2.5 cm (0-5cm), 10 cm (5-15 cm), and 22.5 cm (15-30 cm). Proportional variance of linearity represented by R2, ANOVA with letters representing significant difference ($p<0.1$).

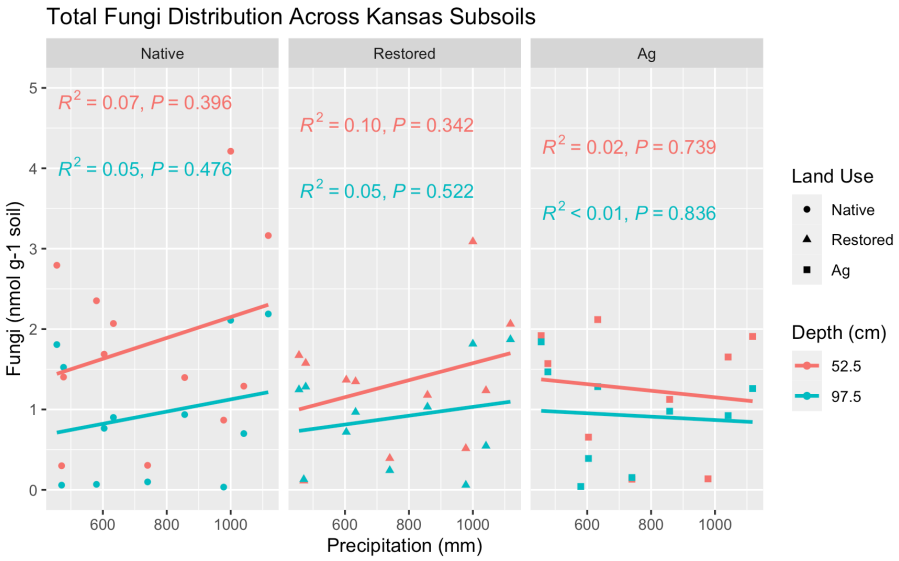

Figure 3.6 Total fungal biomass in the subsoils of the Kansas precipitation gradient by land use and bottom 2 depths of 52.5 cm (30-75 cm), and 97.5 cm(75-120cm). Proportional variance of linearity represented by R2, ANOVA with letters representing significant difference (p<0.1).

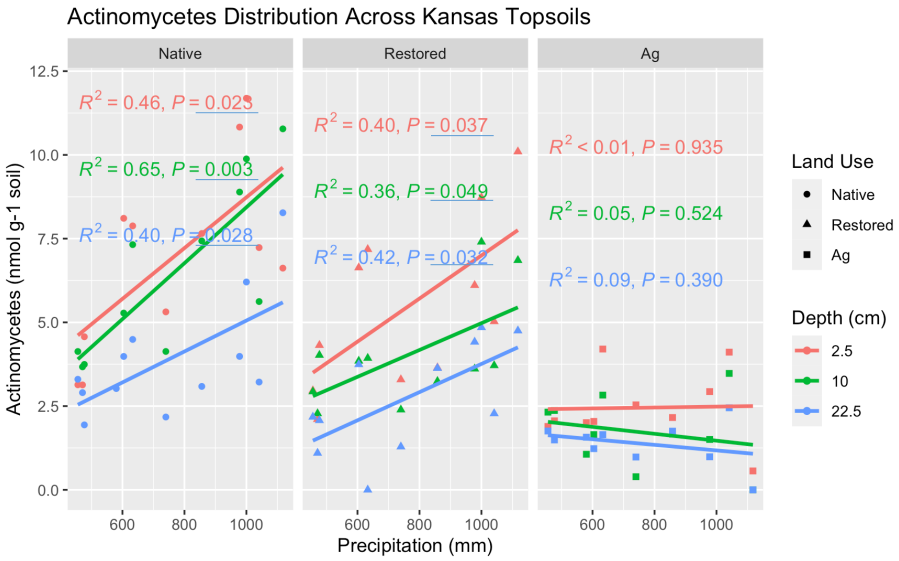

Figure 3.7 Actinomycete bacteria biomass in the topsoil of the Kansas precipitation gradient by land use and top 3 depths of 2.5 cm (0-5cm), 10 cm (5-15 cm), and 22.5 cm (15-30 cm). Proportional variance of linearity represented by R2, ANOVA with letters representing significant difference ($p<0.1$).

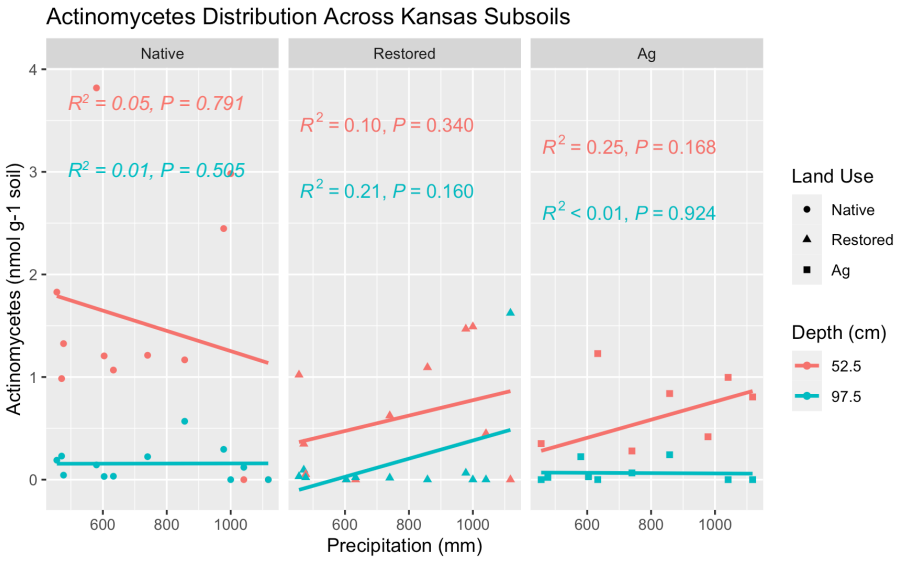

Figure 3.8 Actinomycete bacteria biomass in the subsoils of the Kansas precipitation gradient by land use and bottom 2 depths of 52.5 cm (30-75 cm), and 97.5 cm(75-120cm). Proportional variance of linearity represented by R2, ANOVA with letters representing significant difference ($p<0.1$).

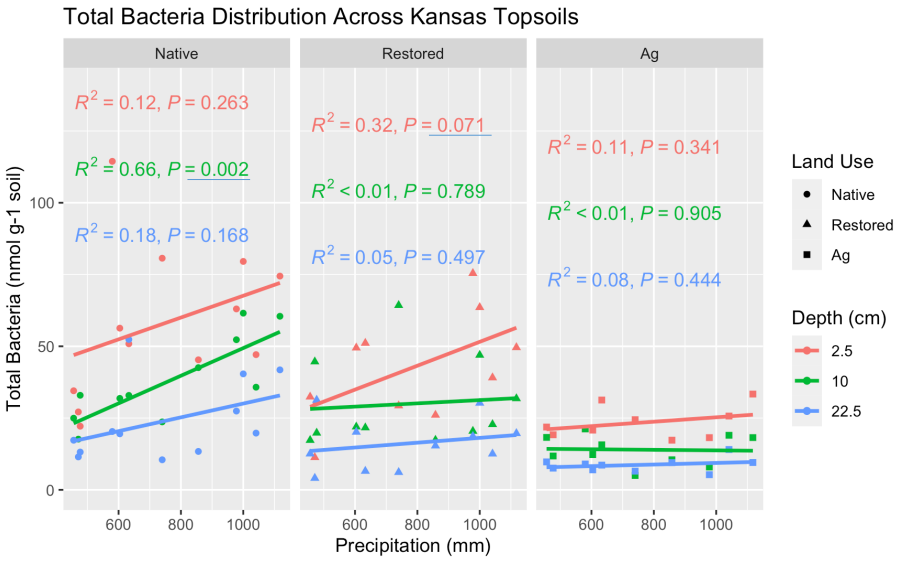

Figure 3.9 Total bacteria biomass in the topsoil of the Kansas precipitation gradient by land use and top 3 depths of 2.5 cm (0-5cm), 10 cm (5-15 cm), and 22.5 cm (15-30 cm). Proportional variance of linearity represented by R2, ANOVA with letters representing significant difference ($p<0.1$).

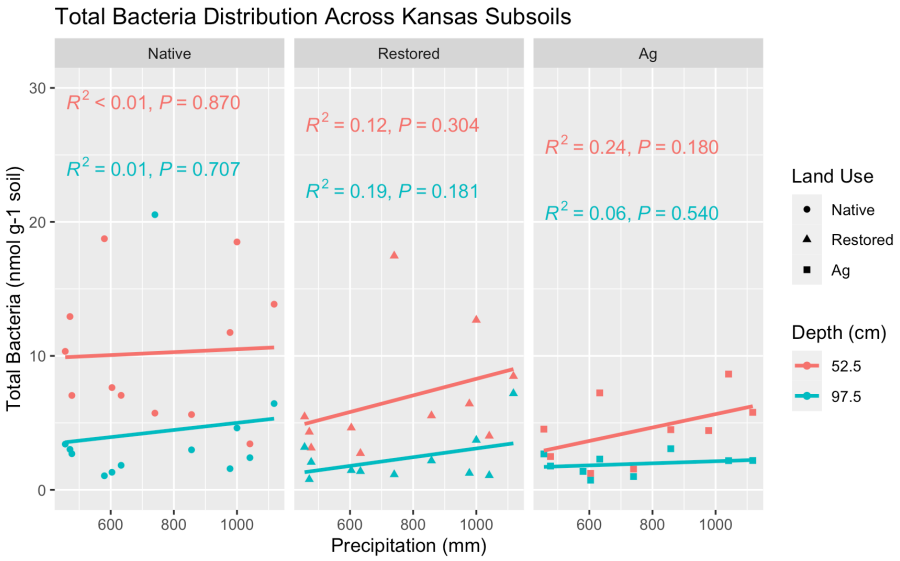

Figure 3.10 Total bacteria biomass in the subsoils of the Kansas precipitation gradient by land use and bottom 2 depths of 52.5 cm (30-75 cm), and 97.5 cm(75-120cm). Proportional variance of linearity represented by R2, ANOVA with letters representing significant difference ($p<0.1$).

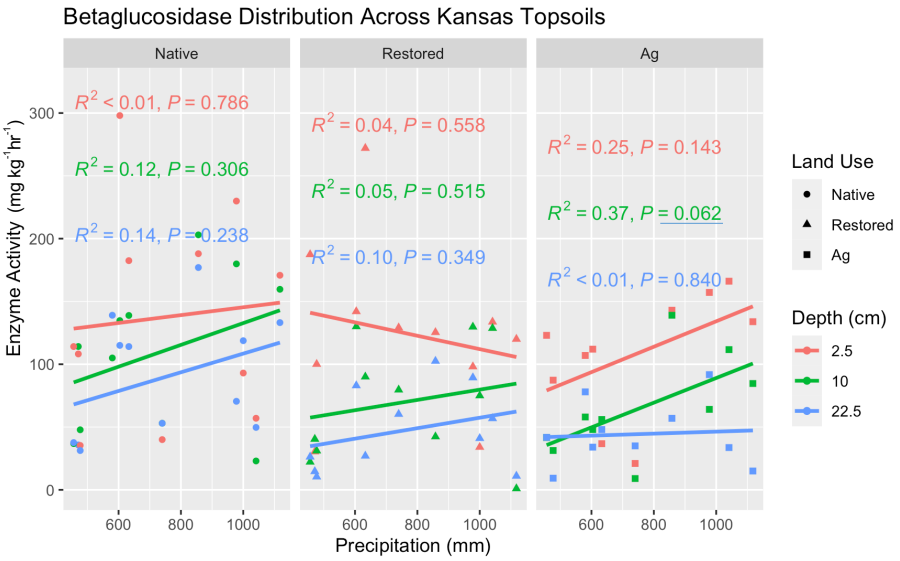

Figure 3.11 β-1,4-glucosidase activity in the topsoil of the Kansas precipitation gradient by land use and top 3 depths of 2.5 cm (0-5cm), 10 cm (5-15 cm), and 22.5 cm (15-30 cm). Proportional variance of linearity represented by R2, ANOVA with letters representing significant difference ($p<0.1$).

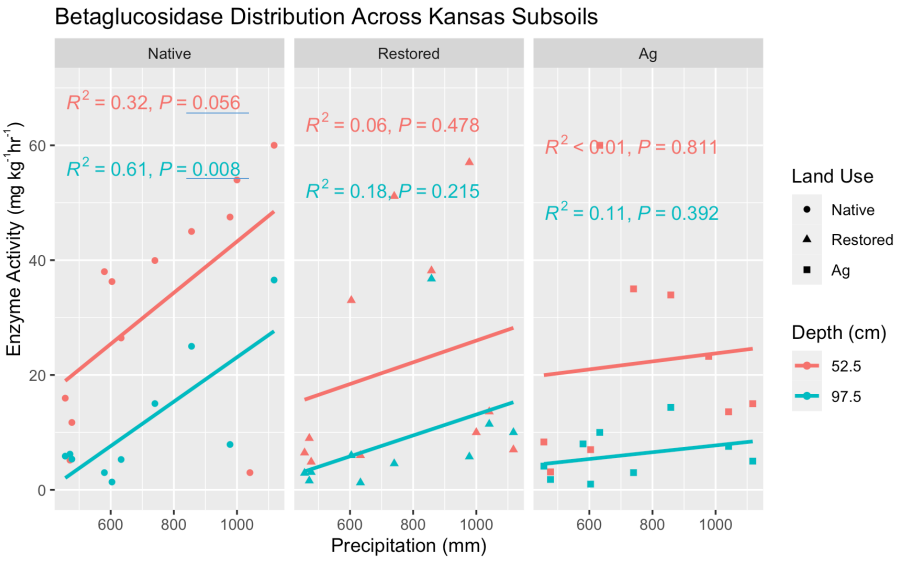

Figure 3.12 β-1,4-glucosidase activity in the subsoils of the Kansas precipitation gradient by land use and bottom 2 depths of 52.5 cm (30-75 cm), and 97.5 cm(75-120cm). Proportional variance of linearity represented by R2, ANOVA with letters representing significant difference ($p<0.1$).

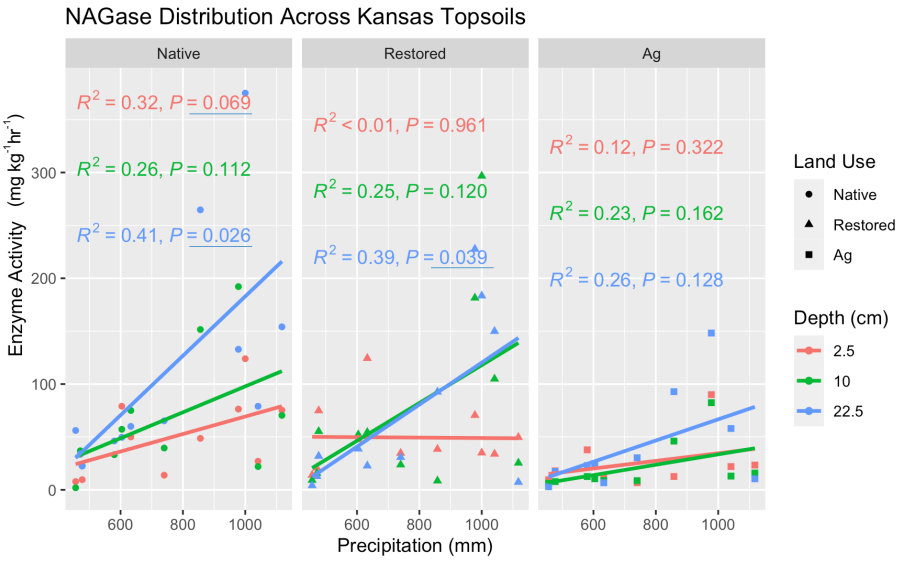

Figure 3.13 N-acetyl-β-D-glucosaminidase activity in the topsoil of the Kansas precipitation gradient by land use and top 3 depths of 2.5 cm (0-5cm), 10 cm (5-15 cm), and 22.5 cm (15-30 cm). Proportional variance of linearity represented by R2, ANOVA with letters representing significant difference ($p<0.1$).

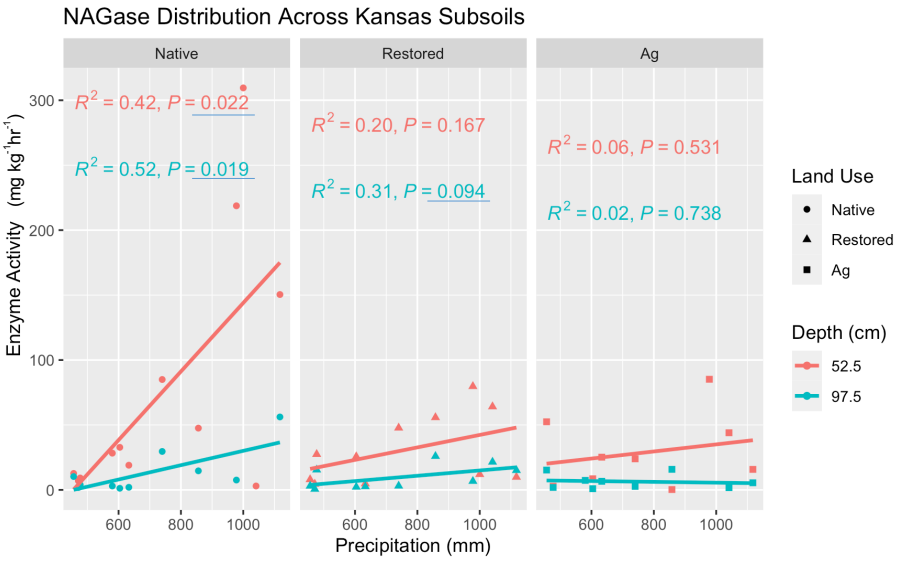

Figure 3.14 N-acetyl-β-D-glucosaminidase activity in the subsoils of the Kansas precipitation gradient by land use and bottom 2 depths of 52.5 cm (30-75 cm), and 97.5 cm (75-120 cm). Proportional variance of linearity represented by R2, ANOVA with letters representing significant difference ($p<0.1$).

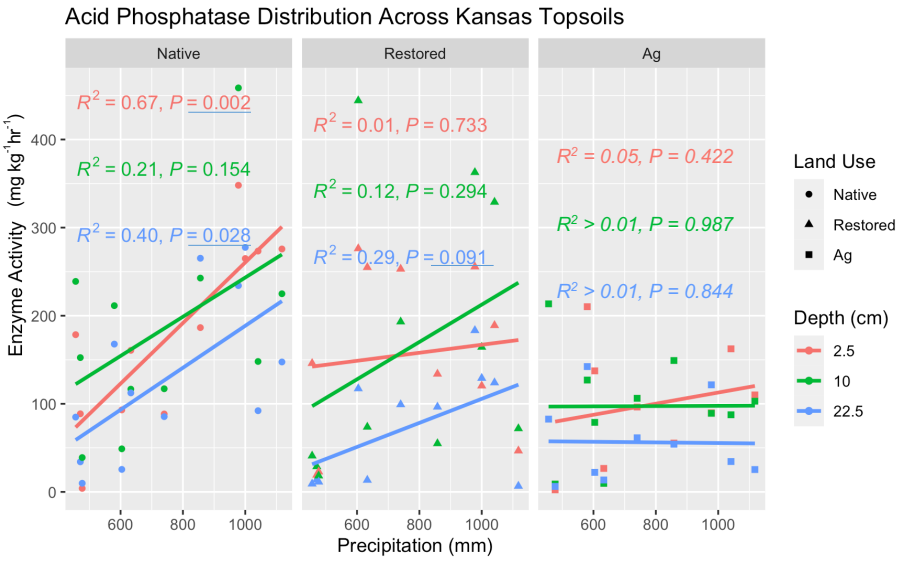

Figure 3.15 Acid phosphatase activity in the topsoil of the Kansas precipitation gradient by land use and top 3 depths of 2.5 cm (0-5 cm), 10 cm (5-15 cm), and 22.5 cm (15-30 cm). Proportional variance of linearity represented by R2, ANOVA with letters representing significant difference ($p<0.1$).

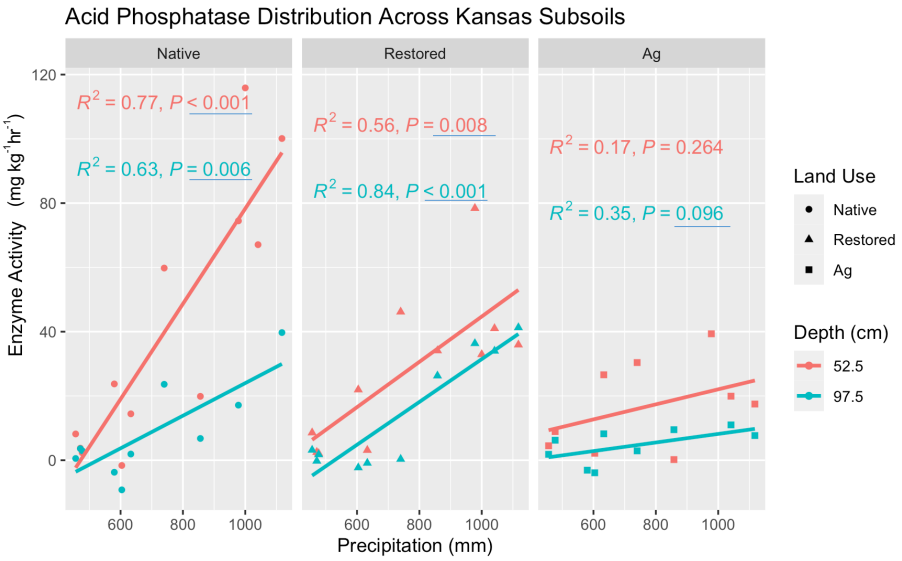

Figure 3.16 Acid phosphatase activity in the subsoils of the Kansas precipitation gradient by land use and bottom 2 depths of 52.5 cm (30-75 cm), and 97.5 cm (75-120 cm). Proportional variance of linearity represented by R2, ANOVA with letters representing significant difference ($p<0.1$).

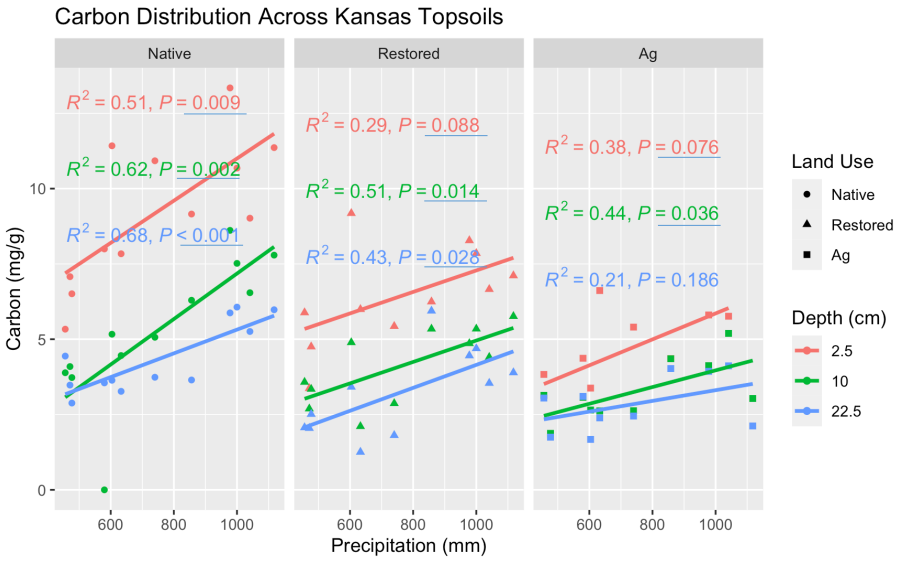

Figure 3.17 Soil organic C in the topsoil of the Kansas precipitation gradient by land use and top 3 depths of 2.5 cm (0-5cm), 10 cm (5-15 cm), and 22.5 cm (15-30 cm). Proportional variance of linearity represented by R2, ANOVA with letters representing significant difference ($p<0.1$).

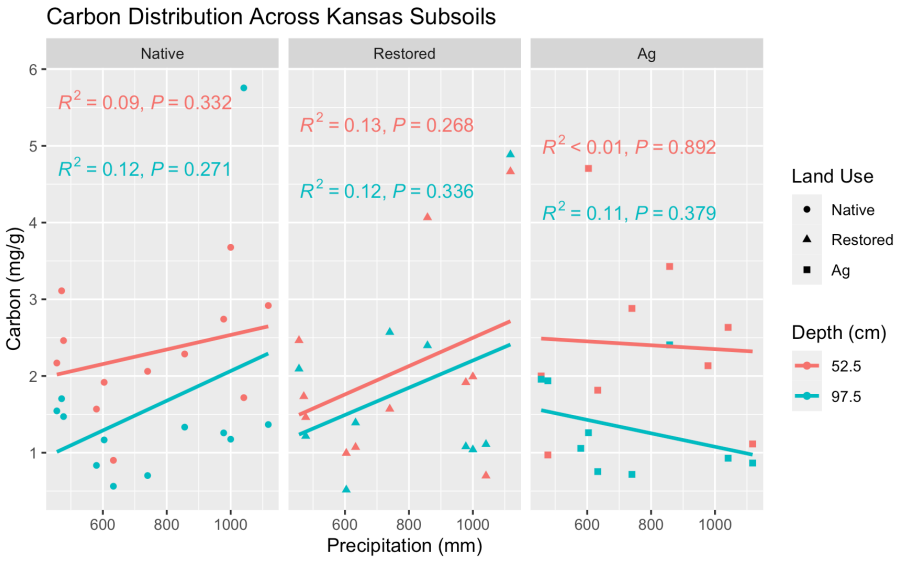

Figure 3.18 Soil organic C in the subsoils of the Kansas precipitation gradient by land use and bottom 2 depths of 52.5 cm (30-75 cm), and 97.5 cm (75-120cm). Proportional variance of linearity represented by R2, ANOVA with letters representing significant difference ($p<0.1$).

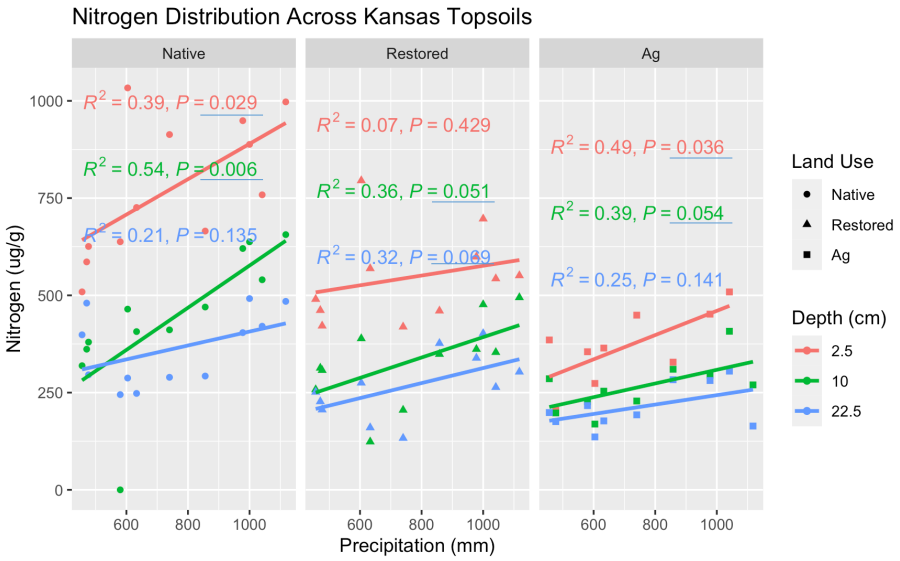

Figure 3.19 Soil nitrogen in the topsoil of the Kansas precipitation gradient by land use and top 3 depths of 2.5 cm (0-5cm), 10 cm (5-15 cm), and 22.5 cm (15-30 cm). Proportional variance of linearity represented by R2, ANOVA with letters representing significant difference ($p<0.1$).

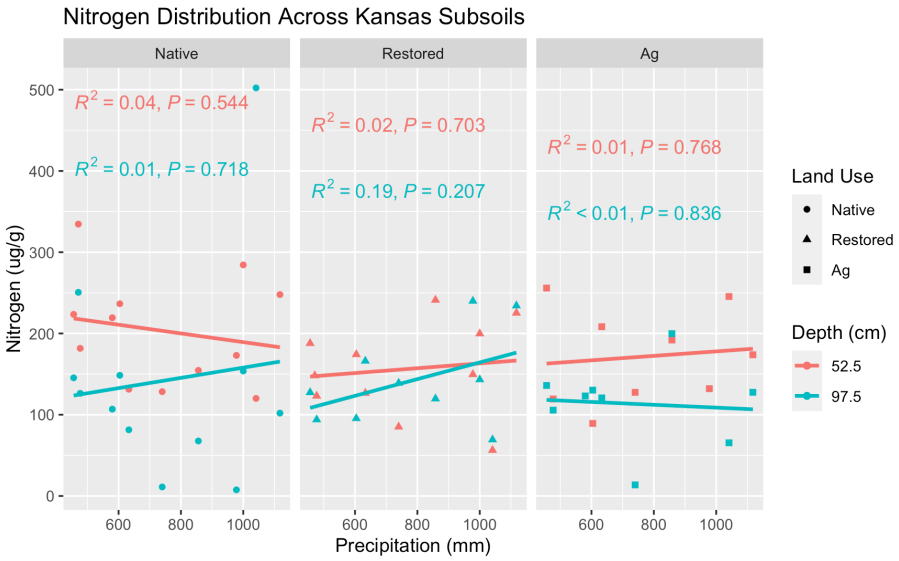

Figure 3.20 Soil nitrogen in the subsoils of the Kansas precipitation gradient by land use and bottom 2 depths of 52.5 cm (30-75 cm), and 97.5 cm(75-120cm). Proportional variance of linearity represented by R2, ANOVA with letters representing significant difference ($p<0.1$).

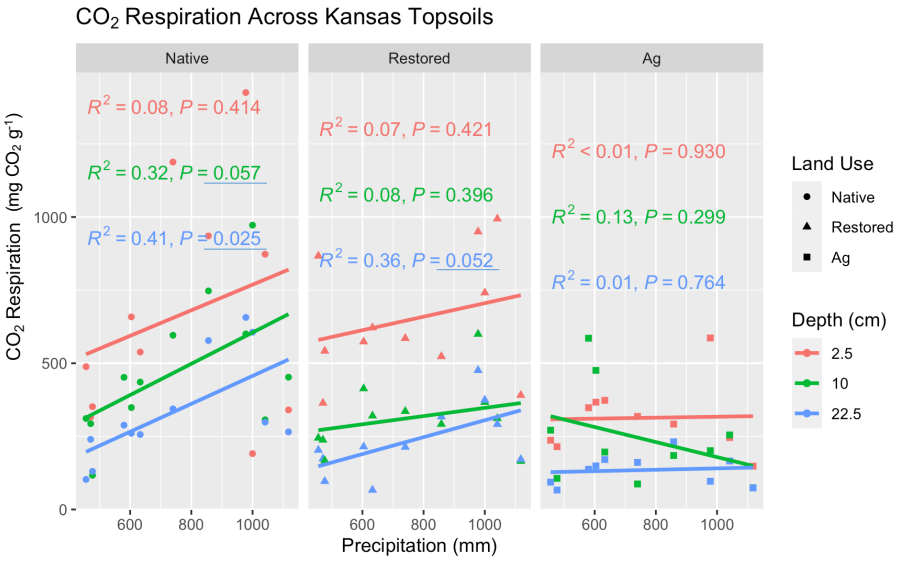

Figure 3.21 Soil CO2 respiration in the topsoil of the Kansas precipitation gradient by land use and top 3 depths of 2.5 cm (0-5cm), 10 cm (5-15 cm), and 22.5 cm (15-30 cm). Proportional variance of linearity represented by R2, ANOVA with letters representing significant difference ($p<0.1$).

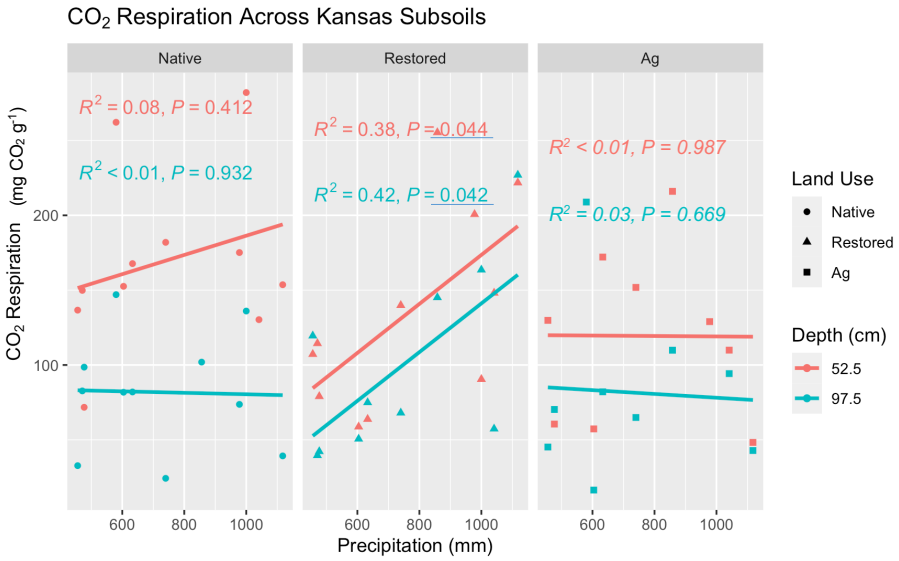

Figure 3.22 Soil CO2 respiration in the subsoils of the Kansas precipitation gradient by land use and bottom 2 depths of 52.5 cm (30-75 cm), and 97.5 cm(75-120cm). Proportional variance of linearity represented by R2, ANOVA with letters representing significant difference ($p<0.1$).

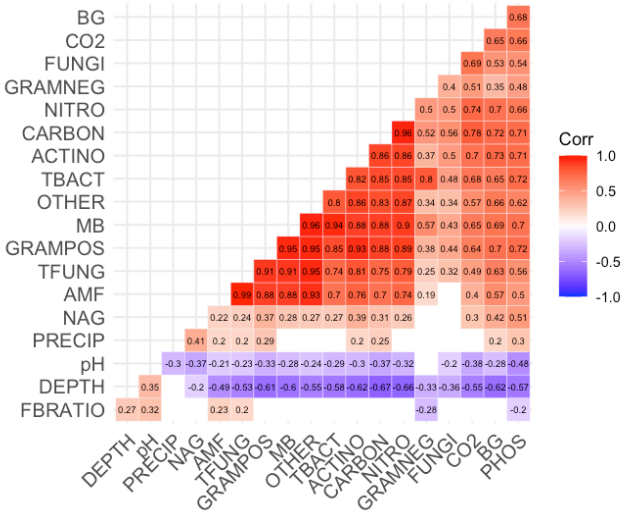

Figure 3.23 Correlation matrix of soil health properties for Native and Ag land uses using. CARBON: soil organic carbon (mg/g); NITRO: total nitrogen (***μ***g/g); pH; BG: β- glucosidase (mg kg-1hr-1); NAG: N-acetyl-β-D-glucosaminidase (mg kg-1hr-1); PHOS: acid phosphatase (mg kg-1hr-1); CO2: soil respiration (mg CO2 g-1); MB: PLFA microbial biomass (nmol PLFA g-1); GRAMPOS: Gram-positive bacteria (nmol PLFA g-1); GRAMNEG: Gram-negative bacteria (nmol PLFA g-1); ACTINO: actinomycete (nmol PLFA g-1); AMF: arbuscular mycorrhizal fungi (nmol PLFA g-1); FUNGI: fungi (nmol PLFA g-1); FB: fungi to bacteria ratio; DEPTH: average depth increments (cm); OTHER: unidentified microbial biomass (nmol PLFA g-1); TFUNG: total fungi (nmol PLFA g-1); TBACT: total bacteria (nmol PLFA g-1).

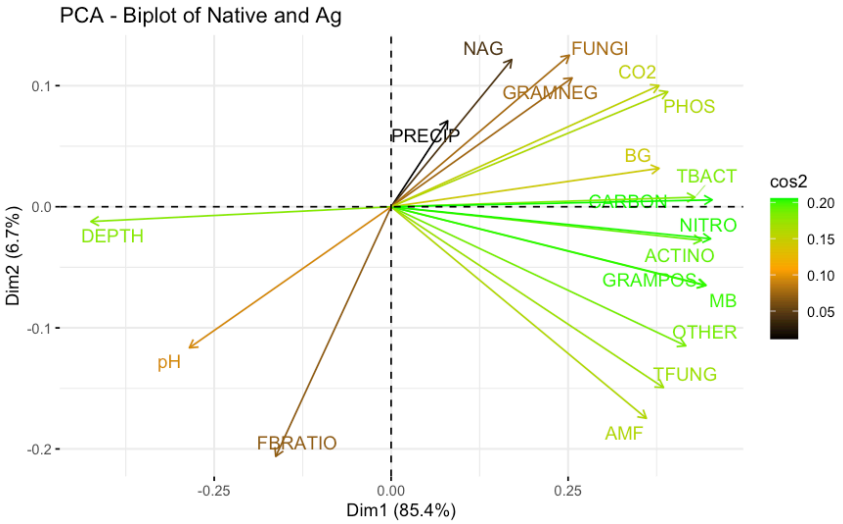

Figure 3.24 Principal component analysis biplot of physical and chemical soil health properties in Native and Ag soils.

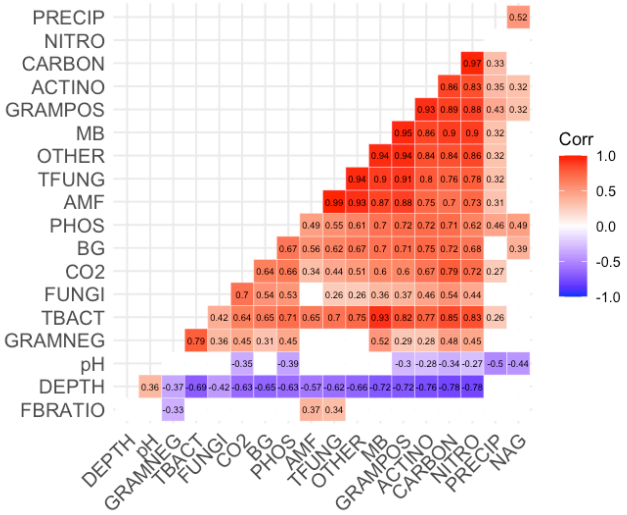

Figure 3.25 Correlation matrix of soil health properties for complete soil profile of native prairie using Spearman correlation coefficient given inside squares ($p<0.1$).

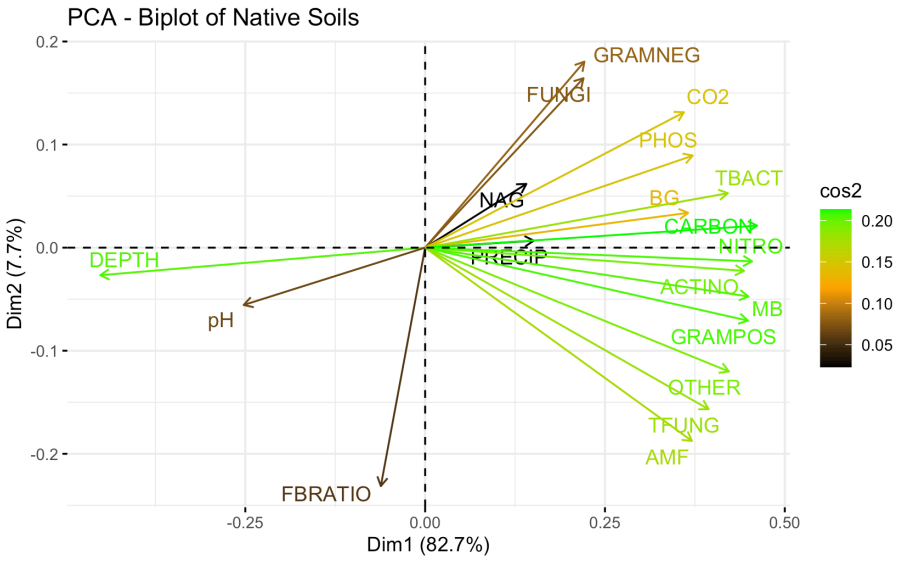

Figure 3.26 Principal component analysis biplot of physical and chemical soil health properties for complete soil profile of native prairie.

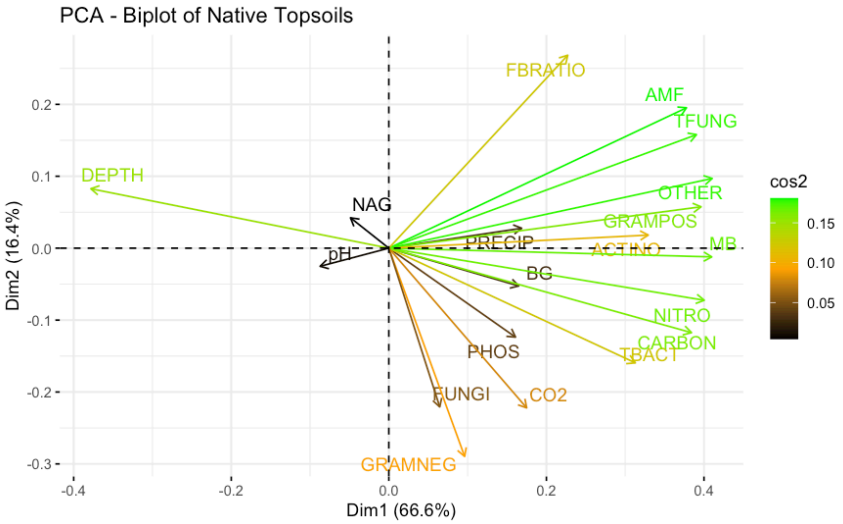

Figure 3.27 Principal component analysis biplot of physical and chemical soil health properties for native topsoil using top three depths (0-5cm, 5-15 cm, 15-30 cm) of native prairie.

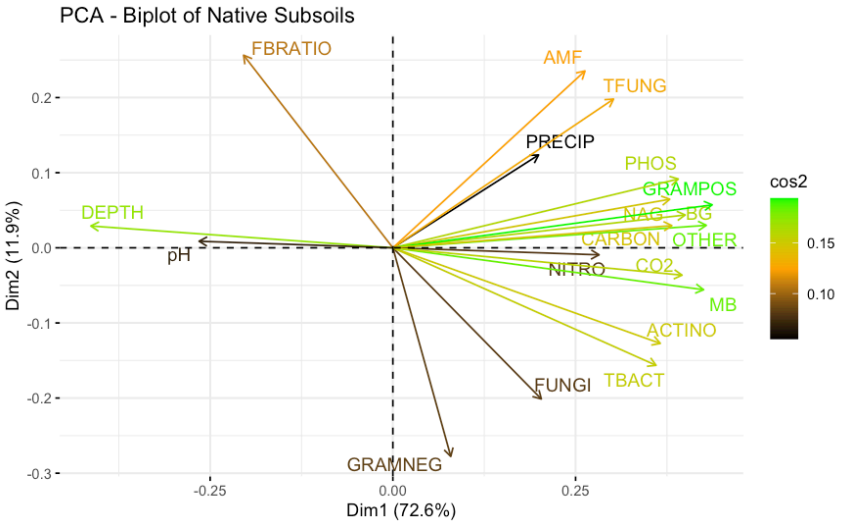

Figure 3.28 Principal component analysis biplot of physical and chemical soil health properties for native subsoils using bottom two depths (30-75 cm, 75-120 cm) of native prairie.

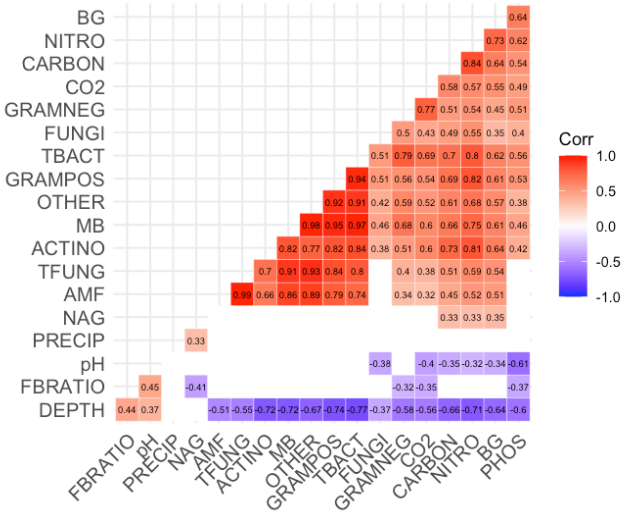

Figure 3.29 Correlation matrix of soil health properties for complete soil profile of agricultural soils using Spearman correlation coefficient given inside squares ($p<0.1$).

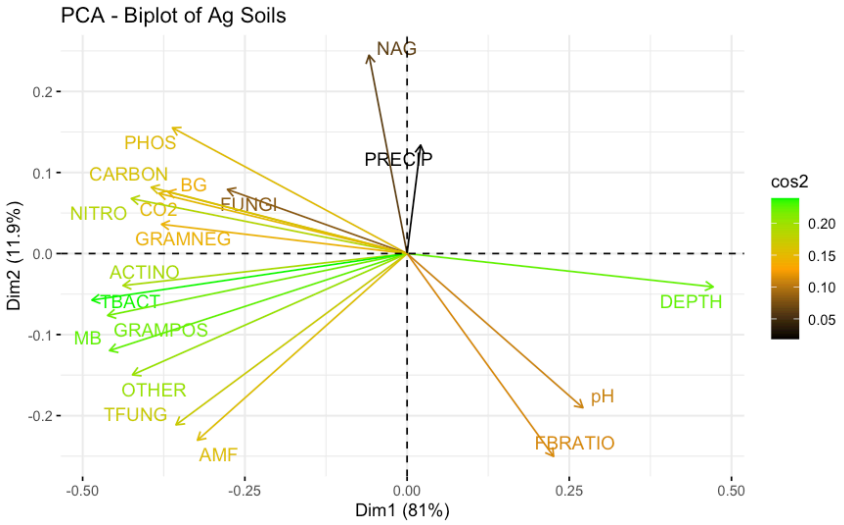

Figure 3.30 Principal component analysis biplot of physical and chemical soil health properties for complete soil profile of for the agricultural soils.

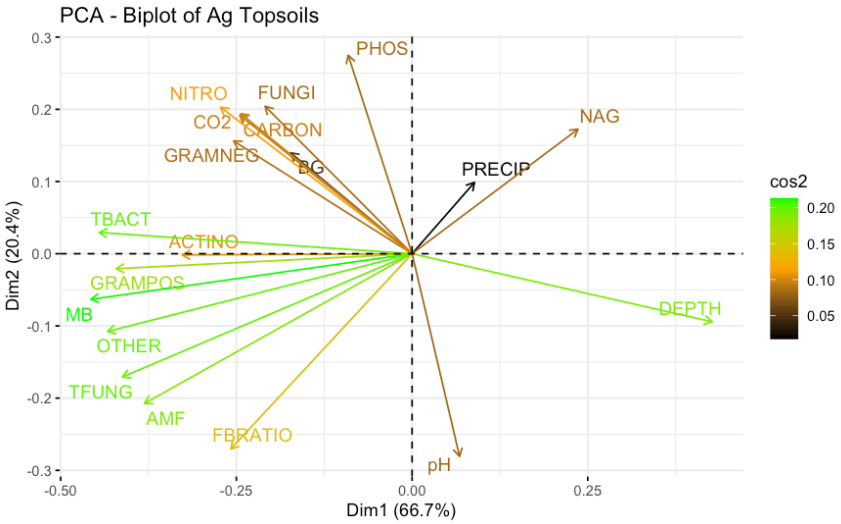

Figure 3.31 Principal component analysis biplot of physical and chemical soil health properties for the agricultural topsoil using top three depths (0-5cm, 5-15 cm, 15-30 cm).

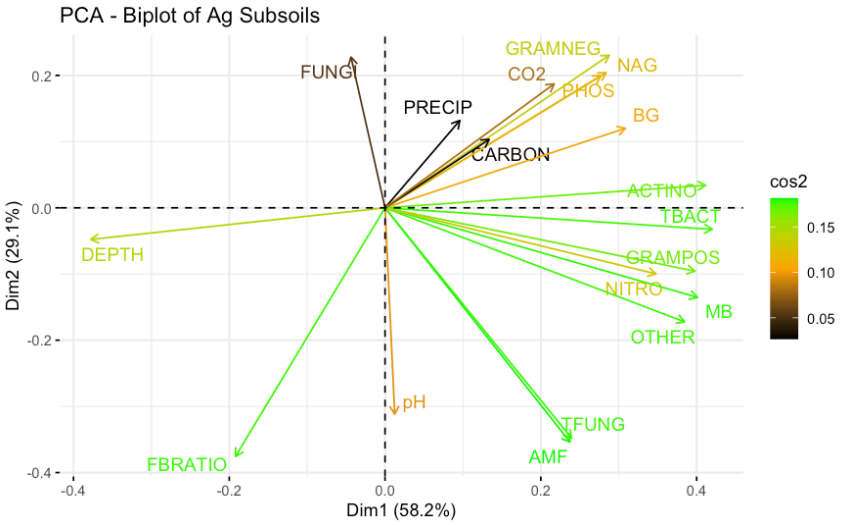

Figure 3.32 Principal component analysis biplot of physical and chemical soil health properties for the agricultural subsoils using bottom two depths (30-75 cm, 75-120 cm).

Chapter 4 - Discussion

Precipitation

Precipitation was a major driver of the measured soil properties especially in grassland systems of both the native and restored prairie (Nielsen et al., 2010; Akbar et al., 2018; Wang et al., 2021) Native and restored prairie sites in the topsoil, showed consistently that microbial abundance, activity, and nutrients consistently increased with precipitation (Fig 3.1—Fig 3.22). As water availability increased, microbial biomass increased (Wan et al., 2021). In general, microbial biomass significantly increased with precipitation in the topsoil of native prairies, demonstrating the importance of water availability in the determination of vegetative capability of production (Li et al., 2017; Fradera-Sola et al., 2019). The only soil indicators that did not follow this trend, were those associated with microbial activity and labile carbon at 0-5 cm, specifically, BG and CO_2.

In contrast, in the rainfed Ag sites, there was neither a decrease nor increase in relation to precipitation and microbial biomass at any depth. Soil nutrients and microbial activity also did not vary across the precipitation gradient, but a relationship with precipitation was found with BG, C, and N. Soil moisture has been identified as being a factor influencing the coupling of the dynamics between land and atmosphere (Schwingshackl et al., 2017; Seneviratne et al., 2010; Akbar et al., 2018). Land use is known to disrupt soil water relations (Niu et al., 2015). The conversion of native land to ag land decoupled the relationship between precipitation and microbial biomass. As Ag sites are under a current effect of disturbance from tillage, this renders the soil health properties of this land use the most vulnerable to human influences, impacting the active layer of the microbial community (Seitz et al., 2022) and altering its stability, structure, and functioning (Liu et al., 2014; Vries et al., 2018).

Both native and restored prairie had multiple correlations with precipitation that demonstrated the influence of climate on their ecosystem productivity (Ganjurjav et al., 2017), but for restored prairie soils, the degree of relationship was affected by legacy effects of disturbance. This can be seen by the differences in the ranges of soil health properties measured as well as less correlations and significance in the restored prairie topsoil. As the restored soils have prior use as conventional ag sites, the original soil structure, microbial diversity, and plant diversity have been lost or altered (Dou et al., 2023), thus requiring the restoration of healthy soil properties. The legacy effects of agriculture in restored or previously disturbed land as the effect of tillage, erosion, and invasive species of microorganisms and plants deter growth and the reintroduction of native microorganisms or plants (Grman & Suding, 2010; Nsikani et al., 2018). In the topsoil of restored prairies, these legacy effects appeared to have caused a slightly weaker relationship with precipitation and a decrease in the soil health properties of restored soils, in comparison to the native sites, but an increase in comparison to Ag.

The precipitation effect on microbial groups in different land uses in the topsoil was dependent on the microbial group. Microbial groups in the topsoil had the strongest relationship with precipitation in the native prairie and the restored prairie. Individually measured fungal groups did not have as strong of a relationship to precipitation, as they did when combined. AMF is known to respond to land use modification and can be useful in assessing changes in the soil ecosystem (Liang et al., 2012; Herzberger et al., 2014; Liu et al., 2021). AMF responds to environmental changes due to the association of its fungal network with its host plant (Akhter et al., 2015). This may insinuate that AMF is more impacted by land use as a soil health indicator than precipitation, but the total fungal biomass may rely more on precipitation. For bacterial biomass, the most significant relationship between precipitation in the prairie ecosystems was in

gram + bacteria, but more specifically actinomycetes. Actinomycetes are major nutrient cyclers and is reliant on soil water (AbdElgawad et al., 2020). For all topsoil depths measured in the prairie ecosystems, actinomycetes responded to precipitation (Fig 3.7), though there was no relationship with precipitation in the subsoils.

Commented [A1]: They do not assist in N fixation except Frankia for certain tree species

Like microbial biomass, soil organic carbon and total nitrogen, increased along the precipitation gradient in the topsoils but did not respond to precipitation in the subsoil. For both carbon and nitrogen, precipitation had a significant relationship within all land uses. Climate variability has been shown to lead spatial variations in the acquisition of SOC stocks (Zhang et al., 2010). As mentioned before, increased precipitation leads to increased grassland biomass production, which benefits microbial growth, and thus nutrient cycling. The increase in the nutrient stores of grassland ecosystems is facilitated by increased water, the limiting resource in grassland ecosystems. As the main nutrient cycled throughout the soil, SOC benefits from increased precipitation (Zhang et al., 2010; Çelik, 2017). This shows that in perennial grassland ecosystems, increased precipitation increased soil C stocks (Çelik, 2017).

For CO_2 respiration, as the direct product of microbial biomass (Zhao et al., 2016), the relationship between it and precipitation closely resembled that of microbial biomass in the topsoil. CO_2 respiration is a combination of the influence of microbial biomass and water availability (Zou et al., 2023). This makes it influenced by climatic influences that may increase or decrease stress or activity in the soil.

The relationship between SOC and precipitation was present for all land uses. For CO_2 respiration and MB, however, there was no effect of precipitation across Ag sites. CO_2 respiration has a direct relationship with the easily decomposed labile fraction of SOC (Lagomarsino et al., 2008; Luo et al., 2023), indicating that the decoupled relationship between

precipitation and CO_2 respiration in Ag may be due to a loss of labile C. Despite a loss in labile carbon in the Ag sites, SOC was related to precipitation. This is likely due to a majority of SOC reside in the non-bioavailable, recalcitrant fraction (Jamala, 2013; Canarini et al., 2017; Cotrufo et al., 2019).

Nitrogen was related to precipitation in the topsoil, but not the subsoil. Grassland ecosystems are nitrogen limited. This is due to the slow acquisition of atmospheric nitrogen deposits over the span of the grassland's existence (Phoenix et al., 2011). When precipitation increases, not only nitrogen content in the soil increases, but nitrogen movement does as well (Olin et al., 2015; Feyissa et al., 2021). Disturbance, soil type, and SOM are known contributors to the change in quantity of nitrogen in the soil, especially when it is coupled with low or no soil cover (Abdalla et al., 2019). As the restored prairies have a legacy effect that disturbs nutrient recovery and biomass output (Hansen & Gibson, 2013), it is likely that the top depth was more vulnerable in comparison to lower depths that are protected from external influences.

Microbial Activity

In all three land uses, BG was not significantly influenced by precipitation and BG activity did not differ in the 0-5 cm depth. The BG activity in the 0-30 cm depths of the native prairie was higher than the restored prairie, supporting disturbance being a factor affecting BG (Pandey et al., 2014). Importantly, BG activity did not have the same relationship with precipitation as MB or soil C. This went against expectations, as BG and other enzymes have a substrate induced activity (Zeng et al., 2021). There was an expectation that in ecosystems with more substrate availability, there would be more BG activity. Additionally, BG is strongly influenced by soil moisture than that of temperature (Zhang et al., 2011). Cregger et al. (2012) suggests that seasonal variability may play a significant role in BG activity. Xu et al. (2020)

highlighted that the preexisting soil moisture influences BG activity. As there is no change in BG activity with precipitation, this suggests that there are other factors than precipitation that are influencing BG activity. Much more needs to be known about the relationship between BG, and precipitation.

The PHOS activity was significantly related with precipitation, even in lower depths. The significant increase in PHOS in relation to precipitation may indicated that there is a higher demand for P with the increased precipitation in grassland ecosystems (Huang et al., 2011). Additionally, factors such as pH, plant diversity, precipitation (Wan et al., 2020; Margalef et al., 2021). Ag topsoil saw no significant interaction with PHOS, which can be significantly influenced by P fertilizers PHOS (Spohn et al., 2015), but at 75-120 cm, a significant interaction with precipitation was shown, which may indicate a maximum range in which P fertilizers may influence PHOS activity.

NAG was similar to PHOS. The activity of NAG did not follow the same trend as the other soil health indicators, where it decreased with depth. Mori et al. (2021), suggested that the ratio of BG:NAG may be an indicator of the stage of decomposition. Cellulose, which is degraded by BG, is primarily added to the soil through vegetative biomass, while chitin and peptidoglycan primarily comprised of necromass is degraded by NAG. NAG activity closely resembled the relationship of precipitation with MB.

Ag has a similar amount of BG activity in comparison to other land uses but did had lower activity of NAG and PHOS. The lower BG activity in the AG sites may be attributed to lower cellulose production (Schimel & Schaeffer, 2003; Niu et al., 2021; Raiesi & Khadem, 2019).

Precipitation and Disturbance

In the Ag sites, the microbial properties were not related to precipitation. Many have found that tillage reduces microbial activity and diversity (Smith et al., 2016; Wagg et al., 2014; Zeiss et al., 2022). This led to decreased microbial biomass in the ag sites, even in the subsoil. The effect of precipitation was confined to the topsoil (0-30 cm). The subsoil is constrained by multiple factors, including low oxygen, nutrient availability, pH, which could restrict the effect of precipitation (Stone et al., 2014; Piotrowska-Długosz et al., 2021; Chen et al., 2021). Actinomycetes, AMF, and T. had a significant relationship with precipitation in the topsoil. All three of these groups have known relationships of increasing with water availability or increasing water absorption in the soil (Wan et al., 2021; Osborne et al., 2010). Agricultural land use is known to impact the soil pH, which affects AMF abundance, diversity, and colonization (Pan et al., 2020). The significant interaction between precipitation and land use was due to the lack of response to precipitation in the ag sites.

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Enzyme activity, in comparison to microbial biomass, varied more in response to soil disturbance. This can first be seen by analyzing the relationship BG has with precipitation in native and Ag sites. For BG, in native and ag, activity was similar and BG activity in the native prairie did not respond to precipitation. This relationship was also found in Yang et al. (2022). Others have found that BG is impacted strongly by agricultural land use (Li et al., 2014). In the subsoil of native prairie there was a strong relationship between precipitation and BG activity, but not in the restored prairie or ag. The relationship between BG activity in the subsoil appears to be decoupled by disturbance.

In NAG, there is a resemblance to microbial biomass, in that there is relationship with precipitation and land use that is disturbed by land use. The trend of disturbance continued in the

subsoil, where ag had no association with precipitation, while native and restored did. This shows that NAG was influenced by precipitation throughout both the topsoil and subsoil and the influence of disturbance can be seen at much greater depths than in MB.

PHOS was more like NAG, as it had linear relationship with precipitation that appeared throughout the topsoil and subsoil. PHOS activity has the highest significance regarding its relationship with land use, precipitation and the interaction of it with precipitation, especially at lower depths (Tab 3.4). The PHOS activity was significantly lower in the ag sites with no relationship to precipitation, which was likely due to the application of P fertilizers and tillage (Margalef et al., 2021).

Enzyme activity abundance is dependent on the relative importance of the enzyme to the soil community (Mori et al., 2021; Margenot and Wade, 2023). Creating ratios of enzymes are a commonly utilized idea, that speculates the relative importance of nutrient or decomposition source in the soil. For BG and NAG, the ratio, or the relationship between these two has been indicators of where energy resource is allocated, but more precisely, we should consider this to be the stage in which decomposition is in (Mori et al., 2021). When comparing BG and NAG, the activity of the two enzymes was most abundant at different depths in all, which may suggest different decomposition stages, especially in ag. This, in combination with the increasing relationship of enzyme activity with depth, may indicate a possible depth dependence of enzyme activity.

Ecosystem Dynamics

The topsoil of native and ag sites proved to be less uniform than the whole soil profile. When comparing the dynamics of native and ag ecosystems in the topsoil, the greatest difference between land uses shows as a 180-degree shift along the x axis, that causes the vectors of ag and

native go in opposite directions. This type of change is consistent with finding that agree, that under similar soil types (Gömöryová et al., 2020; Zhao et al., 2016), land use has a confounding influence on microbial community structure (Leeuwen et al. 2017; Seuradge et al. 2016). Additionally, Cao et al., (2017) observed that this influence is greater in the topsoil than in the subsoil. Grouping among soil health indicators remained similar. This is likely due to land use and precipitation consistently having the same relationship with soil health indicators. Luo et al. (2020), found that in the top 10 cm of the soil land use had a significant influence on both microbial community and enzymatic activity, assumed to be due to the differences in plant diversity loss as well as physical and chemical soil properties. Additionally, while many have found that fungal to bacterial ratio is impacted by tillage and disturbance (Ng et al., 2012; Schmidt et al., 2019), there was no significant difference found between this ratio, precipitation, and land use (Table 3.3), as the ratio of microbial activity in native and ag sites remained similar.

Most research on the impact of precipitation gradients on microbial dynamics focus on grasslands, that in turn have been found to have significant relationships with respiration, biomass, soil total C, and (Nielsen & Ball, 2015; Xu et al., 2016). When modeling Ag along with native through correlation matrixes, the greatest loss in correlation is between that of Ag with precipitation.

The shift along the x axis, can be attributed to the loss of measured soil health properties caused by disturbance. As native soil's lack human disturbance, they have higher seed density and species richness in comparison to other land uses (Thakur et al., 2015), which in turn supports a higher microbial biomass, especially AMF (Xiang et al., 2014; Duley & Boselli, 2022). Tillage negatively effects soil structure (Navas et al., 2020), microbial community

structure (Sun et al., 2018), and biomass (Dou et al., 2023), leading to decreased soil health properties.

In the subsoil, ag soil meets back with native prairie, visible in the way the PCA biplot has flipped along the x axis, causing the two land uses to mirror each other more. This may suggest that Ag subsoils are less influenced by external influences. In Hao et al. (2020), different cropping systems stopped differing in microbial diversity after 60 cm and Cao et al. (2017) found that under different land uses, deeper depths had had more similar microbial structure. While it is understood that microbial diversity, activity, and biomass decrease with depths (Hu et al., 2019; Hao et al., 2020), less is known about subsoil community dynamic itself. As the soil health indicators for native and ag subsoils are similar in their relative abundance, this supports that the movement of vectors is attributed to differences in abundance and variability.

To test the combined effect of soil indicators at each depth, all soil health indicators, excluding summary indicators, were used in a MANOVA, to find how Use, Precip, and Use*Precip changed in accordance with increasing depth. In this, the effect concluded at 75 cm. The soil properties of subsoil, favor slower growing microorganisms (Eilers et al, 2012; Uksa et al, 2017) as there is less availability of needed properties for growth. While this effects 30-75 cm abundance, about individual soil health indicators, 75-120 cm has shown a near uniform lack of effect from any external influence, likely signaling a maximum range of influence for external soil health influences on effect the internal dynamics in the soil.

As soil depth increased, more significance as well as interactions can be seen between that of soil health indicators and external influence in the MANOVA. This could be attributed to many factors but is most likely due to the way that the biproducts of external factors can leach into the subsoil, controlling its ecosystem (Liu et al., 2022). Not much is known about the

influence that precipitation has on lower depths as most research stops at 30 cm (Yang et al., 2022). Increased precipitation could lead to increased total microbial activity in the subsoil, due to nutrient leaching and infiltration, similar to how increased precipitation changes the pH of the soil (Liu et al., 2022). This may influence community composition in the subsoil, especially in relation to enzyme activity (Yang et al., 2022).

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Chapter 5 - Summary

Inconsistencies

Some inconsistencies restricted the range of cover for the soil health of each site, specifically the physical soil indicators. The key difference between land uses that influences the way precipitation influences soil health is attributed to soil disturbance but a physical metric of the way each site differed were not present, due to a lack of soil. This required speculation of the soil physical condition using known factors that are influenced by soil disturbance: microbial activity, biomass, and carbon content (Meurer et al., 2020; Ezeokoli et al., 2020).

Additionally, while all restored prairie locations have been out of ag use for over a decade, not all sites had an exact year in which restoration occurred. The degree in which each restored prairie was either actively or passively restored is also unknown. It is known that active restoration can far accelerate the recovery of restored sites in comparison to passively restored sites (Allek et al., 2022; Parkhurst et al, 2021), and passive restoration can produce both negative and positive results (Leon & Osorio, 2014; Miao et. al, 2016). Due to the variability of restored prairie, restored prairie was excluded on more technical analysis.

Recommendations

In any rainfed soil ecosystem, the optimal soil health of a soil would be similar to native, having a strong correlation with precipitation. But as seen in restored, disturbance may always have a strong legacy effect on soil health. When it comes to ag, it can be seen that active disturbance is the most destructive to the relationship between the soil and its ecosystem. Without the elimination of disturbance to the soil, the relationship between the soil and precipitation will be unable to persist. But, by introducing conservation ag practices, there may

be a way to reestablish a reconnection between precipitation and soil health indicators that are less effected by disturbance, such as BG.

Appendix

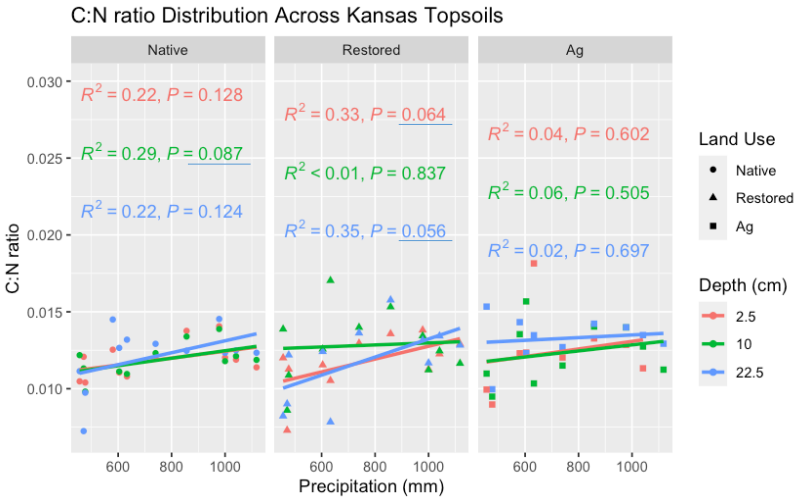

Figure 5.1.A. Soil carbon to nitrogen ratio in the topsoil of the Kansas precipitation gradient separated by land use and top 3 depths of 2.5 cm (0-5cm), 10 cm (5-15 cm), and 22.5 cm (15-30 cm). Proportional variance of linearity represented by R^2, ANOVA with letters representing significant difference ($p<0.1$).

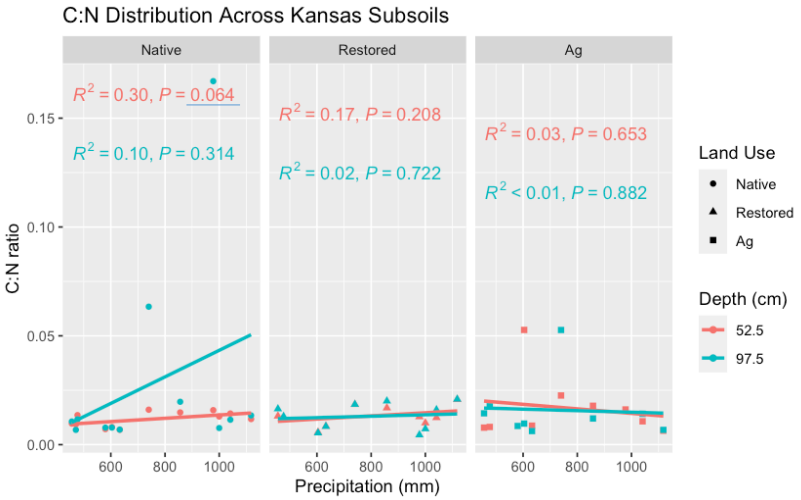

Figure 5.2.A Soil carbon to nitrogen ratio in the subsoils of the Kansas precipitation gradient, separated by land use and bottom 2 depths of 52.5 cm (30-75 cm), and 97.5 cm(75-120cm). Proportional variance of linearity represented by R^2, ANOVA with letters representing significant difference ($p<0.1$).

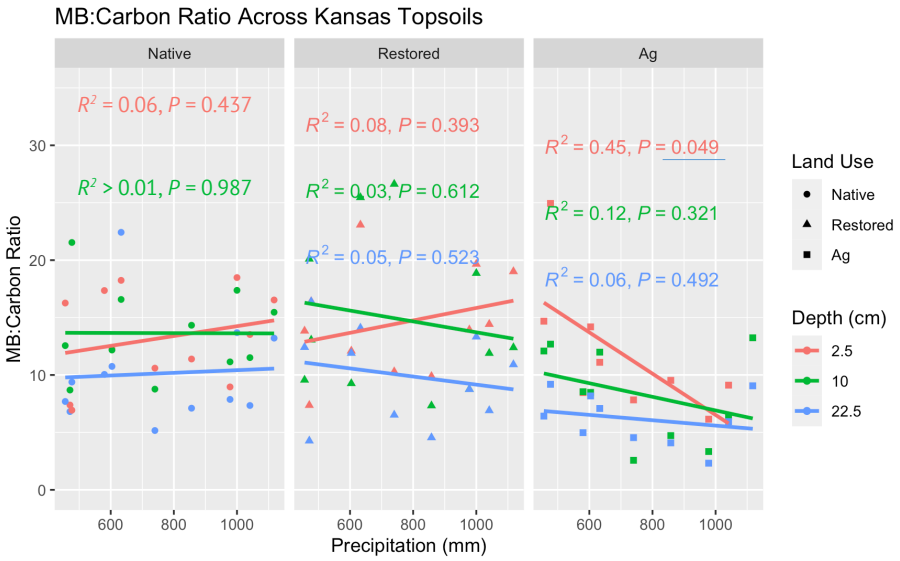

Figure 5.3.A Microbial biomass to soil C ratio in the topsoil of the Kansas precipitation gradient by land use and top 3 depths of 2.5 cm (0-5cm), 10 cm (5-15 cm), and 22.5 cm (15-30 cm). Proportional variance of linearity represented by R^2, ANOVA with letters representing significant difference ($p<0.1$).

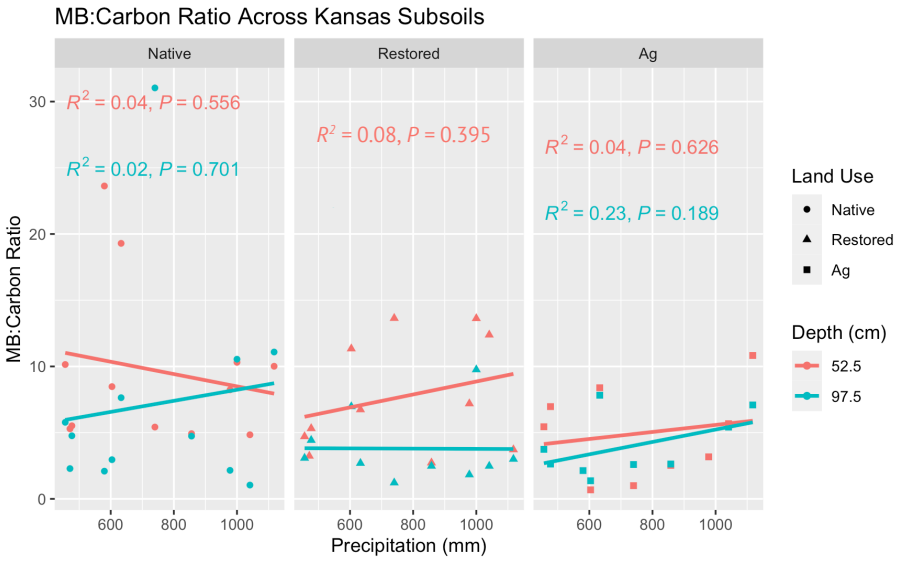

Figure 5.4.A Microbial biomass to soil C ratio in the subsoils of the Kansas precipitation gradient by land use and bottom 2 depths of 52.5 cm (30-75 cm), and 97.5 cm(75-120cm). Proportional variance of linearity represented by R^2, ANOVA with letters representing significant difference ($p<0.1$).

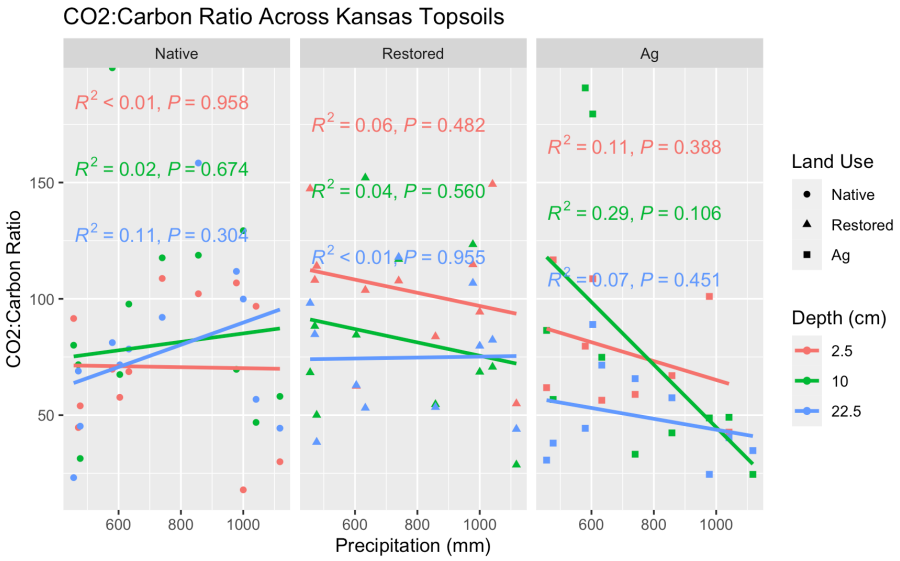

Figure 5.5.A CO_2 activity to soil C ratio in the topsoil of the Kansas precipitation gradient by land use and top 3 depths of 2.5 cm (0-5cm), 10 cm (5-15 cm), and 22.5 cm (15-30 cm). Proportional variance of linearity represented by R^2, ANOVA with letters representing significant difference ($p<0.1$).

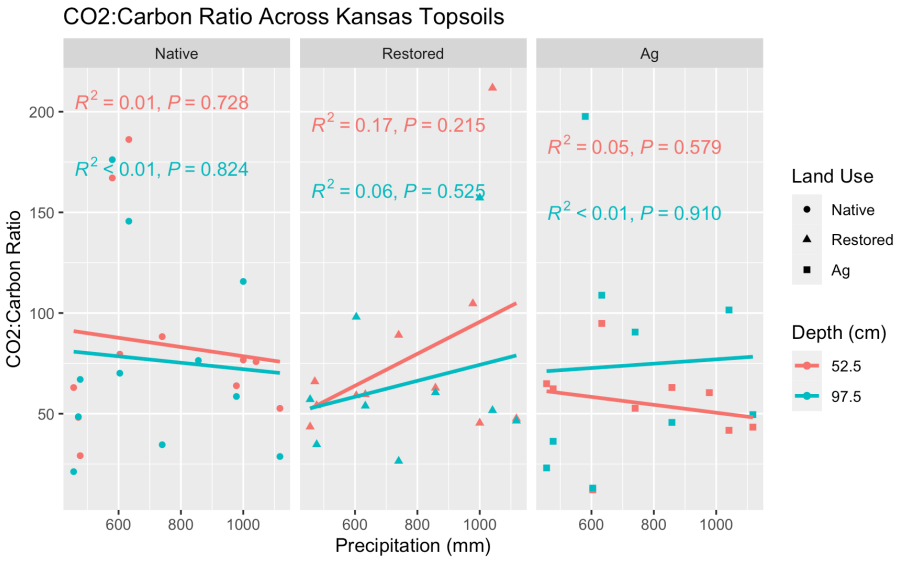

Figure 5.6.A CO2 respiration to soil C ratio in the subsoils of the Kansas precipitation gradient by land use and bottom 2 depths of 52.5 cm (30-75 cm), and 97.5 cm(75-120cm). Proportional variance of linearity represented by R2, ANOVA with letters representing significant difference (p<0.1).

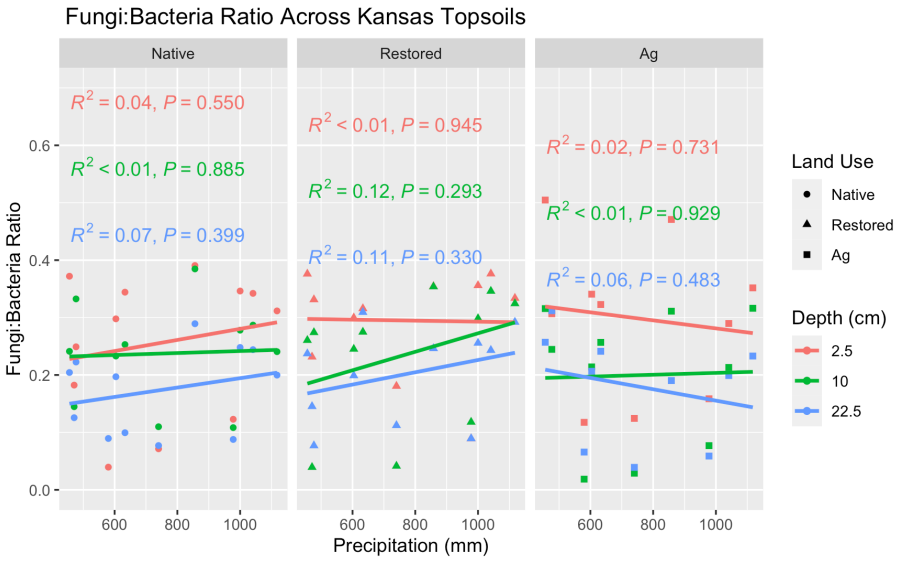

Figure 5.7.A Fungal to Bacteria ratio in the topsoil of the Kansas precipitation gradient by land use and top 3 depths of 2.5 cm (0-5cm), 10 cm (5-15 cm), and 22.5 cm (15-30 cm). Proportional variance of linearity represented by R2, ANOVA with letters representing significant difference (p<0.1).

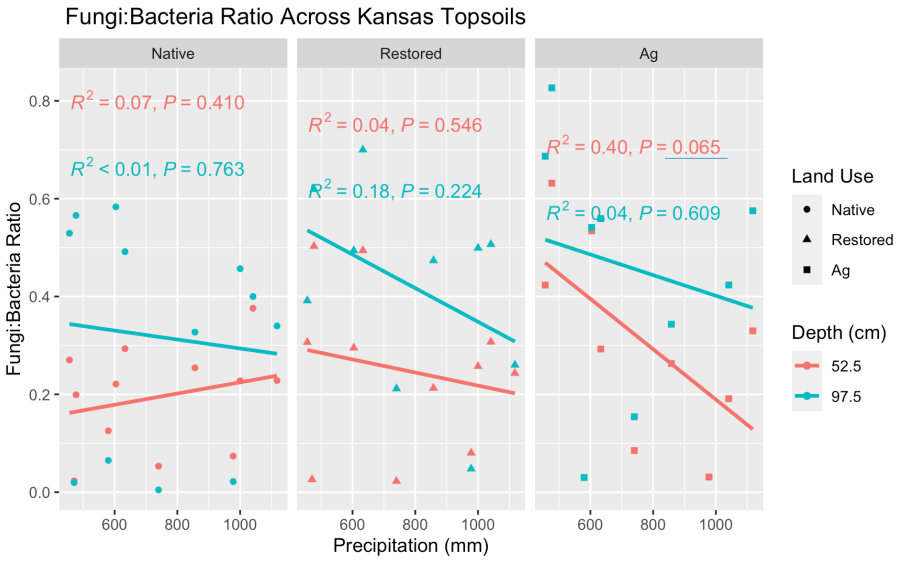

Figure 5.8.A Fungal to Bacteria ratio in the subsoils of the Kansas precipitation gradient by land use and bottom 2 depths of 52.5 cm (30-75 cm), and 97.5 cm(75-120cm). Proportional variance of linearity represented by R2, ANOVA with letters representing significant difference (p<0.1).