Influence of legume species on soil health indicators in an organic corn intercropping system in Tennessee

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ABSTRACT

Organic production has expanded worldwide in recent years. In the United States, organic grain production has been expanding, driven by increasing demand from the organic livestock and food industries. Despite this growth, organic crop production has several limitations, especially for corn. The prohibition of the use of agricultural inputs such as synthetic fertilizers and crop protection products (such as herbicides, insecticides and fungicides) makes the management and profitability of the system challenging. Two of the most persistent constraints are weed competition and nitrogen supply, due to the high demand of corn crops for this specific nutrient. In organic row-crop systems, an alternative method to supply N and mitigate weed emergence is the use of living mulches. Living mulches are cover crops planted between the rows and grown simultaneously within the cash crop, which can provide benefits throughout a part or the entire growing season. This study evaluated four legume species either managed as cover crops or living mulches within an organic corn intercropping system. A field experiment with six treatments and four replications under a randomized complete block design (RCBD) was conducted to assess the effects of legume species on soil health indicators during the 2024 and 2025 growing seasons in Spring Hill and Knoxville, Tennessee. Soil samples were taken in different corn growth and development stages to evaluate the soil health indicators. Results showed that legume-based cover crops and living mulches in organic intercropping systems had limited short-term effects on soil chemical and biological properties, but improved early-season nitrogen availability, soil moisture, permanganate-oxidizable carbon (POXC), and short-term soil physical conditions, with responses strongly influenced by site-specific and

interannual variabilities. While legume-based cover crops and living mulches improved early-season nitrogen availability and some soil health indicators, their long-term impacts remain uncertain. Extended-duration studies are needed to determine their consistency in enhancing soil properties and quality, while maintaining crop productivity under different conditions.

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Chapter One: Introduction

Over the past decade, organic production has expanded globally, reaching approximately 72.3 million hectares and representing about 1.5% of the total agricultural land worldwide (Knorr, 2024). In the United States, organic grain production is growing, driven by a rising demand from the organic livestock and food industry. Focusing on the corn (*Zea mays*), a market largely dominated by conventional production, growing interest from organic consumers is creating opportunities for the development of a new market niche.

According to the Certified Organic Survey, there are more than 17,000 certified organic farms in the United States, covering a total area of approximately 2 million hectares, and accounting for an estimated $11 billion in market share (USDA, 2021). In Tennessee, these numbers are summarized in 46 certified farms, totaling 2.8 thousand hectares, and a total market value of $16.3 million (USDA, 2021). Organic corn in Tennessee is mainly directed by the production of sweet corn and grains for animal feed. Despite this upward trend, organic corn acreage still represents a relatively small proportion of total harvested corn area in the United States, accounting for less than 1% in 2019 (USDA, 2021).

Despite being an emerging part of the market, organic crop production has several limitations, especially for corn. The prohibition of the use of agricultural inputs such as chemical fertilizers and crop protection packages such as fungicides, insecticides, and herbicides makes the management and profitability of the system challenging.

This lack of resources to manage corn crops forces growers to adopt physical, biological, and cultural practices as the main tools to control weeds, insects and diseases. Due to the need for intensive mechanical management, organic corn production costs tend to be higher compared to conventional production. McBride et al. (2015) estimate that organic corn costs are between $83–98 higher per acre than conventional production costs.

Organic corn typically trades at a higher price, known as a premium, compared to its conventional counterpart, to encourage and compensate growers for the additional operating costs. According to McConnell et al. (2021), in 2021, the organic corn price was around $2 to $4 per bushel higher than the conventional corn price. However, in order to sell corn as organic, producers must go through the certification process and obtain the USDA organic seal. This organic certification process can take up to 3 years, since it is a requirement that the cropland must be managed organically for at least 36 months, and in order to maintain the organic certification, annual fees must be paid and renewed.

Despite the financial appeal, the profit from organic corn production is often diluted by higher production costs and lower grain yields. The main limitations are weed competition and nitrogen management, due to the high nutritional demand of corn crops for this specific nutrient. In organic farming, manure application is a common practice that aims to meet the N demand of the crops, but it can also lead to nutritional imbalance in the soil due to the high levels of phosphorus and other micronutrients present in the manure.

Organic production systems aim to promote and optimize available natural resources, contributing to the maintenance of agricultural biodiversity, biological

cycles, and soil biological activity, turning the environment more diverse and resilient, minimizing the use of inputs from outside the farm. The main components of an organic production system are crop rotation, crop management (genetic material selection, planting date, population, and row space), soil management (no-till and cover crops), and crop protection, based mainly on the use of biological control.

Cover crops are plants whose primary purpose is to provide physical protection to the soil, mitigating erosion problems, but they also contribute several benefits to chemical and biological soil aspects, such as nutrient cycling, maintaining soil moisture, and increasing soil organic matter. In recent decades, the use of cover crops has been one of the main strategies to stimulate diversity in agricultural systems, improve soil biology, increase carbon sequestration, promote soil health, and consequently benefit cash crop productivity. The symbiotic relation that legume cover crops have with fixing nitrogen bacteria (FNB) has shown great potential to provide N to agricultural organic systems (Baldwin & Creamer, 2006), being capable of fixing atmospheric N, and its N contribution can vary widely, from 50 to 200 kg N ha^{-1} (Fageria et al., 2005). The amount of N cover crops provide is dependent on the amount of biomass produced. However, this may be a limiting factor due to the relatively early termination timing required to plant corn in Tennessee.

The use of cover crops for weed control can be effective depending on plant architecture, phenological cycle, residue quantity and quality, and termination time. This cultural control of weeds by cover crops occurs through the release of allelopathic compounds into the soil or by blocking light, suppressing weed germination (Nichols et al., 2020). The effect of these benefits is correlated with the cover crop biomass accumulation (the greater the biomass, the greater the weed suppression), but the

corn planting period may precede the termination of the cover crop. Legume cover crops also decompose quickly after termination, due to the lower C/N ratio of the residue, which may shorten the period of effective weed suppression. In this scenario, the use of legumes as cover crops is not as efficient for the organic corn production system. Morse et al. (2006) highlighted the importance of producing a thick, dense, and uniformly distributed residue after the cover crop termination to obtain efficient weed suppression and reduced competition with the cash crop.

In sustainable and organic row cropping systems, an alternative method to chemical and mechanical weed control is the use of living mulches. Living mulches are cover crops planted between the rows and grown simultaneously within the cash crop, being able to provide benefits throughout a part or the whole growing season (Mohammadi, 2012). Living mulches contribute for many soil health mechanisms, by increasing nutrient cycling and availability; preventing land degradation by decreasing soil erosion; increasing soil organic matter (SOM), soil organic carbon (SOC) and microbial biomass; reducing nutrient losses, and regulating soil moisture. Compared to a pre-planting cover crop termination, a living mulch allows for greater biomass accumulation for a longer period, turning the N fixation and weed suppression more efficient during the cash crop growing season. In a meta-analysis of legume living mulch, improved weed control was observed in 82% of experimental units compared to non-weeded controls and 66% of experimental units compared to weeded controls (Verret et al., 2017). For these reasons, the use of living mulch species may become an important practice for organic row crop systems (Bond & Grundy, 2001; Leary & DeFrank, 2000).

Although it has several benefits, the implementation of living mulches requires careful consideration. The main problem with living mulch systems is that the living mulch competes with the cash crop for water and nutrients, which can compromise cash crop growth and yield. These yield losses are dependent on the compatibility of the living mulch/cash crop species combination, making the living mulch species selection a very important decision to take.

Chapter Two: Literature Review

2.1. Soil Health

Soil can be defined as a medium composed of mineral fractions (clay, silt, and sand), organic matter, water, and air, which together sustain terrestrial life on the Earth (Al-Kaisi et al., 2017; Lal & Shukla, 2004). Jenny (1941) describes that the characteristics of a soil depend on soil formation factors such as parent material, climate, organisms (fauna, flora, and microorganisms), topography, and time.

The relevance and interest in the concept of soil health have been growing significantly over the years. Since the 1970s, with the beginning of warnings about the finiteness of natural resources, national and international governmental agencies have been promoting and encouraging farmers to produce food by adopting sustainable practices and inputs (Doran & Parkin, 1994; Karlen & Stott, 1994; Lal, 2016; Larson & Pierce, 1991). In 2015, the United Nations launched the 2030 Agenda for Sustainable Development, which has 17 goals (Sustainable Development Goals), which aim to promote peace, prosperity, health, education, equality, and economic growth for the U.N. Member States. Within these 17 goals, it is possible to relate them with promotion of soil health, such as poverty reduction, hunger eradication, human health and well-being, access to clean water, economic development, sustainable cities and communities, and combating climate change. Thus, to preserve the world and agriculture for future generations, it is necessary to develop sustainable production systems that protect and enhance soil quality and health.

Often used as synonyms, the concepts of soil health and soil quality differ in their approach, focus, and emphasis. While soil quality is defined as the fitness of the

soil for a specific function, the concept of soil health is broader, encompassing a greater view of the ecosystem, considering the soil as a finite and living resource, emphasizing mainly the biological and ecological relations, whose functions are mediated by a diversity of organisms that require management and conservation (Lal, 2016; Maikhuri & Rao, 2012). According to Doran and Zeiss (2000), soil health can be defined as "the capacity of soil to function as a vital living system, within ecosystem and land-use boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and promote plant and animal health". Van Bruggen and Semenov (2000) credit the resistance and resilience capacity of a soil to disturbances (internal or external) to the term soil health.

It is estimated that anthropogenic action has already caused degradation to approximately 40% of the agricultural areas around the world, resulting in soil erosion, air pollution, salinization, and desertification (Oldeman, 1994). Field mechanization in particular, combined with the monoculture row cropping production, contributes significantly to the physical degradation of the soil, leading to soil organic matter losses and consequently, releasing CO_2 into the atmosphere (Houghton et al., 1983). Soil health assessment using soil quality indicators is a tool that can be used by farmers, professionals, government, and research agencies capable of identifying problems in agricultural areas, estimating yield increases or reductions, and monitoring changes in soil quality, allowing a greater perception of environmental sustainability and land use (Granatstein & Bezdicek, 1992).

These soil quality indicators used to assess soil health are based on their utility to understand the ecosystem processes and the integration of the physical, chemical, and biological soil properties (Doran & Parkin, 1994). Doran and Zeiss (2000) and Parisi

et al. (2005) lists 5 criteria for selecting a good soil quality indicator: (I) sensitivity to variations in management (the indicator must be sensitive to capture medium to long-term changes in soil quality, but cannot be influenced by short-term weather changes); (II) well correlated with essential soil functions; (III) useful for elucidating ecosystem processes (the indicator should help to identify why the soil is or is not performing its essential functions, and the link between the causes and effects with the type of land management); (IV) comprehensible and useful to land managers (the indicator must be easy to understand and help in decision making); (V) easy and inexpensive to measure (the indicator must be accessible and economic in terms of both time and money).

The soil essential functions mentioned before are: (I) providing physical support, serving as a medium for plant roots growth of terrestrial species; (II) regulation of hydrological flow and storage in the environment, (III) sustaining plant and animal life, (IV) filtering and buffering potential, by elimination of the harmful effects of contaminants by means of physical, chemical and biological processes, (V) storing and cycling nutrients, playing a key role in the biogeochemical cycles from essential elements such as carbon, nitrogen, phosphorus, sulfur and many others, as result of the decomposition of animals, plants or industrial residues (Lal, 2016; Maikhuri & Rao, 2012). These soil functions, separately and through interaction, create conditions that are conducive to life.

Soil health is the basis for sustainable agricultural development as influenced by farm management practices. Healthy soils have improved infiltration and water use efficiency, resistance to compaction and erosion, and favors nutrient cycling and natural biological processes. Well-structured soils have stable aggregates that are not easily dispersed by water, and pores (macro and micro) that promote aeration and

water infiltration, making it easier for root penetration and root system development (Ngosong et al., 2019). Ideal healthy soils support biological life and promote the diversity (fungi, bacteria, nematodes, earthworms and insects) with considerable benefits for soil physical, chemical, and biological properties, promoting natural regeneration of soils and providing an adequate supply of essential elements to improve crop performance (Ngosong et al., 2019).

Mulching is a practice used in sustainable agriculture for soil management, depending on the cash crop and climatic conditions (Kader et al., 2017). Mulching uses organic or inorganic materials to improve soil health by retaining moisture, regulating temperature, suppressing weeds, improving fertility, and preventing erosion, pests, and diseases (Montenegro et al., 2013). According to Hartwig and Ammon (2002), living mulches are plants grown in association with the cash crop that provide various benefits to the ecosystem and to the cash crop. There are four main attributes of effective living mulches in agricultural systems, which include the ability to suppress weeds without affecting the cash crop germination and development, fast soil coverage, reduce pest pressure by favoring natural enemies, and nitrogen addition to soil via biological N-fixation (Kołota & Adamczewska-Sowińska, 2013). Living mulches are usually fast-growing nitrogen-fixing legumes that grow in the inter-row spacing. Such plants form symbiosis with the cash crop and contribute significant amounts of nitrogen through biological fixation, and also influence the carbon, nitrogen, and phosphorus pools of the microbial biomass (Duda et al., 2003). Overall, soil organic matter is considered the most determinant of soil health, which can be enhanced by the use of living mulch species, leading to better soil physical, chemical, and biological properties (Albiach et al., 2000; Ali et al., 2022).

Soil health is more pertinent to global issues now than ever before. Preserving and enhancing soil health is essential to human health, ecosystem functions, and nature conservation, influencing deeply on the atmosphere, biosphere, and the hydrosphere. Its management is essential to advancing sustainable farming practices, guaranteeing food security and production, critical to mitigate modern climatic change challenges, promote soil biodiversity, and reduce soil/water pollution and contamination (Lal, 2016). Despite the benefits and ecological biases originating from sustainable practices, the adoption of these measures is only valid for farmers with the appropriate economic return, such as increasing in yield, profitability, and stability, leading to the viability of the adoption of these sustainable practices (Doran & Zeiss, 2000).

2.2. Chemical Indicators

The determination of chemical soil indicators and the continuous monitoring of their changes is of great importance to farmers, given the direct relationship between the availability of essential nutrients, plant development, and crop yield. The chemical properties of a soil are strongly influenced by its physical and biological properties. Measuring soil pH, cation exchange capacity (CEC), nitrogen, and other macro and micronutrient levels are common and accessible assessments made by farmers and researchers that provide important data for assessing soil chemical quality.

Cation exchange capacity is a measure of the total negative charges within the soil that adsorb cations (Saha, 2022). The CEC depends on several factors such as pH, mineralogy (clay type and quantity), characteristics of the parent material, and the organic matter content (Al-Kaisi et al., 2017; Haynes & Swift, 1990). Soil clay minerals and organic matter tend to be negatively charged, thus attracting positively charged

ions (cations), such as calcium (Ca^{2+}), magnesium (Mg^{2+}) and potassium (K^{+}), ammonium (NH_4^{+}), sodium (Na^{+}), hydrogen (H^{+}), aluminum (Al^{3+}), iron (Fe^{2+} or Fe^{3+}), manganese (Mn^{2+}), copper (Cu^{2+}) and zinc (Zn^{2+}), being able to held them on their surfaces by electrostatic forces (Saha, 2022). This soil property is important not only for plant's nutrient supply, but also for the controlling and filtering of some organic and inorganic chemical compounds that leach into groundwater reservoirs (Al-Kaisi et al., 2017). In most soil reports, CEC is expressed by the term milliequivalents (meq), mole of positive charge absorbed per unit mass (per 100 grams of soil (meq/100g or as cmol/kg when using International Scientific Units)) (Al-Kaisi et al., 2017; Saha, 2022).

Soils with higher CEC tend to retain more nutrients when compared to soils with lower CEC. Understanding this concept, it is possible to expand it to the base saturation (BS) term, which is the percentage of CEC that is occupied by basic cations (Ca^{2+}, Mg^{2+}, and K^{+}), nutrients that are essential for plant growth and development. Acid cations (H^{+} and Al^{3+}) are related to soil acidity and have a toxic effect on plant development. Soils with a higher percentage of BS are generally more fertile, due to the greater amount of essential nutrients and a higher pH (Saha, 2022).

The soil acidity or alkalinity can be determined by measuring the pH, that is, the negative log of the concentration of the hydrogen ions in the soil solution, on a scale from 0 to 14. By rule, pH values between 6.0 and 7.5 are considered optimum for plant growth (O'Kennedy, 2022). This soil chemistry quality indicator is important due to the effects that it exercises on nutrient availability, mineral solubility, and microbial activity, where most of them present greater efficiency and functionality in a pH-neutral environment (Arias et al., 2005). Variation in pH can also cause disturbances in the CEC and consequently in the BS of the soil, where acidic soils will present a higher

concentration of H^+ and Al^{3+} in the CEC, limiting the adsorption of basic cations on the surface of soil colloids. This pH effect is more severe in weathered soils, where 1:1 minerals, Fe and Al oxides, predominate, and also have low organic matter levels (Al-Kaisi et al., 2017).

Soil pH is considered one of the most important soil variables, because it directly influences the chemical (forms, solubility and availability of nutrients in the soil), physical, and biological properties of the soil, highly affecting plant growth and biomass production (Neina, 2019). Each nutrient has its own optimum pH range in which its availability is enhanced, commonly between 6.0 and 7.0. At this range, the majority of the essential nutrients for crop development are in their most soluble form and readily available for root absorption, and the presence of toxic elements is minimized (Khaled & Sayed, 2023). Variations in pH values can lead to plant nutritional problems, whether due to deficiency or toxicity, directly affecting their development and yield. In acidic soils (pH < 5.5), the solubility of toxic metals such as aluminum (Al) and manganese (Mn) increases, (inhibiting root growth and function) and essential nutrients such as phosphorus (P), calcium (Ca), and magnesium (Mg) become less available due to precipitation or adsorption onto soil particles (Khaled & Sayed, 2023). On the other hand, in alkaline soils (pH > 7.5), the availability of micronutrients such as iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu) decreases due to the formation of insoluble hydroxides and carbonates, also compromising crop productivity (O'Kennedy, 2022).

Among several benefits, intercropping with legume living mulches has been suggested as a sustainable method that can reduce the dependence on N from synthetic fertilizers. Living mulches can benefit soil chemistry by increasing soil

ammonium (NH_4^+) levels and reducing nitrate (NO_3^-) leaching (Parkin et al., 2006; Xie et al., 2017; Żelazny & Licznar-Małańczuk, 2018). However, in some systems, living mulches can compete with crops for water and nutrients, leading to reductions in growth and yield of the target crop (Granatstein & Sánchez, 2009; Sosna & Fudali, 2023; Żelazny & Licznar-Małańczuk, 2018). Clovers are a group of legumes that are often used as cover crops and living mulch in many different systems. Crimson clover is a winter season legume species commonly used throughout the southeastern United States. Crimson clover has been shown to contribute 78 to 168 kg of N per hectare when grown in pecan orchards in Georgia (Wells, 2017). McAmis et al. (2024) studying tea production in Florida, simulating different systems using mulching (weed barrier cloth, perennial peanut and crimson clover), noticed that the soil under peanut had significantly higher concentrations of NO_3^-, and Ca^{2+} than soil under crimson clover and barrier cloth. In addition, the treatments using legume living mulches presented significantly higher OM and pH than the barrier cloth treatment. No differences in K^+ or Mg^{2+} levels were observed among the cover crops treatments.

The nutrient balance in the soil system is dynamic and constantly changing as a result of plant growth (where available nutrients are absorbed by the root system) and land management (tillage system, erosion, and run-off control) (Al-Kaisi et al., 2017). Soil nutrients income is either from parent materials, addition of organic or chemical sources, or cycled by the decomposition of organic matter, supplying nutrients that are essential to plant and microbial growth, which influence soil health and quality, reflecting in biomass production and crop yield (Al-Kaisi et al., 2017). The plant radicular system and microfauna associated with the roots have a role in mineral weathering, where they can contribute to the solubilization and insolubilization

processes and promote the availability of nutrients such as K, P, Fe, Mg, or adsorbed NH_4^+ in the rhizosphere (Marschner et al., 2011). This interaction and release of nutrients is ruled by numerous chemical, physical, and biological processes of the soil (Swift et al., 1979; Zech et al., 1997).

Regular soil analysis is extremely important for monitoring pH and macro and micronutrient levels. With this information, the farmer is able to detect nutritional problems and assist him in making decisions to correct or maintain the fertility protocol. Sustainable agricultural practices, such as crop rotation and no-tillage, aiming to add organic matter to the soil, can be used as strategies to manage soil pH, nutrient availability, and promote microbial activity. The incorporation of organic matter in the soil system has a buffering effect on pH changes, improving soil structure, nutrient cycling, and plant growth (Khaled & Sayed, 2023).

2.3. Physical Indicators

Soils are composed of 4 components: minerals (derived from the parent material), organic matter (animal or plant origin), water, and air, being present in the soil in 3 different phases, solid, liquid, and gas (Al-Kaisi et al., 2017; Lal & Shukla, 2004). Under ideal crop growing conditions, it is expected that 50% of the soil volume will be composed of solids (minerals and SOM), and both the liquid and gaseous phases will make up 25% each (Lal & Shukla, 2004). The soil volume occupied by water or air can increase or decrease, depending on soil moisture and precipitation (Lal & Shukla, 2004).

Correct management of soil physics is essential for sustainable agricultural production, affecting plant growth directly and indirectly. Soil physical properties such as bulk density, particle size, soil structure, porosity, among others, impact soil

processes such as compaction, erosion, water movement and storage, aeration, and heat transfer, which consequently reflect on biomass productivity (Lal & Shukla, 2004). The understanding and application of soil physics principles assist farmers in the control of several environmental problems, including soil erosion and runoff, soil compaction, soil salinity, water drainage, storage, and irrigation (Lal & Shukla, 2004).

Soil physical properties can be defined by the two main characteristics: soil texture and soil structure. Soil texture refers to the relative proportions of the sand, silt, and clay particles in the soil, determining the textural class of a soil (Al-Kaisi et al., 2017; Moreno-Maroto & Alonso-Azcárate, 2022). The term soil structure relates to the arrangement of the primary soil particles, in addition to organic matter and other organic compounds, creating soil aggregates that can vary by size, shape, type, and grade. The formation of soil aggregates determines the soil structure and geometric characteristics of pore spaces in which water and air movements occur (Tisdall & Oades, 1982). The four main soil structure types are platy, blocky, columnar (or prismatic), and granular. Platy structures are characterized by thin, horizontal aggregates and may form under compaction, particularly in clayey soils subjected to heavy machinery. Blocky structures consist of irregular, roughly cube-shaped aggregates, typically found in B-horizons, where they enhance drainage, aeration, and root penetration. Columnar or prismatic structures are defined by vertically oriented, prism-like aggregates commonly occurring in subsurface horizons. Granular structures are composed of loosely arranged, spheroidal aggregates, usually present in A horizons, and are therefore the most susceptible to management practices (Al-Kaisi et al., 2017). Land management, cropping systems and duration of vegetative cover have great influence on soil aggregate formation and stability (Horne & Sojka, 2002; Lal,

1993). Plant root systems and fungal hyphae associations are more likely to relate to the formation of macroaggregates (> 0.25mm), while organic compounds are responsible for the formation of microaggregates (<0.25 mm) (Kloepper & Metting, 1993; Tisdall & Oades, 1982).

The ground vegetation is significantly important in the role of soil aggregate formation and stability, where perennial species, in particular grasses, produce more stable and well-formed soil aggregates (Dıaz-Zorita et al., 2002) as compared to annual species cropping systems (Bronick & Lal, 2005; Cambardella, 2006). The difference between perennials and annual crops on soil aggregates lies in the root system, where the root intensity and morphology can significantly impact soil aggregation (Dıaz-Zorita et al., 2002). Horne and Sojka (2002) describe an increase in soil aggregate strength in no-tillage soil management allied with a diverse cropping system with perennial crops.

Soil physical properties are variable over time and space, and are highly affected by human interference. This happens due to the dynamics and sensitivity of the soil's physical properties to changes in soil management and organic matter. Small-scale variability is changes that occur in meters or centimeters, are difficult to measure, and visualize. These small-scale changes are often noticeable in biological activity (macro and microorganisms), due to their greater sensitivity to soil changes. Fertility patches are also common examples of small-scale variability, being affected by some input application problem or the decomposition of some residue. Medium-scale variability is notable changes between tens and hundreds of meters. The topography of a location can be affected depending on the land use and agricultural practices adopted, which can lead to soil erosion. Variations in soil fertility are also examples of medium-scale variability. Large-scale variations are affected by soil formation factors

(climate, parent material, weather, topography, and vegetation), causing regional differences and biome diversity.

Lal and Shukla (2004) list 12 physical properties of the soil as the main ones for determining its quality in relation to specific soil functions. Assessing soil density (particle density, bulk density, relative density, and dry specific volume), soil porosity (total porosity, air-filled porosity, void and air ratio), and soil moisture content (gravimetric soil moisture, volumetric soil moisture, liquid ratio, and degree of saturation) can generate important data and information for determining the physical quality of a soil. In this study, the physical properties evaluated are bulk density, soil moisture, and subsurface hardness.

Bulk density (ρb) is the ratio of the mass of solid (Ms) to the total volume (Vt). Soil bulk density can be defined as dry (ρb) and wet (ρ' b), which includes the mass of water. Wet soil bulk density is an ever-changing entity because of soil evaporation at all times under natural conditions. Therefore, soil bulk density is preferably reported as a dry soil bulk density. Its units are also those of mass/volume as g/cm^3 or Mg/m^3 (Lal & Shukla, 2004). It generally depends on soil texture and is affected by management practices that include tillage, field equipment, travel pattern, and crop rotation (Al-Kaisi et al., 2017).

$$(\rho b) = Ms / Vt$$

$$(\rho' b) = Ms + Mw / Vt$$

Soil moisture is the term used to denote water contained in the soil. Soil moisture can exist in three forms: gravitational, capillary, and hygroscopic (Briggs, 1897). Gravitational moisture is defined as the free water that moves through the soil due to the force of gravity, found in the macro-pores, and its movement is quite rapid

in well-drained soil, draining out in 2–3 days after the rainfall. A sandy soil has a bigger proportion of macro-pores compared to a clay soil, resulting in better drainage capacity. Capillary moisture is the moisture present in the micro-pores of the soil and is held within the soil due to cohesion and adhesion against the force of gravity. This moisture is the available water source for most of the physical, chemical, and biological interactions between the soil and the environment. Hygroscopic moisture forms a very thin film on the surface of soil particles, making it very difficult to remove due to the extremely high adhesion forces. A clay soil has a bigger proportion of micro-pores and higher particle surface area compared to a sandy soil, reflecting in higher water storage capacity (Lekshmi et al., 2014).

The amount of soil moisture available to be held in the soil depends on the soil density, soil texture and structure, particle size and type, meteorological factors (precipitation and temperature), soil organic matter, and soil-plant-atmosphere interactions (Rasheed et al., 2022). The soil moisture has great variability in relation to soil depth, with the surface layer (0-10 cm) being considered the most representative portion of the soil profile in agricultural water management studies, due to the greater concentration of the root system (Rasheed et al., 2022).

Growing corn in an intercropping system with living mulch can provide ecological benefits, but competition for water between the main crop and the living mulch crop often reduces corn yields, preventing the widespread use of such a cropping system (Duiker & Hartwig, 2004). Yield losses have been mostly attributed to poor corn emergence and hydric stress as the living mulch competes with the corn crop for water (Box Jr et al., 1980; De Haan et al., 1997). Researchers at the University of Wisconsin-Madison have developed a corn intercropping system using kura clover

as living mulch, where corn is planted on suppressed or killed bands of the kura clover, enhancing corn emergence and development, but still allowing regrowth and recovery of the living mulch (Zemenchik et al., 2000). Swenson et al. (2004) and Borowy (2012) observed higher soil moisture under living mulch cover due to lower evaporation rate in comparison to conventional cultivation in tomato production. The opposite impact was reported by Brainard et al. (2012) those who indicated a strong competition and significant reduction of soil available water in an asparagus and rye as living mulch intercropping system. Zemenchik et al. (2000) did not find differences in soil moisture among treatments at either the 0- to 22.5-cm or the 22.5- to 45-cm depth in a two-year experiment with corn-kura clover intercropping system. Ziyomo et al. (2013) observed that the use of drought-tolerant corn is an option to combine the environmental and economic benefits of this intercropping system. Drought-tolerant hybrids had statistically equal mean yields of 12.90 Mg ha^{-1} in the killed mulch treatment and 13.69 Mg ha^{-1} in the living mulch treatment. In contrast, mean yields of drought-susceptible hybrids were significantly reduced from 4.53 Mg ha^{-1} in the killed mulch treatment to 3.48 Mg ha^{-1} in the living mulch treatment.

Soil compaction is a problem that has become more frequent for farmers, due to the increase in the size and weight of agricultural equipment over the past few decades, increasing the severity and depth of compacted zones in the soil profile (Nawaz et al., 2023). The soil compaction is caused by a downward pressure that squeezes the soil and increases the soil's bulk density (Wolkowski & Lowery, 2008). The main concern about soil compaction is the negative impact on crop yields and soil degradation. Soil compaction can easily reduce crop yields by 10 percent, and can lead to water and soil quality degradation due to runoff losses and soil structure

destruction (Duiker, 2002). Soil compaction has a negative impact on water drainage and flow, crop yield, and root growth, development, and distribution (Duiker, 2002; Kumar et al., 2012; Unger & Kaspar, 1994).

Soil compaction can be distinguished into two types: surface and subsurface (Duiker, 2002). Surface compaction (0 – 10 cm) is related to higher bulk density and hardness on the topsoil zone, leading to seed emergence, water infiltration, and surface crust problems, which are partly alleviated with normal tillage operations. Subsurface compaction (> 15 cm) occurs especially because of heavy machinery traffic, and leads to increases in bulk density and penetration resistance on deeper layers of the soil profile, leading to root growth restriction, affecting water and nutrient absorption.

The measurement of soil compaction can be performed by using a penetrometer reading, which measures soil resistance and the degree of compaction (Duiker, 2002). Although the limit of zero root growth may not be exactly at 2,000 kPa, the higher penetrometer readings indicate that root growth will be greatly inhibited, being visible on both wet and dry soil conditions, and is independent of soil texture (Duiker, 2002). Although soil compaction problems are more severe and frequent in clay soils and under wet conditions (Kumar et al., 2012).

Reducing soil tillage, increasing SOM, adopting wheel traffic control, and subsoiling operations are some tools that farmers can use to prevent and mitigate soil compaction problems (Kumar et al., 2012). Conservative tillage systems, such as strip-till and no-till should have preference, and operations such as plowing must be conducted only when necessary to correct some compaction problem, and the soil must have the appropriate moisture level. The addition of organic matter helps

prevent surface compaction by enhancing soil structure, increasing water infiltration, improving soil water-holding capacity, and promoting friability. These benefits can be achieved through practices such as manure application, the use of cover crops, and maintaining plant residues on the soil surface (Blanco-Canqui & Lal, 2009). Machine traffic control is another strategy to mitigate compaction problems.

2.4. Biological Indicators

The organisms that inhabit the soil are vital components of the soil system, playing crucial roles in maintaining life and ecosystem functions (Coleman et al., 2017). Their importance has made them a focal point of research and study in the field of soil health. Soil organisms are recognized as one of the most sensitive biological markers available and a reliable tool for assessment of disturbed or contaminated systems (Turco et al., 1994). They can be correlated with beneficial soil and ecosystem functions, including water storage, organic matter decomposition and nutrient cycling, environmental detoxification, and suppression of pathogenic organisms, being highly sensitive to land management practices and climate changes (Doran & Zeiss, 2000). Microbial populations can provide evidence of subtle changes in soil long before they can be accurately measured by changes in organic matter (Powlson, 1987). In the recovery of a disturbed system, microbial activity is one of the first factors to improve soil conditions, thus allowing plant development (Turco et al., 1994).

Part of the biological fraction of the soil is composed of living and widely diverse organisms. In a study on bacterial diversity, Torsvik et al. (1990) using samples from the top 10 cm of the soil, the presence of 1.5×10^{10} bacteria cells per gram of dry soil, indicates the complexity of the soil microbial community. In addition to prokaryotes (bacteria and archaea), eukaryotes (fungi, nematodes, protozoa, algae,

and oligochaetes) and viruses also stand out in the complexity of organisms that inhabit the soil (Brady & Weil, 2009). These different groups of organisms are commonly separated according to their size, giving rise to the division into the soil fauna: macrofauna (organisms larger than 2 mm), mesofauna (organisms between 0.1 and 2 mm), and microfauna (organisms smaller than 0.1 mm) (Giller, 1996).

Soil organisms play a crucial role in degradation of organic compounds and consequent nutrient cycling, soil structure and pedogenesis, nitrogen cycling (fixation, nitrification and denitrification), mutualistic relations (mycorrhizae), support to the trophic chain (predation), bioremediation (detoxification) and biotechnology (antibiotics and Bt proteins) (Pimentel et al., 1997; Smith & Paul, 2017). In addition, microorganisms promote physicochemical changes in the soil, such as the stabilization of soil organic matter, N_2 fixation, and other alterations in soil properties necessary for plant growth (Turco et al., 1994). Due to their critical role in a wide range of functions within the soil ecosystem, these organisms are recognized as key agents in promoting and sustaining soil quality and health (Bartz et al., 2013; Coleman et al., 2017; Lima et al., 2017; Turco et al., 1994).

According to Holloway and Stork (1991), for the selection of good biological indicators, it must be sensitive and accurate to show prompt perturbations, reflect aspects of the functioning of the ecosystem, economically accessible and able to be broadly used, yet show individual specificity to temporal or spatial patterns in the environment. A set of abiotic (oxygen, temperature, water, pH, redox potential, and nutritional sources) and biotic factors (genetics, biological interactions, and human interference) govern the development of soil organisms. The interaction of these factors influences the diversity, distribution, quantity, ecological role, and dynamics of

the soil macro, meso, and micro fauna (Filser et al., 2016). Water directly influences biological activity, being fundamental in the process of nutrient diffusion, biochemical reactions, mobility, thermal regulation, and habitat (Filser et al., 2016; Moreira & Siqueira, 2006). Soil pH affects the composition and activity of microbial communities. Acidic soils tend to have lower microbial activity and diversity, affecting processes such as nitrogen fixation and organic matter decomposition. In contrast, neutral to slightly alkaline soils generally support a more diverse and active microbial community (O'Kennedy, 2022). The ideal pH varies between species of soil microorganisms, which can be classified as acidophilic (acidic pH), neutrophilic (neutral pH), or basophilic (basic pH) (Moreira & Siqueira, 2006). Temperature directly influences (accelerating or attenuating) the occurrence of microbial metabolic processes, and just like pH, each microorganism has an optimal temperature value for its replication and development. Depending on the optimal temperature range for its growth and activity, microorganisms can be classified as psychrophilic (less than 20°C), mesophilic (between 20 and 40°C), thermophilic (more than 40°C), and hyperthermophilic (above 60°C) (Madigan et al., 1997).

The oxygen concentration in soil (macro and micropores) determines the occurrence of aerobic, microaerobic, and anaerobic environments, allowing the proliferation of aerobic (aerobic cellular respiration), microaerophilic (requires low concentrations of oxygen to develop), and anaerobic organisms (using sulfate, nitrate, or organic compounds to obtain energy) (Cardoso & Andreote, 2016). Related to oxygen availability, redox potential determines the environment's tendency to gain or lose electrons. In soils, determining this value serves as a biochemical indicator of the predominant metabolic process. In oxidizing conditions (high redox potential), the high

oxygen availability favors oxidation reactions. In this scenario, chemical compounds tend to lose electrons, and electronegative compounds act as final electron acceptors, favoring the occurrence of aerobic microorganisms and facultative aerobiosis processes, stimulating the decomposition of organic matter and nutrient cycling. On the other hand, in reduced conditions (low redox potential), with low or no oxygen available, biochemical processes are dominated by reduction reactions, in which compounds gain electrons. This scenario is typical of soils saturated with water, such as in flooded and drainage problem areas. These environments favor the activity of anaerobic microorganisms, influencing the availability of nutrients and metals, affecting ecological dynamics and environmental quality.

Soil organic matter (SOM) consists of plant and animal residues at various stages of decomposition, living and dead organisms, and substances synthesized by the organisms and plant roots (Baldwin & Creamer, 2006). The top 20 cm layer of the soil is the one with the greatest biological activity, due to the accumulation of soil organic matter through the deposition of plant and animal residues, in addition to the rhizosphere influence area. Doran (1980) observed that microbial activity drastically differed with depth, with the greatest microbial activity occurring near the no-till surface. The same was mentioned by Konopka and Turco (1991), mentioning that the number of microbial populations usually decreases with increasing depths of soil. Subsurface microbial density, activity, and diversity, appear to be correlated with organic matter content and water presence in the deeper layers of the soil profile (Turco et al., 1994). Depending on the composition and abundance of the residues to be decomposed, organic matter can be classified as labile or easily degradable (mainly carbohydrates, amino acids, and lipid fractions), slowly decomposing (lignin

compounds), relatively recalcitrant (aliphatic molecules such as suberin, waxes, and tannins), and relatively stable, with persistence in soil ranging from months to decades or even centuries, corresponding to the humic fraction (Lal, 2016). Organic matter influences the chemical and physical properties of soil, the availability of nutrients for microbial replication, and regulates carbon cycling and its stabilization. Changes in soil organic matter content deeply affect soil microbial diversity.

Soil carbon content refers to the carbon reserves stored within the soil, which can be categorized into two distinct forms: soil organic carbon (SOC) and soil inorganic carbon (SIC) (Lal, 2016). The SIC is related to soil minerals, especially the primary and secondary carbonates. The SIC consists of elemental C, carbonate-bearing minerals (secondary carbonates, formed through reaction of HCO_3^- and CO_3^{2-} with Ca^{2+} or Mg^{2+}, Na^+, and K^+), and gaseous CO_2 as a product of heterotrophic respiration, having the role of pH regulation and nutrient availability, soil buffer capacity, and sequestration of atmospheric CO_2 (Jansson et al., 2010). The SOC is the main fraction of the soil organic matter, representing about 45 – 60% of the SOM mass. SOC is a heterogeneous mixture of organic materials, including fresh litter, root exudates, animal and plant residues, organic compounds (carbohydrates, sugar, proteins, and amino acids), and the microbial biomass and their by-products. Due to the nature of SOC being a highly reactive and dynamic component, it's considered the basis of numerous pedogenic processes and its permanence depends on the degree of protection (physical, chemical, biological, and ecological) within the soil matrix (Lal, 2016). SOC is more susceptible to form stable microaggregates and organo-mineral complexes due to the nature of its high surface area and charge density, which reacts with clay and minerals, creating a protection against microbial processes and being transferred to deeper

layers of the subsoil, away from the zone of natural and anthropogenic perturbations (Lal, 2016).

Different SOC fractions exist and are often classified based on their turnover rates and the means of laboratory determination. Three common analyses to describe the active fractions of SOC are permanganate-oxidizable C (POXC), microbial biomass C (MBC), and water-extractable organic C (WEOC) (Decker, 2021). The total SOC is typically determined by combustion analysis. Permanganate oxidizable C is considered a useful measure of biologically-active SOC, which represents active and decomposed SOC with a turnover rate of 2-5 years (Weil et al., 2003). Microbial biomass carbon (MBC) represents organic C compounds in microbial biomass, reflecting the effects of soil management on soil microbial populations, and water-extractable organic carbon (WEOC) represents water-soluble root exudates and decomposition by-products (typically amino acids and carbohydrates) with a maximum turnover rate of 6 months (Dungait et al., 2012).

Maintenance of soil organic carbon (SOC) is essential for: (1) soil structure and aggregation, which influence aeration and water flow; (2) water storage and use efficiency, thereby increasing tolerance to water stress; (3) nutrient retention and cycling; (4) rhizospheric processes that regulate elemental transformations and contribute to the development of disease-suppressive soils; and (5) gaseous emissions (e.g., CO_2, CH_4, N_2O), which affect biogeochemical cycles and play a role in climate regulation (Lal, 2016). Soil biological activity is directly related to soil management. Conservation agricultural practices such as no-tillage (which encourage crop rotation, minimize soil disturbance, and residue maintenance on the soil surface) are tools that can be used by farmers to create a positive C balance to increase and enhance the SOC

content (Cardoso & Andreote, 2016). No-till land management, integrated and diverse cropping systems, and the use of organic inputs (manure, litter, green manure, mulching) are options that improve soil ecosystem functions. In general, soil health is affected more by the dynamics of SOC than SIC (Lal, 2016).

Soil respiration is the term used to estimate the biological conversion of organic matter, by oxidation, into water and gaseous carbon dioxide (CO_2), due to microbial activity (both aerobic and anaerobic organisms) (Alef, 1995), serving as an indicator of global carbon balance and terrestrial carbon exchange with the atmosphere (Vallotton et al., 2023). Soil respiration is a good indicator of the soil food web stability and is essential in the C biogeochemical cycle (Vallotton et al., 2023). Soil respiration can be related to SOM and fine SOC content, microbial biomass and activity, soil management, and multiple environmental variables, such as temperature, soil moisture, and texture (Arias et al., 2005; Singh et al., 2021; Vallotton et al., 2023). Besides being able to represent microbial activity, the soil microbial respiration is one of the major terrestrial CO_2 emissions pools to the atmosphere, with it being estimated that around 60 to 80 Pg of C are emitted globally annually (Jian et al., 2018; Warner et al., 2019).

Several factors exert great influence on soil respiration. As soil temperature rises, the soil respiration also increases (up to 40°C), and the opposite also occurs, with soil respiration reducing as temperature decreases (Vallotton et al., 2023). As any biochemical process requiring water, soil moisture is also a limiting factor. When the soil pore space is 50% water-filled, it's considered ideal for maximal respiration rate (Franzluebbers, 1999; Vallotton et al., 2023). Singh et al. (2021) observed lower respiration rates in three different soil types (ultisol, alfisol, and vertisol) when they

were in the lowest moisture content (air-dried conditions). In higher soil moisture scenarios, the water excess can lead to anaerobic conditions and, due to the lack of oxygen, reduce the soil aerobic respiration (Vallotton et al., 2023). Singh et al. (2021) noticed that each soil type has a unique optimal moisture level that will enhance the soil respiration rate, being strongly controlled by the soil texture and clay content.

Carbon and nutrient availability are also variables that influence soil respiration. As the majority of the soil microbes are heterotrophic, they depend on the presence of carbon-based substrate as a source of energy for their metabolic reactions (Cardoso & Andreote, 2016). The more readily available the carbon source, the faster soil microbes will utilize it, thereby accelerating their metabolism and increasing soil respiration and CO_2 emissions (Vallotton et al., 2023). Sugars are a good example of an efficient source of carbon that can be immediately used as an energy source, leading to an immediate increase in respiration rates. Other complex carbon compounds, such as cellulose, chitin, and lignin, that are less available to microorganisms need to be degraded by more specific organisms, such as fungi (which possess specific enzymes that can degrade this complex organic compound), leading to longer degradation and slower utilization, and consequently reducing respiration rate (Vallotton et al., 2023). Rather than C, other nutrients, especially N and P, are essential for the production and functioning of microbial cells. N is an essential component for protein synthesis and enzymatic activity, whereas P is highly related to energy production (i.e., ATP) (Vallotton et al., 2023).

Soil tillage also has great influence on soil respiration, due to disrupting soil aggregates and exposing the soil organic matter to microbial activity, leading to soil C losses by CO_2 emissions (Cherubin et al., 2021). N et al. (2006) observed higher CO_2

emissions in sugarcane field over conventional tillage, when compared to reduced-tillage and no-tillage treatments, suggesting losses of around 30% of soil carbon input in crop residues after plowing operations in tropical soils, when compared to the no-till plot emissions, in a period of one month after tillage.

In the present study, white clover (*Trifolium repens*), red clover (*Trifolium pratense*) and crimson clover (*Trifolium incarnatum*) were used strictly as living mulches. Hairy vetch (*Vicia villosa*) was used as a living mulch and cover crop. All living mulch and cover crop species were planted and managed to be part of an organic corn intercropping system, at two different locations in the state of Tennessee. A treatment without living mulch or cover crop was used as the control. The objective of this study was to determine the effects of these living mulch and cover crop species on the soil chemical, physical and biological aspects and their influences on soil health.

Chapter Three: Materials and Methods

3.1. Field Experiment

This study was conducted at two different locations, one at the University of Tennessee's Middle Tennessee AgResearch and Education Center (MTREC) in Spring Hill, and the other at the University of Tennessee's East Tennessee AgResearch and Education Center – Organic Crops Unit (ETREC) in Knoxville. The soil type at each location is defined as Alfisol Udalf Typic Paleudalf (MTREC) and Ultisol Udult Paleudult (ETREC). The experimental design used was a randomized complete block design with four replicates and six treatments. The treatments consisted of the following: 1) the control (no living mulch or cover crop, soil tilled prior to corn planting); 2) hairy vetch as a cover crop (HVCC), soil tilled prior to corn planting; 3) hairy vetch as living mulch (HVLM); 4) red clover as living mulch (RCLM); 5) crimson clover as living mulch (CCLM); and 6) white clover as living mulch (WCLM). All legume treatments were planted by September 13rd to October 10th of 2023, and October 17th of 2024 using a hege drill. Legumes seeding rates were: hairy vetch 33.6 kg ha^{-1}, white clover 8.96 kg ha^{-1}**,** red clover 13.44 kg ha^{-1}, and crimson clover 22.4 kg ha^{-1}. Plots were 6 meters wide by 9 meters long at each location. Every year, prior to the corn planting date, poultry litter was applied at a rate of 89.7 kg N ha^{-1}. On April 18th and 25th of 2024, and April 23rd and 30th of 2025, corn was planted at a row spacing of 0.76 meter, with a target population of 69,000 plants per hectare. Plots were manually harvested in both years at both locations. Weather data were collected from local weather stations.

3.2. Soil Sampling

The initial soil samples were taken on September 14th, 2023, a composite soil

sample of 12 cores (2.5-cm diameter, 2 from each plot), collected in 0-15 cm from each replicate before cover crop and living mulch planting, and analyzed for basic soil properties: pH, organic matter and nutrients.

During the summer of 2024, 0-15 cm soil samples were collected from each plot when the corn was at V6 (May 23rd in Knoxville and May 30th in Spring Hill), V10 (June 13th in Knoxville and June 20th in Spring Hill), and R1 (June 27th in Knoxville and July 1st in Spring Hill), respectively. During the summer of 2025, 0-15 cm soil samples were collected from each plot when the corn was at V6 (May 29th in Knoxville and May 28th in Spring Hill), V10 (June 20th in Knoxville and June 18th in Spring Hill), and R1 (July 9th in Knoxville and July 8th in Spring Hill), respectively. Soil moisture readings were determined with a moisture meter (Dynamax TH150 Portable Soil Moisture Probe, Houston, Texas), also at the same corn growth stages and dates from the respective years.

During the fall of 2024 (October 22nd in Knoxville and October 24th in Spring Hill), after the corn harvest, a composite soil core sample by depths of 0-15, 15-30 and 30-60 cm was collected from each plot, using a hydraulic soil probe, and analyzed for plant-available nitrogen (NO_3^--N and NH_4^+-N) in the soil profile. The identical soil sampling procedure was repeated at the end of the experiment in the fall of 2025 (September 30th in Knoxville and October 1st in Spring Hill).

3.3. Soil Chemical Analysis

3.3.1. pH

Soil pH was determined according to the methodology described by McLean (1982). This pH measurement method consists of weighing 10 g of air-dried soil, sieved through a 2-mm mesh, transferring it to a container, in which 10 mL of water is added

(1:1 soil/water ratio). The mixture is then stirred for 5 minutes and left to rest for 30 minutes. After resting, pH readings are taken with the electrode properly calibrated with pH 4.0 and 7.0 buffer solutions. The Brookside Laboratories at New Bremen in Ohio performed the pH analysis.

3.3.2. Macro and Micronutrients

The soil macro and micronutrient contents were determined with the Mehlich-3 method, which is one of the most used methods for extracting nutrients from the soil due to its practicality and ability to extract a wide range of nutrients with a single extracting solution. The method developed by Mehlich (1984) makes use of the Mehlich-3 solution, which consists of acetic acid (0.2 mol L^{-1} CH_3COOH), ammonium nitrate (0.25 mol L^{-1} NH_4NO_3), ammonium fluoride (0.015 mol L^{-1} NH_4F), nitric acid (0.013 mol L^{-1} HNO_3), and ethylenediaminetetra-acetic acid (0.001 mol L^{-1} EDTA). The process begins with weighing 2.5 g of air-dried soil, sieved through a 2-mm mesh, and then adding 25 mL of Mehlich-3 solution for every 2.5 g of soil. The mixture is then stirred for 5 minutes. The suspension is filtered or centrifuged to separate the soil residue from the liquid extract, which contains the available nutrients. The nutrients in the extract are then determined in the following ways: P and S by UV-Vis spectrophotometry; K, Na, Ca, and Mg by flame photometry or ICP-OES; and the micronutrients (Cu, Zn, Mn, Fe, and B) by ICP-OES or AAS (atomic absorption spectrometry). The Brookside Laboratories at New Bremen in Ohio performed the macro and micronutrient analyses.

3.3.3. Ammonium-N and Nitrate-N

The soil available nitrogen (NO_3^--N and NH_4^+-N) were determined based on the methodology described by Keeney and Nelson (1982). Briefly, this measurement

method consists of weighing 10 g of air-dried soil, sieved through a 2-mm mesh, transferring it to a container, in which 50 mL of potassium chloride (KCl, 2 mol L^{-1}) is added (1:5 soil/solution ratio). The mixture is then stirred for 30 minutes and filtered. Measurements of the level of ammonium-N (NH_4^+-N) and nitrate-N (NO_3^--N) are taken with the use of Skalar Continuous Flow Analyzer (Mulvaney, 1996). The Brookside Laboratories at New Bremen in Ohio performed the available nitrogen analyses.

3.4. Soil Physical Analysis

3.4.1. Bulk Density

During both years at V6 corn growth stage, and by the end of the experiment in 2025, 3 soil core samples were collected from each plot at each location. The soil bulk density was determined using the Grossman and Reinsch (2002) methodology, using the dry mass of a known volume soil sample. Using a core sampler, soil was collected from the field and dried in an oven at 105ºC for 24 hours. After drying and cooling, the soil's dry mass weight was determined. Bulk density was calculated with the equation:

$$\text{Bulk Density (g/cm}^3\text{)} = \text{Dry Mass of Soil (g)} / \text{Volume of Core (cm}^3\text{)}$$

3.4.2. Soil Moisture

Three soil moisture readings were taken from each plot, during both years, at each location at V6, V10, and R1 corn growth stages, using the Dynamax TH150 Portable Soil Moisture Probe, Houston, Texas. This device measures the volumetric water content percentage.

3.4.3. Soil Penetration Resistance

The soil physical indicator surface hardness (PR15) was measured using a penetrometer (FieldScout SC 900 Soil Compaction Meter, Spectrum Technologies, Inc, Aurora, Illinois) (Duiker, 2002). Compaction readings (3 readings per plot in the center

rows) were taken after the termination of the experiment in the fall (September 30th in Knoxville and October 1st in Spring Hill) of 2025.

A penetrometer consists of a circular stainless-steel rod connected to a pressure gauge. This device is designed to mimic a plant's root and simulate root growth. The tip is slightly wider and sharper, making it easier to penetrate the soil. The pressure gauge indicates pressure in pounds per square inch, and the readings taken with the penetrometer are called the code index (CI). Root growth is limited and decreases with the increasing penetration resistance, when the pressure is above 2,000 kPa (Duiker, 2002). Ideally, penetrometer measurements should be taken with soil at the field capacity of water, simulating ideal conditions for root growth, with spring being the best time of the year for this measurement. When the soil is too wet, penetrometer readings can be underestimated, and the opposite happens when the soil is too dry, with the readings being overestimated.

For the detection of compacted zones in the soil profile, two points are required (top and bottom), starting by pushing the penetrometer into the soil, and recording the depth at which the CI exceeds 2,000 kPa, indicating the top of the compacted zone. While still pressing the penetrometer down, record the depth at which the CI falls below 2,000 kPa, indicating the bottom of the compacted zone. If the penetrometer never records values above 2,000 kPa which indicates that the area does not have severe root-limiting compaction. On the other hand, if the penetration resistance increases above 2,000 kPa, but never falls below 2,000 kPa, the bottom of the compaction zone is deeper than the penetrometer rod length (Duiker, 2002).

3.5. Soil Biological Analysis

3.5.1. Soil Organic Carbon (SOC)

Organic carbon is present in the soil organic fraction, which consists of the cells of microorganisms, plant and animal residues at various stages of decomposition, and inorganic carbon, is typically in the form of carbonates, such as calcite and dolomite (Nelson & Sommers, 1996). Inorganic carbon differs from organic carbon because it is not an accessible form of carbon for biological systems. Total organic carbon (TOC) analysis, therefore, provides essential information about microbiological activity and organic matter to evaluate soil health (Elementar, 2023).

The direct method of soil TOC determination is based on high-temperature combustion at temperatures above 900°C, where carbon is converted into CO_2 in the presence of oxygen and then measured by a thermal conductivity or infrared detector. First, the inorganic carbon needs to be removed, so the sample is acidified (HCl) prior to the measurement. For this process, it has weighed the homogenized sample into a silver cup (record down the weight, since this is the weight which will finally be used for the calculation of the element concentrations) and slowly add diluted acid (1M HCl) to the sample until the reaction stops. Then slowly add concentrated acid (10M HCl) until any visible reaction stops. For some samples, this procedure has to be repeated several times in order to remove all the inorganic carbon from the sample. The carbonate in the sample is converted into CO_2, which is removed during a drying step for over 4 hours at 130°C. Finally, the silver cup has to be closed and packed into a tin foil cup, in order to enhance the combustion temperature to well over 1000°C. The remaining carbon content is bound to organic compounds (TOC) and can be measured directly using the vario EL cube or vario MACRO cube. The vario EL cube is configured

in CN mode (in CNS mode, the S column will be destroyed). The vario EL cube can be operated with the standard combustion and reduction tubes when TOC samples are measured alternately with normal CN measurements. The Brookside Laboratories at New Bremen in Ohio performed the TOC analysis.

3.5.2. Permanganate Oxidizable Carbon (POXC)

The permanganate oxidizable carbon (POXC) is considered a useful measure of biologically-active SOC, which represents active and decomposed SOC, and it was measured using the methodology developed by Weil et al. (2003). Each air-dried soil sample (2.5 g) was taken in a 50-mL polypropylene tube. Then 20 mL of 0.02 mol L^{-1} of potassium permanganate ($KMnO_4$) solution was added to each tube, and the tubes were shaken for exactly 2 minutes on a shaker, then left to decant at room temperature for 10 minutes. After 10 minutes, 0.5 mL of the supernatant from each sample was transferred to a new 50-mL centrifuge tube containing 49.5 mL of water. The absorbance of the samples was measured at 550 nm using a spectrophotometer. The Brookside Laboratories at New Bremen in Ohio, performed the POXC analysis.

3.5.3. Soil Respiration (24 hours Infrared Gas Analyzer)

The soil respiration can be quantified with the concentration of CO_2 evolved from air-dried soil after rewetting (Franzluebbers et al., 2000). This method uses an Infrared Gas Analyzer (IRGA) unit to measure the concentration of CO_2 released from the soil. The method involves weighing 20-30 g of air-dried soil into a 473 mL jar and adding water to reach 50% of the soil water-holding capacity, then letting the sample incubate at 25^{o}C for 24h. For the CO_2 measures, start by turning on the CO_2 tank (1000 ppm) and setting the outflow pressure to 10-20 PSI, and then, using 2 different sets of needles, one for gas flow and the other for outflow from the CO_2 tank into a glass vial.

While CO_2 is flowing, draw 3 mL of air from the sample and inject it into the IRGA and record the maximum reading and the exact time (24h) of measurement per sample (Schindelbeck et al., 2016). For this experiment, samples were read as close to the 24h mark after adding water to the sample. The Brookside Laboratories at New Bremen in Ohio performed the soil respiration (24 hours IRGA) analysis.

3.5.4. Earthworm Population

Linden et al. (1994) proposed a concept that earthworms serve as indicators of soil quality by acting as biological catalyst that transform plant residues into soil organic matter. Crop residues have been reported as the major limitation to a sustained earthworm population (Jensen, 1985). When earthworms are present, and the soil does not have a major limiting chemical or physical condition, then the factor most often limiting earthworms is food supply. The population of earthworms was estimated by counting the number of individuals in each plot at the V10 and R3 corn growth stages, in 2024 and 2025, respectively. A hole measuring approximately 0.093 m^2 and 5 cm in depth was dug at the center of each plot, and the soil was used to make the counting.

3.6. Statistical Analysis

Comparisons of soil parameters among treatments and sampling times were analyzed by year and location. Data was submitted to analysis of variance (ANOVA) at $\alpha = 0.05$. Significant effects were separated using least square means with the Student's t-test. Analyses, graphical presentations, and data visualization were performed using JMP Student Edition 18 (JMP Statistical Discovery, Cary, North Carolina).

Chapter Four: Results

4.1. Chemical Indicators

4.1.1 Chemical Characterization

By the end of the experiment in the fall of 2025, the soil chemical analysis obtained in Spring Hill did not demonstrate differences among the treatments in any of the chemical indicators (Table 1). All treatments presented adequate pH levels for agricultural practices, with a variation of only 0.33 from the highest value (WCLM) to the lowest (HVCC). Low variations were also observed in CEC values, where the WCLM treatment exhibited the highest CEC value (14.92), while RCLM presented the lowest (13.25). The same trend of small variations with no differences was observed for macro and micronutrient contents.

Table 1 pH, cation exchange capacity, and macro and micronutrient concentrations of soils under a corn intercropping system in 2025 in Spring Hill, TN

	CONTROL	HVCC [a]	HVLM [a]	RCLM [a]	CCLM [a]	WCLM [a]	P value
pH [ns b]							
	6.40	6.17	6.42	6.20	6.22	6.50	0.8566
CEC (cmolc kg^{-1}) [ns b]							
	14.52	14.49	15.15	13.25	13.67	14.92	0.3135
Macronutrients (mg kg^{-1}) [b]							
P [ns]	245.20	288.00	257.00	262.00	246.00	262.00	0.7893
K [ns]	374.00	350.20	365.00	343.50	347.50	344.20	0.9933
Ca [ns]	1,968.50	1,852.00	2,112.00	1,712.70	1,780.50	2,072.70	0.4245
Mg[ns]	197.20	195.50	193.70	172.20	179.50	197.20	0.6447
S [ns]	13.00	14.25	13.75	12.50	12.50	13.25	0.5706
Micronutrients (mg kg^{-1}) [b]							
B [ns]	0.80	0.70	0.70	0.71	0.70	0.76	0.1623
Fe [ns]	182.00	199.75	188.00	191.25	184.75	181.75	0.8418
Mn[ns]	86.00	82.25	93.00	78.25	82.75	96.00	0.9039
Cu [ns]	1.44	1.44	1.58	1.21	1.26	1.64	0.4722
Zn[ns]	2.48	2.87	2.80	2.29	2.70	3.18	0.7558

[a] Treatments: HVCC (hairy vetch cover crop), HVLM (hairy vetch living mulch), RCLM (red clover living mulch), CCLM (crimson clover living mulch), and WCLM (white clover living mulch)
[b] Means in a row followed by ns were not significantly different from the ANOVA test (α=0.05).

By the end of the experiment in the fall of 2025, the soil chemical analysis obtained in Knoxville showed differences among the treatments in pH and copper concentrations (Table 2). Although all treatments presented adequate pH levels for agricultural practices, the HVCC treatment exhibited the highest pH value (6.12), differing from the RCLM (5.72) and HVLM and WCLM (both with 5.57) treatments, while the control and CCLM treatments did not differ from the other treatments. Analyzing the copper content, the RCLM treatment presented the highest copper content (1.67), while the HVLM obtained the lowest content value (1.20).

Table 2 pH, cation exchange capacity, and macro and micronutrient concentrations of soils under a corn intercropping system in 2025 in Knoxville, TN

	CONTROL	HVCC [a]	HVLM [a]	RCLM [a]	CCLM [a]	WCLM [a]	P value
pH [b]							
	5.87 ab	6.12 a	5.57 b	5.72 b	5.80 ab	5.57 b	0.0363
CEC (cmolc kg^{-1}) [ns b]							
	10.13	10.53	10.47	10.97	10.80	11.74	0.0963
Macronutrients (mg kg^{-1}) [b]							
P [ns]	23.00	20.00	16.25	30.50	23.25	24.00	0.2609
K [ns]	221.00	222.25	181.75	263.25	222.25	223.50	0.7576
Ca [ns]	1,036.25	1,166.50	944.50	1,044.50	1,067.25	1,046.50	0.3536
Mg[ns]	221.50	258.00	202.00	216.50	229.00	221.50	0.1869
S [ns]	10.00	8.75	10.25	9.75	10.75	9.50	0.1904
Micronutrients (mg kg^{-1}) [b]							
B [ns]	0.36	0.36	0.34	0.37	0.38	0.36	0.7753
Fe [ns]	75.00	70.75	76.00	77.00	75.25	78.75	0.2408
Mn[ns]	538.00	552.50	499.75	532.00	527.25	513.00	0.6555
Cu	1.39 abc	1.61 ab	1.20 c	1.67 a	1.59 ab	1.34 bc	0.0448
Zn[ns]	2.96	3.35	2.33	3.40	3.10	2.97	0.0613

[a] Treatments: HVCC (hairy vetch cover crop), HVLM (hairy vetch living mulch), RCLM (red clover living mulch), CCLM (crimson clover living mulch), and WCLM (white clover living mulch)
[b] Data were submitted to an ANOVA test, and means were separated according to Student's t-test (α=0.05); means in a row followed by the same letter or ns were not significantly different.

4.1.2. Ammonium-N and Nitrate-N

At the V10 corn growth stage in Spring Hill 2024, differences in soil nitrate-N levels were observed among the treatments applied (Table 3). Specifically, hairy vetch treatments, both LM and CC, demonstrated significantly higher nitrate-N levels than the control. Among the living mulch treatments, HVLM exhibited the highest nitrate-N level, outperforming the other living mulch treatments. However, no differences were detected in ammonium-N or the sum of available nitrogen levels across all treatments. When comparing the nitrogen forms at post-harvest, no differences were observed in nitrate-N, ammonium-N, or the sum of available nitrogen levels among the treatments.

Table 3 Soil inorganic nitrogen concentration (mg kg^{-1}) across different corn growth stages in 2024 in Spring Hill, TN

	Control	HVCC [a]	HVLM [a]	RCLM [a]	CCLM [a]	WCLM [a]	P value
V10 [b]							
Nitrate-N	0.85 c	3.12 ab	3.90 a	2.57 b	2.15 bc	1.87 bc	0.0028
Ammonium-N [ns]	8.32	10.17	9.12	13.90	11.60	10.32	0.3927
Sum of N [ns]	9.17	13.30	13.02	16.47	13.75	12.20	0.2317
Post Harvest [b]							
Nitrate-N [ns]	19.02	27.85	29.00	23.25	29.12	27.00	0.5937
Ammonium-N [ns]	3.95	3.90	7.05	4.95	4.52	5.32	0.2604
Sum of N [ns]	22.97	31.75	36.05	28.20	33.65	32.32	0.5017

[a] Treatments: HVCC (hairy vetch cover crop), HVLM (hairy vetch living mulch), RCLM (red clover living mulch), CCLM (crimson clover living mulch), and WCLM (white clover living mulch)

[b] Data were submitted to an ANOVA test and means were separated according to Student's t-test (α=0.05); means in a row followed by the same letter or ns were not significantly different.

By the fall of 2024, in Spring Hill, differences in the distribution of nitrogen available forms across the soil profile were detected (Figure 1). The nitrate-N ($P < 0.0001$) and ammonium-N ($P < 0.0001$) levels, and consequently the sum of nitrogen ($P < 0.0001$), were significantly higher in the top 15 cm of soil than in the deeper layers, as this zone is the primary area influenced by organic matter inputs and microbial

activity. In contrast, no differences were detected between the 15-30 cm and 30-60 cm depths, although there was a trend of decreasing nitrogen levels with increasing soil depth. These findings highlight the concentration of nitrogen near the surface, likely influenced by factors such as organic matter decomposition, microbial activity, and nutrient cycling processes that are more active in the upper soil layer.

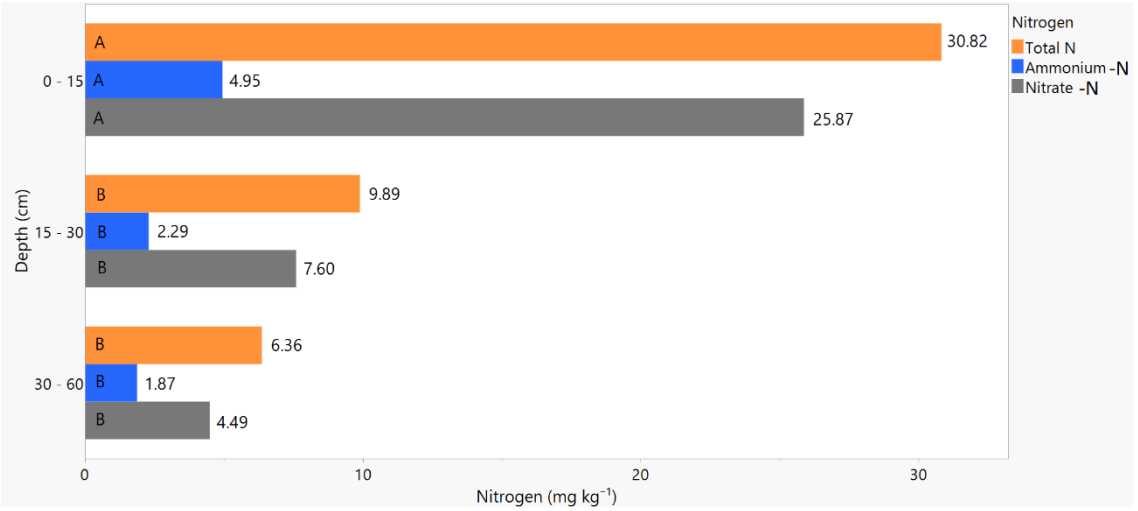


Figure 1 Comparison of available nitrogen forms behavior across the soil profile in the fall of 2024 in Spring Hill, TN; means of each measurement among depths were separated according to Student's t-test (α=0.05); means of each measurement among depths followed by the same letter were not significantly different.

The last observation obtained in Spring Hill during 2024 were the differences in levels of available nitrogen forms comparing the V10 corn growth stage to the post-harvest period (Figure 2). Nitrate-N ($P < 0.0001$) level was higher during the post-harvest period, whereas ammonium-N ($P < 0.0001$) level was greater at the V10 stage. Additionally, the sum of nitrogen ($P < 0.0001$) was higher at post-harvest. This pattern highlights the potential of organic management, combined with the incorporation of legumes, to enhance nitrogen fixation and availability over time.

At the V10 corn growth stage in Spring Hill during 2025, no differences were observed in the levels of available nitrogen or the sum of nitrogen among the treatments (Table 4). When comparing the nitrogen forms at post-harvest, there were

no differences observed in nitrate-N, ammonium-N and the sum of nitrogen levels among the treatments.

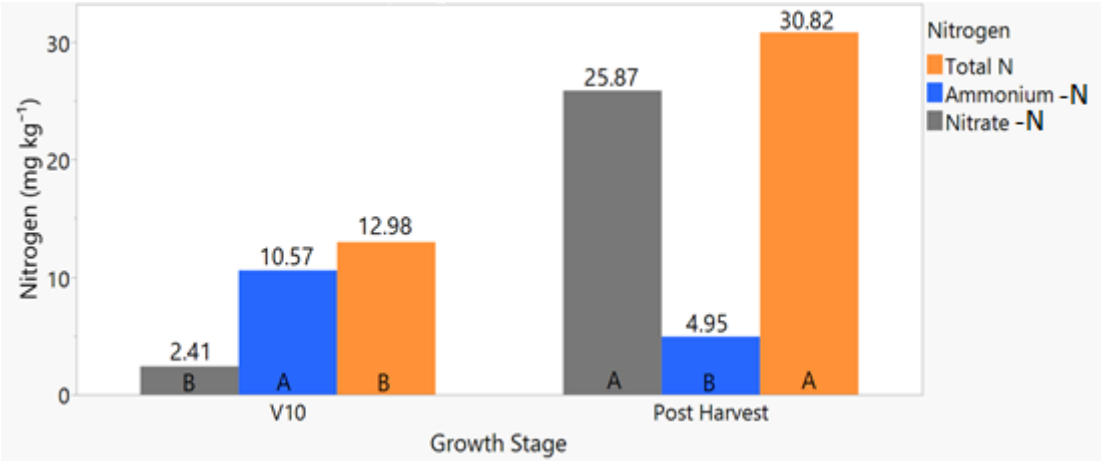


Figure 2 Comparison of available nitrogen forms by corn growth stage during the 2024 growing season in Spring Hill, TN; means of each measurement between growth stages were separated according to Student's t-test (α=0.05); means of each measurement between growth stages followed by the same letter were not significantly different.

Table 4 Soil inorganic nitrogen concentration (mg kg^{-1}) across different corn growth stages in 2025 in Spring Hill, TN

	Control	HVCC [a]	HVLM [a]	RCLM [a]	CCLM [a]	WCLM [a]	P value
V10 [b]							
Nitrate-N [ns]	5.85	7.75	5.52	4.10	6.20	4.57	0.5268
Ammonium-N [ns]	6.40	14.55	4.07	8.05	13.02	5.80	0.4271
Sum of N [ns]	12.25	22.30	9.60	12.15	19.22	10.37	0.3378
Post Harvest [b]							
Nitrate-N [ns]	15.97	19.67	18.85	12.97	20.00	23.10	0.4694
Ammonium-N [ns]	1.67	2.12	1.47	1.62	1.37	2.25	0.6029
Sum of N [ns]	17.65	21.80	20.32	14.60	21.37	25.35	0.4387

[a] Treatments: HVCC (hairy vetch cover crop), HVLM (hairy vetch living mulch), RCLM (red clover living mulch), CCLM (crimson clover living mulch), and WCLM (white clover living mulch)

[b] Means in a row followed by ns were not significantly different according to ANOVA test (α=0.05).

As observed in the previous year, by the fall of 2025, in Spring Hill, differences in the distribution of nitrogen available forms across the soil profile were detected (Figure 3). The nitrate-N ($P < 0.0001$) and sum of nitrogen ($P < 0.0001$) levels were

significantly higher within the top 15 cm of soil than in the deeper layers. For the sum of nitrogen, no differences were detected between the 15-30 cm and 30-60 cm depths, although there was a trend of decreasing nitrogen levels with increasing soil depth. For ammonium-N (P = 0.0130), the levels obtained at the 30-60 cm depth were statistically similar to the top 15 cm, and higher when compared to the 15-30 cm. For nitrate-N level, the trend observed is decreasing nitrate-N level with increasing soil depth. These findings highlight the concentration of nitrogen near the surface, as observed at the same location in the previous year.

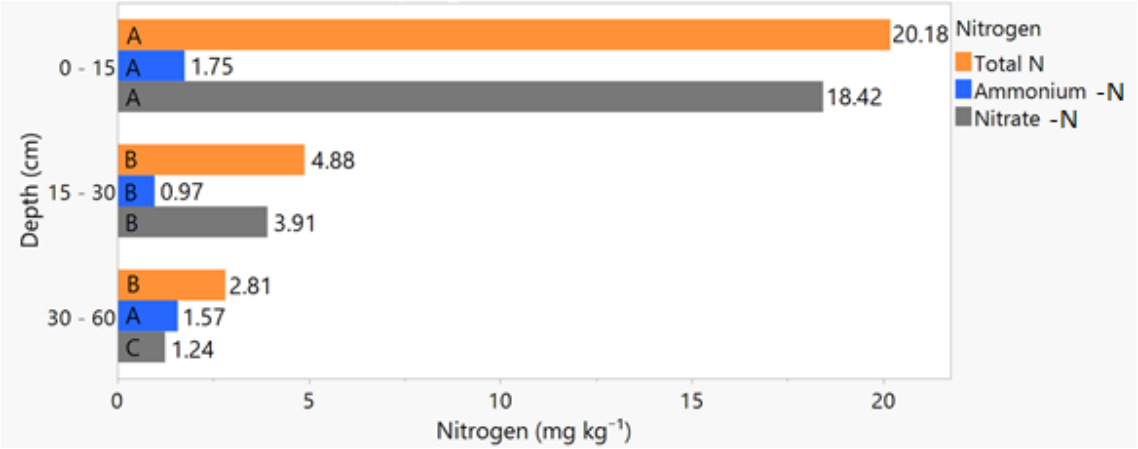


Figure 3 Comparison of available nitrogen forms concentration across soil profile in the fall of 2025 in Spring Hill, TN; means of each measurement among depths were separated according to Student's t-test (α=0.05); means of each measurement among depths followed by the same letter were not significantly different.

Finally, when comparing levels of available nitrogen forms in Spring Hill during 2025, differences were detected comparing the V10 corn growth stage to the post-harvest period (Figure 4). As recorded in the previous year, the nitrate-N (P < 0.0001) level was higher during the post-harvest period, whereas ammonium-N (P = 0.0002) level was greater at the V10 stage. Additionally, the sum of nitrogen (P = 0.0217) was higher at post-harvest. This pattern highlights the potential of organic management, combined with the incorporation of legume species, to enhance nitrogen fixation and availability over time.

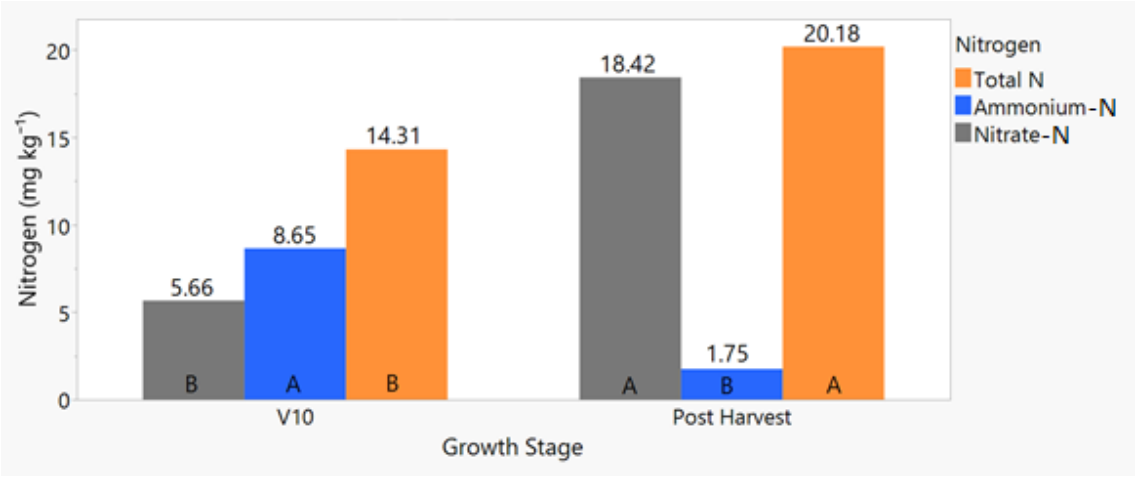


Figure 4 Comparison of available nitrogen forms concentration across corn growth stages during the 2024 growing season in Spring Hill, TN; means of each measurement between growth stages were separated according to Student's t-test (α=0.05); means of each measurement between growth stages followed by the same letter were not significantly different.

When comparing available nitrogen forms measured in 2024 and 2025, in Spring Hill, differences were detected across all three-soil layers evaluated (Table 5). Nitrate-N, ammonium-N, and total inorganic nitrogen concentrations were consistently higher in 2024 than in 2025 across all three-soil depth intervals (0-15, 15-30, and 30-60 cm).

Table 5 Soil inorganic nitrogen concentration (mg kg^{-1}) across the soil profile in 2024 and 2025 in Spring Hill, TN

Depth (cm) [a]	Nitrogen	2024	2025	P value
0 -15	Nitrate-N	25.87 a	18.42 b	0.0026
	Ammonium-N	4.95 a	1.75 b	<0.0001
	Total N	30.82 a	20.18 b	<0.0001
15 - 30	Nitrate-N	7.60 a	3.91 b	<0.0001
	Ammonium-N	2.29 a	0.97 b	<0.0001
	Total N	9.89 a	4.88 b	<0.0001
30 - 60	Nitrate-N	4.49 a	1.24 b	<0.0001
	Ammonium-N [ns]	1.87	1.57	0.2217
	Total N	6.36 a	2.81 b	<0.0001

[a] Data were submitted to an ANOVA test, and means were separated according to Student's t-test (α=0.05); means in a row followed by the same letter or ns were not significantly different.

At the V10 corn growth stage in Knoxville during 2024, differences in nitrate-N, ammonium-N, and the sum of nitrogen levels were observed across the treatments

(Table 6). The HVLM treatment stood out as the only one to demonstrate a statistically significant increase in nitrate-N levels compared to the control. For ammonium-N level, the RCLM, WCLM, and HVLM treatments presented the highest values compared to the control, with WCLM recording the highest ammonium-N level among all treatments. When assessing the sum of nitrogen, both HVLM and WCLM treatments demonstrated differences from the control, with HVLM presenting the highest total nitrogen content overall. In contrast, when evaluating nitrogen forms during the post-harvest period, no differences in nitrate-N, ammonium-N, or the sum of nitrogen level were detected among the treatments.

Table 6 Soil inorganic nitrogen concentration (mg kg^{-1}) across different corn growth stages in 2024 in Knoxville, TN

	Control	HVCC [a]	HVLM [a]	RCLM [a]	CCLM [a]	WCLM [a]	P value
V10 [b]							
Nitrate-N	4.75 b	5.02 b	9.67 a	4.77 b	5.90 b	5.77 b	0.0027
Ammonium-N	3.75 c	3.77 c	6.50 a	5.27 ab	4.70 bc	6.65 a	0.0012
Sum of N	8.50 c	8.80 c	16.17 a	10.05 bc	10.60 bc	12.42 b	0.0001
Post Harvest [b]							
Nitrate-N [ns]	34.25	28.97	27.05	35.12	29.47	42.97	0.2032
Ammonium-N [ns]	8.92	6.62	6.55	4.95	6.12	5.80	0.5587
Sum of N [ns]	43.17	35.60	33.60	40.07	35.60	48.77	0.2401

[a] Treatments: HVCC (hairy vetch cover crop), HVLM (hairy vetch living mulch), RCLM (red clover living mulch), CCLM (crimson clover living mulch), and WCLM (white clover living mulch)

[b] Data were submitted to an ANOVA test, and means were separated according to Student's t-test (α=0.05); means in a row followed by the same letter or ns were not significantly different.

Analyzing available nitrogen in the soil profile, differences in the distribution were observed in Knoxville during 2024 (Figure 5). The nitrate-N ($P < 0.0001$), ammonium-N ($P < 0.0001$), and sum of nitrogen ($P < 0.0001$) levels were significantly higher within the top 15 cm of soil. The available nitrogen forms concentrations

tended to decrease with increasing soil depth, with notable differences observed in total nitrogen and nitrate-N levels. However, no differences in ammonium-N level were detected between 15-30 cm and 30-60 cm depths.

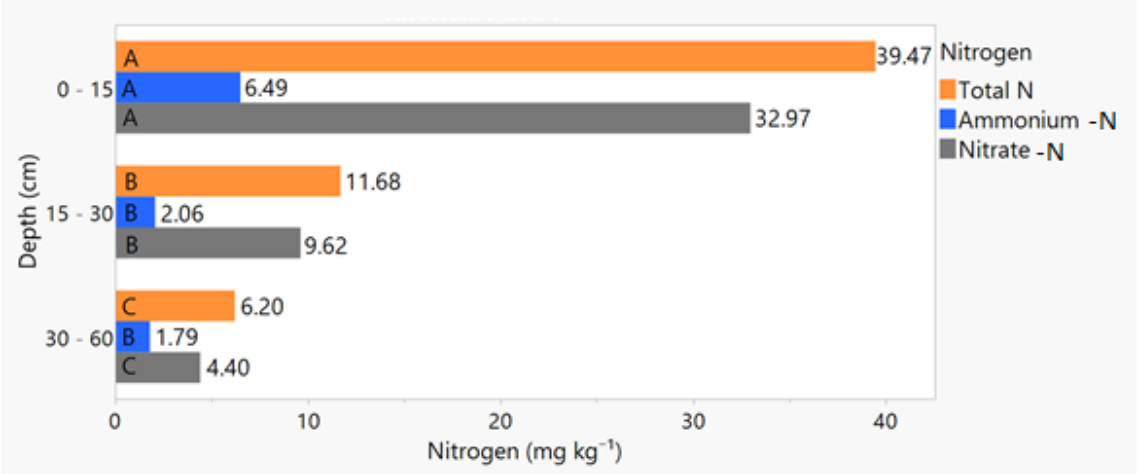


Figure 5 Comparison of available nitrogen forms behavior across the soil profile in the fall of 2024 in Knoxville, TN; means of each measurement among depths were separated according to Student's t-test (α=0.05), means of each measurement among depths followed by the same letter were not significantly different.

When comparing levels of available nitrogen forms in Knoxville during 2024, differences were detected when comparing the V10 corn growth stage to the post-harvest period (Figure 6). The nitrate-N ($P < 0.0001$) and ammonium-N ($P = 0.0430$) levels, and consequently the sum of nitrogen ($P < 0.0001$), were higher during the post-harvest period than during the V10 growth stage. This pattern highlights the ability of the organic management, combined with the use of legume species, to enhance nitrogen fixation during a short-term period.

At the V10 corn growth stage in Knoxville during 2025, differences were observed only in the soil nitrate-N level, with no differences being observed ammonium-N or the sum of nitrogen among the treatments (Table 7). For nitrate-N, the RCLM treatment was the only one that statistically differ from the control, presenting the highest nitrate-N level relative to all other treatments. When evaluating

the available nitrogen forms at the post-harvest period in 2025, differences were detected in the nitrate-N and the sum of nitrogen levels. For nitrate-N, the WCLM, HVLM, and RCLM treatments were the ones that differed from the control and the HVCC; with the WCLM exhibiting the highest nitrate-N level overall. For the sum of nitrogen, the WCLM, HVLM, and RCLM treatments differed from the control and HVCC, with WCLM once again exhibiting the highest value overall.

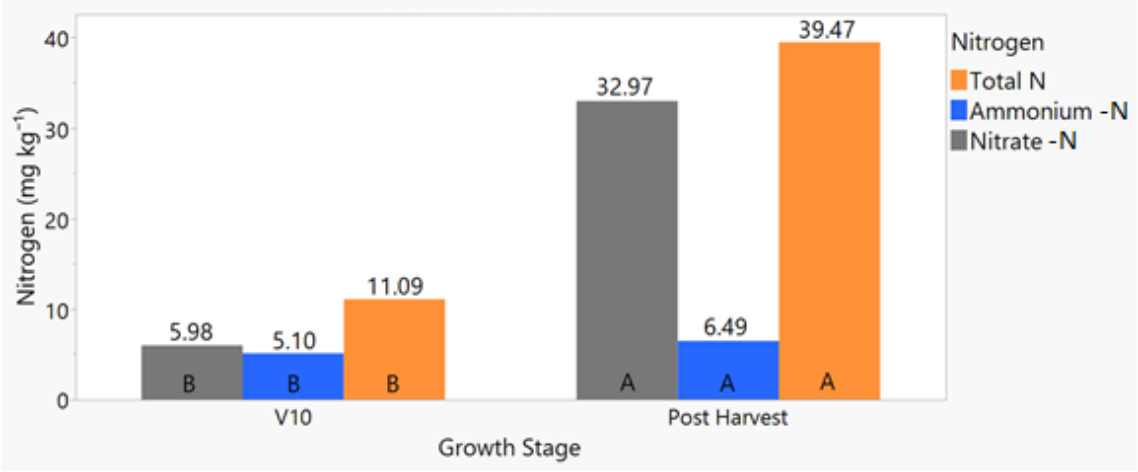


Figure 6 Comparison of available nitrogen forms across corn growth stages during the 2024 growing season in Knoxville, TN; means of each measurement between growth stages were separated according to Student's t-test (α=0.05); means of each measurement between growth stages followed by the same letter were not significantly different.

Table 7 Soil inorganic nitrogen concentration (mg kg^{-1}) across corn growth stages in 2025 in Knoxville, TN

	Control	HVCC [a]	HVLM [a]	RCLM [a]	CCLM [a]	WCLM [a]	P value
V10 [b]							
Nitrate-N	2.62 bc	2.67 bc	2.05 c	4.35 a	4.20 ab	3.75 ab	0.0353
Ammonium-N [ns]	6.75	7.15	7.50	7.72	7.55	7.80	0.9023
Sum of N [ns]	9.37	9.82	9.55	12.07	11.75	11.55	0.2754
Post Harvest [b]							
Nitrate-N	14.57 c	13.45 c	23.05 ab	22.92 ab	19.15 bc	27.25 a	0.0066
Ammonium-N [ns]	1.10	1.22	1.02	1.12	1.02	1.60	0.3186
Sum of N	15.67 c	14.67 c	24.07 ab	24.05 ab	20.17 bc	28.85 a	0.0053

[a] Treatments: HVCC (hairy vetch cover crop), HVLM (hairy vetch living mulch), RCLM (red clover living mulch), CCLM (crimson clover living mulch), and WCLM (white clover living mulch)
[b] Data were submitted to an ANOVA test, and means were separated according to Student's t-test (α=0.05); means in a row followed by the same letter or ns were not significantly different.

Similar to the previous year, when analyzing the available nitrogen in the soil profile, differences in the distribution were observed in Knoxville during 2025 (Figure 7). The nitrate-N ($P < 0.0001$) and sum of nitrogen ($P < 0.0001$) levels exhibited a similar behavior; recording higher levels in the top 15 cm of the soil profile, and reducing accordingly with increasing the soil depth, even without differences between the 15-30 cm and 30-60 cm. For the ammonium-N level, no differences were detected ($P = 0.6767$).

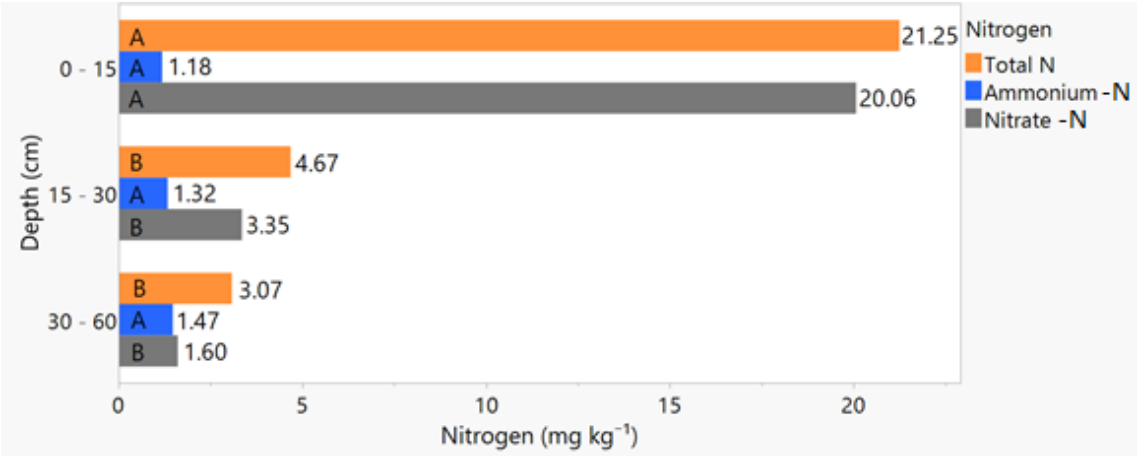


Figure 7 Comparison of available nitrogen forms behavior across the soil profile in the fall of 2025 in Knoxville, TN; means of each measurement among depths were separated according to Student's t-test (α=0.05); means of each measurement among depths followed by the same letter were not significantly different.

When analyzing levels of available nitrogen forms in Knoxville during 2025, differences were detected comparing the V10 corn growth stage to the post-harvest period (Figure 8). As recorded in the previous year, the nitrate-N ($P < 0.0001$) and sum of nitrogen ($P < 0.0001$) levels were higher during the post-harvest period than during the V10 growth stage. Different from the data recorded in 2024, the ammonium-N ($P < 0.0001$) level decreased from V10 to the post-harvest period.

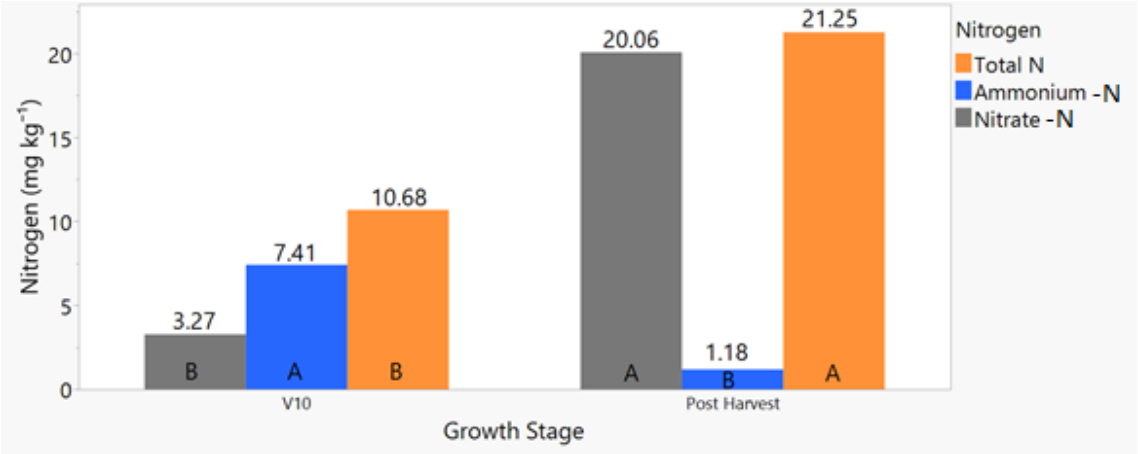


Figure 8 Comparison of available nitrogen forms across corn growth stages during the 2025 growing season in Knoxville; means of each measurement between growth stages were separated according to Student's t-test (α=0.05); means of each measurement between growth stages followed by the same letter were not significantly different.

When comparing available nitrogen forms measured in 2024 and 2025, in Knoxville, differences were detected across all three soil layers evaluated (Table 8). Nitrate-N, ammonium-N, and total inorganic nitrogen concentrations were consistently higher in 2024 than in 2025 in the top 15 cm of the soil profile. In the 15-30 and 30-60 cm soil layers, similar trends were observed across nitrogen forms, with nitrate-N and total nitrogen concentrations significantly higher in 2024 than in 2025, while ammonium-N level did not differ between the two years.

Table 8 Soil inorganic nitrogen concentration (mg kg^{-1}) across the soil profile in 2024 and 2025 in Knoxville, TN

Depth (cm) [a]	Nitrogen	2024	2025	P value
0 -15	Nitrate-N	32.97 a	20.06 b	<0.0001
	Ammonium-N	6.49 a	1.18 b	<0.0001
	Total N	39.47 a	21.25 b	<0.0001
15 - 30	Nitrate-N	9.62 a	3.35 b	<0.0001
	Ammonium-N [ns]	2.06	1.32	0.0527
	Total N	11.68 a	4.67 b	<0.0001
30 - 60	Nitrate-N	4.40 a	1.60 b	<0.0001
	Ammonium-N [ns]	1.79	1.47	0.1549
	Total N	6.20 a	3.07 b	<0.0001

[a] Data were submitted to an ANOVA test, and means were separated according to Student's t-test (α=0.05); means in a row followed by the same letter or ns were not significantly different.

4.2. Physical Indicators

4.2.1. Bulk Density

Bulk density differences were observed among the treatments in Spring Hill during 2024 (Table 9). Specifically, the living mulch treatments demonstrated lower bulk density values than the HVCC treatment, with RCLM, HVLM, and CCLM outperforming the control. These results suggest that the use of living mulch contributed to reduced soil compaction, potentially enhancing soil structure and aeration. However, in the summer of 2025, no differences in bulk density were detected among the treatments, indicating that the effects observed in the previous year may not have persisted over time or under varying seasonal conditions (Table 9). Similarly, during the final bulk density measurement conducted in the fall of 2025 by the end of the experiment, no differences were observed among the treatments. When analysing the average bulk density using data collected in Spring Hill across both years, 2024 and 2025 (summer and fall), no differences were detected among the treatments.

Table 9 Soil bulk density (g cm^{-3}) across 2024 and 2025 in Spring Hill, TN

Year [b]	Control	HVCC [a]	HVLM [a]	RCLM [a]	CCLM [a]	WCLM [a]	P value
2024 Summer	1.185 ab	1.211 a	1.102 c	1.099 c	1.109 c	1.135 bc	0.0212
2025 Summer [ns]	1.046	1.057	1.014	1.027	1.014	1.007	0.4646
2025 Fall [ns]	1.149	1.116	1.131	1.128	1.144	1.109	0.9379

[a] Treatments: HVCC (hairy vetch cover crop), HVLM (hairy vetch living mulch), RCLM (red clover living mulch), CCLM (crimson clover living mulch), and WCLM (white clover living mulch)

[b] Data were submitted to an ANOVA test, and means were separated according to Student's t-test (α=0.05); means in a row followed by the same letter or ns were not significantly different.

When comparing average bulk density across the three different sampling times at the Spring Hill site, differences in mean bulk density values were detected ($P < 0.0001$) (Figure 9). The summer of 2025 presented overall lower bulk density values than the other sampling times, indicating improved soil conditions during this period. In contrast, the fall of 2025 presented bulk density values that were statistically similar to those recorded during the summer of 2024. This observation may suggest that the effectiveness of the legumes in reducing bulk density is more pronounced during the summer months, coinciding with their active growth stages. During this period, legumes are likely contributing to improved soil structure through their root systems, which can enhance soil aeration and reduce compaction.

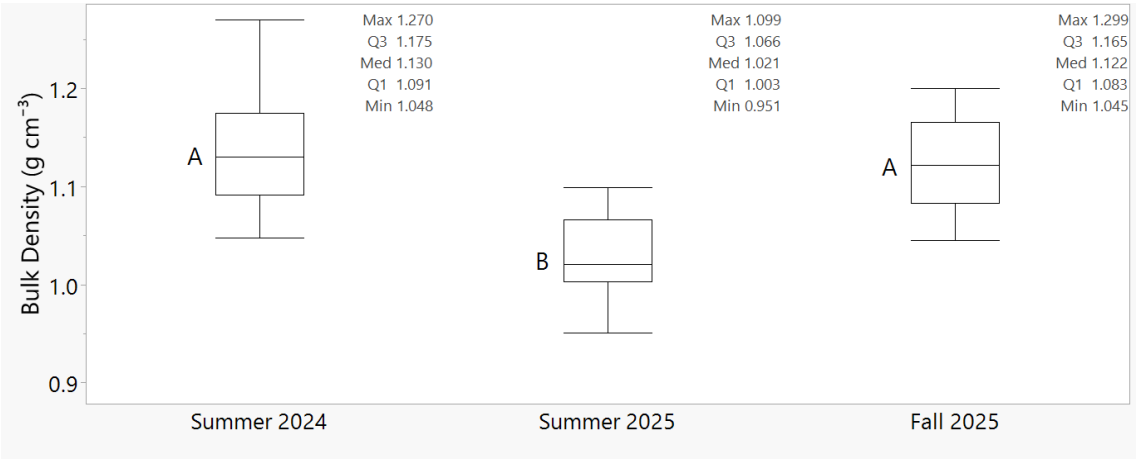


Figure 9 Soil bulk density (g cm^{-3}) across the different sampling times in 2024 and 2025 in Spring Hill, TN; means of different times separated according to Student's t-test (α=0.05); means of different times followed by the same letter were not significantly different.

No differences in bulk density were observed among the treatments at the Knoxville site during both the summer of 2024 and the fall of 2025 (Table 10). During the summer of 2025, adverse weather and field conditions hindered precise soil sampling, resulting in excessive variability in the data, which made the summer 2025 dataset unavailable for analysis. The lack of statistical differences in bulk density

observed during the fall of 2025 in Knoxville may be attributed to the absence of actively growing legume species, a pattern that was also noted at the Spring Hill location.

Table 10 Soil bulk density (g cm^{-3}) across 2024 and 2025 in Knoxville, TN

Year [b]	Control	HVCC [a]	HVLM [a]	RCLM [a]	CCLM [a]	WCLM [a]	P value
2024 Summer [ns]	1.322	1.274	1.253	1.220	1.213	1.173	0.0710
2025 Fall [ns]	1.028	1.056	1.022	1.054	1.050	1.043	0.9221

[a] Treatments: HVCC (hairy vetch cover crop), HVLM (hairy vetch living mulch), RCLM (red clover living mulch), CCLM (crimson clover living mulch), and WCLM (white clover living mulch)

[b] Means in a row followed by ns were not significantly different.

When comparing average bulk density across the two different sampling times at the Knoxville site, differences in mean bulk density values were detected ($P < 0.0001$) (Figure 10). The fall of 2025 presented lower bulk density values than the summer of 2024, indicating improved soil conditions during this period. Although data from the summer of 2025 was unavailable due to the sampling challenges, this observation may suggest that the effectiveness of the legumes in reducing bulk density over time. These findings support that the benefits of legumes on soil bulk density can manifest within relatively short timeframes, emphasizing their role as a viable strategy for improving soil health in organic intercropping systems.

4.2.2. Soil Moisture

Analyzing soil moisture data collected in Spring Hill during 2024, no differences in soil moisture were observed among the treatments during the V6, V10, or R1 corn growth stage (Table 11). A similar pattern was observed in 2025, where no differences in soil moisture were detected across the treatments during any of the sampling times (Table 11).

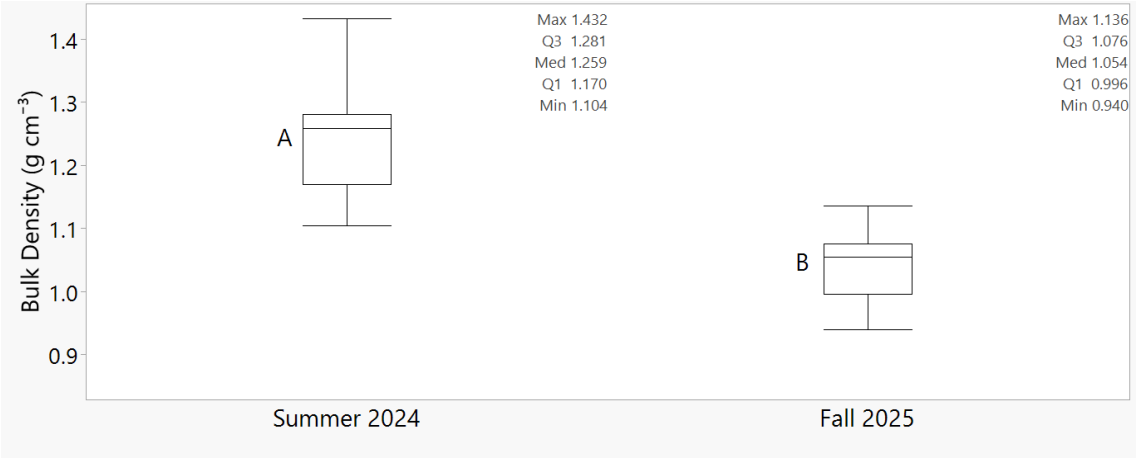


Figure 10 Soil bulk density (g cm^{-3}) across the different sampling times in 2024 and 2025 in Knoxville, TN; means between sampling times were separated according to Student's t-test (α=0.05); means of different sampling times followed by the same letter were not significantly different.

Table 11 Soil moisture content (g kg^{-1}) through different corn growth stages in 2024 and 2025 in Spring Hill, TN

		2024	
Treatment [a]	**V6 [ns b]**	**V10 [ns b]**	**R1 [ns b]**
Control	296.1	221.1	213.5
HVCC	322.1	210.5	209.8
HVLM	314.0	205.6	211.6
RCLM	341.7	227.8	212.1
CCLM	334.3	207.2	220.8
WCLM	336.2	203.5	213.3
P value	0.2445	0.6015	0.9875
		2025	
Treatment [a]	**V6 [ns b]**	**V10 [ns b]**	**R1 [ns b]**
Control	374.1	392.8	209.0
HVCC	369.3	405.3	203.5
HVLM	381.2	409.4	198.5
RCLM	390.9	407.0	187.1
CCLM	387.7	392.2	228.6
WCLM	383.8	401.6	214.0
P value	0.7868	0.6305	0.3279

[a] Treatments: HVCC (hairy vetch cover crop), HVLM (hairy vetch living mulch), RCLM (red clover living mulch), CCLM (crimson clover living mulch), and WCLM (white clover living mulch)

[b] Means in a column within a year followed by ns were not significantly different.

Differences in soil moisture content were observed in Spring Hill across the corn growth stages during both years of the experiment, 2024 ($P < 0.0001$) and 2025 (P

< 0.0001) (Figures 11 and 12). In 2024, soil moisture values were notably higher at the V6 stage than at the V10 and R1 stages, indicating greater water availability during early crop development. Interestingly, the V10 and R1 stages exhibited similar soil moisture levels. In 2025, a similar behavior was observed, with soil moisture levels consistently higher during the vegetative stages than the reproductive stage. Specifically, at the V10 stage, soil moisture content showed 5% increase compared to the V6 stage. Furthermore, soil moisture at the V6 stage was approximately 45% higher than that at the R1 stage, emphasizing the significant decline in water availability during the corn development cycle. These findings demonstrate that soil moisture dynamics vary significantly across corn growth stages, with vegetative phases generally exhibiting higher water availability than the reproductive stage.

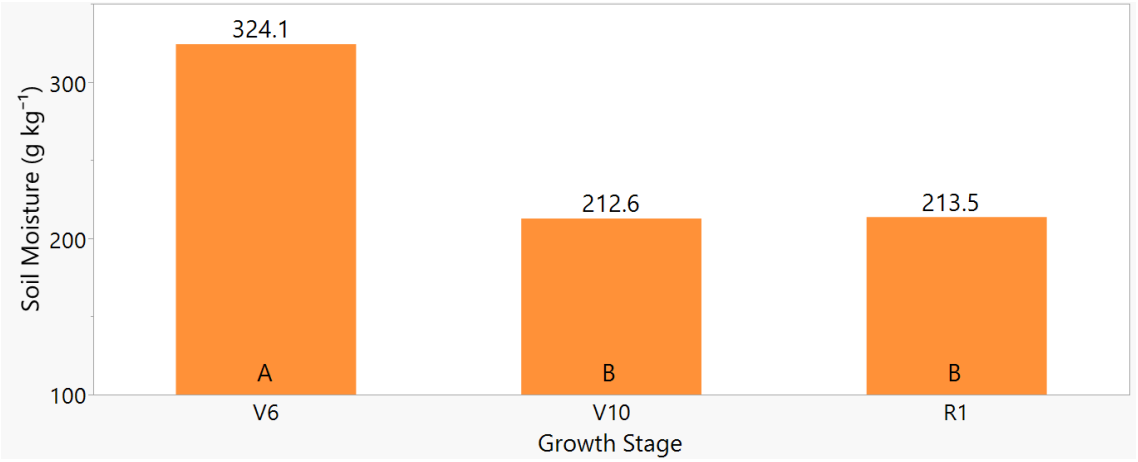


Figure 11 Average soil moisture (g kg^{-1}) by corn growth stage in 2024 in Spring Hill, TN; means among growth stages were separated according to Student's t-test (α=0.05); means of different growth stages followed by the same letter were not significantly different.

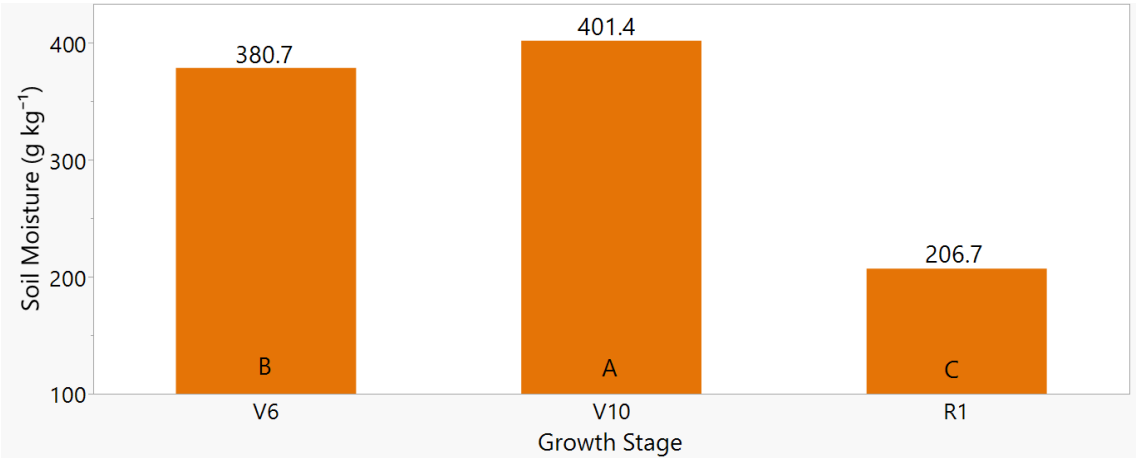


Figure 12 Average soil moisture (g kg^{-1}) by corn growth stage in 2025 in Spring Hill, TN; means among growth stages were separated according to Student's t-test (α=0.05); means of different growth stages followed by the same letter were not significantly different.

Differences in soil moisture levels were observed among the treatments at V6, V10, and R1 corn growth stages in 2024 at the Knoxville location, highlighting the potential for water retention from the legume species (Table 12). At the V6 stage, differences in soil moisture were detected, with HVLM, CCLM, and WCLM treatments exhibiting higher soil moisture levels than the control, suggesting the effectiveness of this species in enhancing early-stage water retention. During the V10 stage, both WCLM and HVLM treatments retained more soil moisture than the control and the HVCC treatment. At the R1 stage, WCLM and CCLM treatments demonstrated higher soil moisture percentages than the control and HVCC treatments. Similar findings were observed in 2025, with differences in soil moisture being detected among the treatments at V6, V10, and R1 corn growth stages (Table 12). At the V6 stage, all treatments utilizing living mulch presented higher soil moisture than the control and the HVCC treatment. During the V10 stage, the HVLM, WCLM, and RCLM treatments demonstrated greater soil moisture retention than both the control and the HVCC

treatment. At the R1 stage, the WCLM, HVLM, and RCLM treatments showed higher soil moisture percentages than the control and the HVCC treatment.

Table 12 Soil moisture content (g kg^{-1}) through different corn growth stages in 2024 and 2025 in Knoxville, TN

		2024	
Treatment [a]	**V6 [b]**	**V10 [b]**	**R1 [b]**
Control	312.1 b	334.3 bc	227.8 b
HVCC	344.0 ab	323.4 c	236.3 b
HVLM	394.5 a	389.9 a	277.3 ab
RCLM	359.5 ab	379.9 ab	276.7 ab
CCLM	382.3 a	375.6 abc	294.8 a
WCLM	379.9 a	421.8 a	325.4 a
P value	0.0310	0.0106	0.0112
		2025	
Treatment [a]	**V6 [b]**	**V10 [b]**	**R1 [b]**
Control	385.9 b	251.1 cd	111.6 bc
HVCC	392.7 b	233.2 d	87.0 c
HVLM	464.5 a	292.3 a	158.5 a
RCLM	435.6 a	277.5 ab	153.7 a
CCLM	452.4 a	257.9 cd	125.7 b
WCLM	450.2 a	276.3 ab	164.3 a
P value	0.0002	0.0002	<0.0001

[a] Treatments: HVCC (hairy vetch cover crop), HVLM (hairy vetch living mulch), RCLM (red clover living mulch), CCLM (crimson clover living mulch), and WCLM (white clover living mulch)

[b] Means were submitted to an ANOVA test, and separated according to Student's t-test (α=0.05); means in a column within a year followed by the same letter were not significantly different.

Differences in soil moisture levels were observed across the corn growth stages in 2024 at the Knoxville location, exhibiting distinct patterns of water retention between vegetative and reproductive stages ($P < 0.0001$) (Figure 13). Notably, soil moisture levels were higher during the vegetative stages than the reproductive stage, with the R1 stage showing approximately a 30% decrease in moisture content relative to the V6 and V10 stages. This decline may be attributed to increased plant water uptake during reproductive growth or/and environmental factors such as lower precipitation rates.

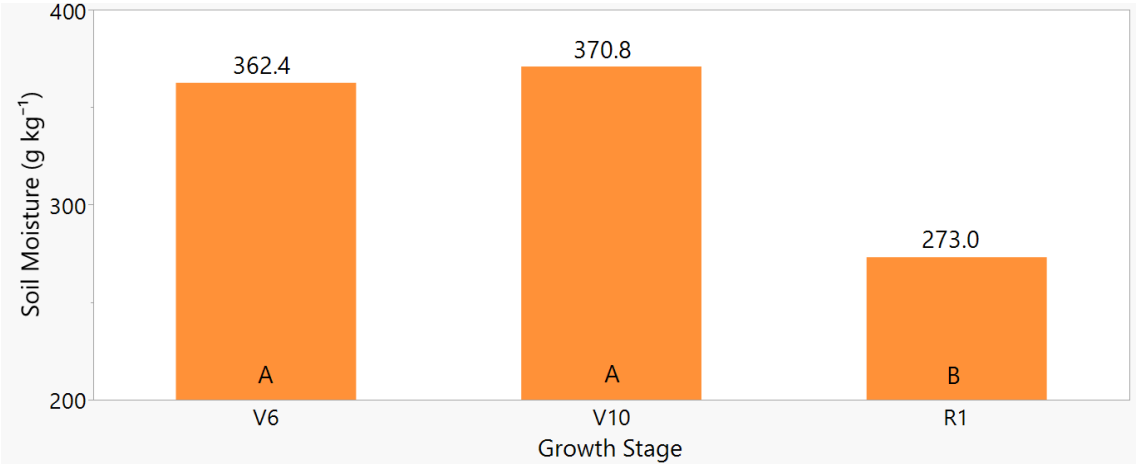


Figure 13 Average soil moisture (g kg^{-1}) by corn growth stage in 2024 in Knoxville, TN; means among different growth stages were separated according to Student's t-test (α=0.05); means of different growth stages followed by the same letter were not significantly different.

Similarly, in 2025, soil moisture dynamics followed a comparable trend compared to the previous year, with vegetative stages demonstrating higher moisture levels than the reproductive stage ($P < 0.0001$) (Figure 14). At the V6 stage, soil moisture content was approximately 40% greater than at the V10 stage, suggesting enhanced water retention during early vegetative growth. Furthermore, soil moisture at the V6 stage was more than three times higher than that observed at the R1 stage. In addition, soil moisture content at the V10 stage was nearly double that recorded at the R1 stage, emphasizing the decrease in soil moisture levels according to the changes during the corn growth stages.

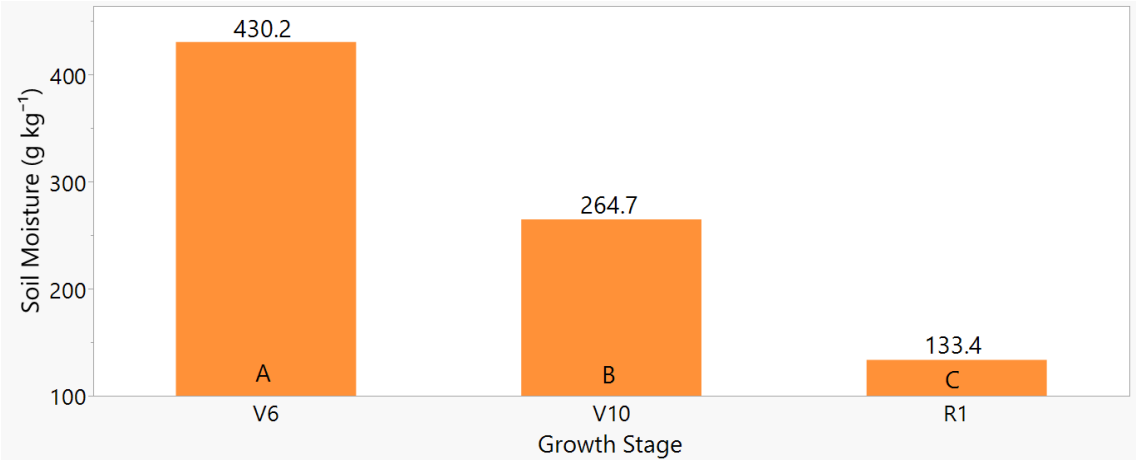


Figure 14 Average soil moisture (g kg^{-1}) by corn growth stage in 2025 in Knoxville, TN; means among different growth stages were separated according to Student's t-test (α=0.05); means of different growth stages followed by the same letter were not significantly different.

4.2.3. Soil Penetration Resistance

No differences in soil penetration resistance were observed among the treatments across all soil depths at the Spring Hill location by the conclusion of the experiment in fall 2025 ($P > 0.05$) (Figure 15). This observation indicates that the applied treatments with legume species as cover crop or living mulches did not have a statistically significant impact on soil compaction or resistance within the tested soil profile.

Differences in soil penetration resistance were observed among the treatments in three different soil depths in Knoxville by the end of the experiment in fall 2025 (Figure 16). This observation indicates that the applied treatments have a statistically significant impact on soil compaction or resistance within certain layers of the soil profile.

At the Knoxville location, differences in soil penetration resistance were observed at the third ($P = 0.0230$), fourth ($P = 0.0104$), and fifth ($P = 0.0062$) soil depths

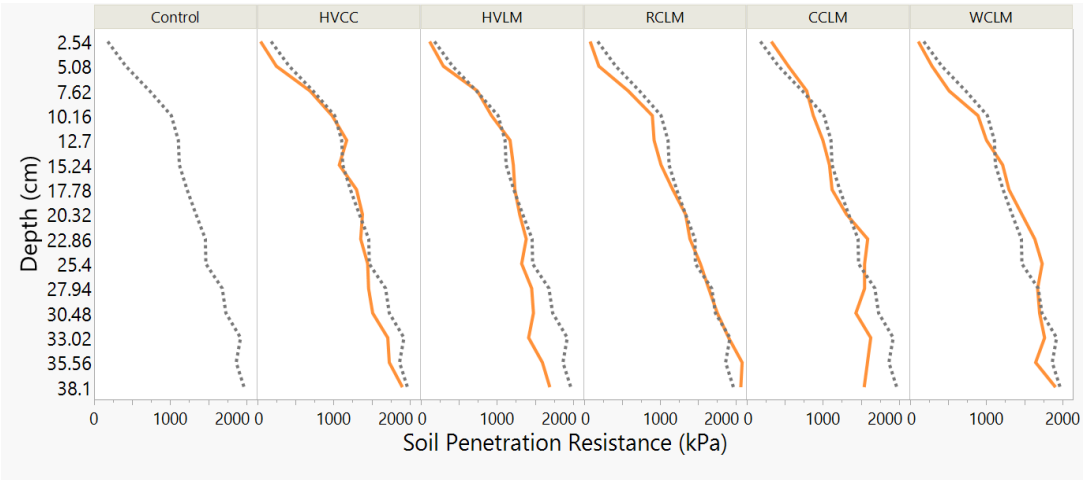


Figure 15 Soil penetration resistance response to treatments across the soil profile in the fall of 2025 in Spring Hill, TN. Different colored lines represent the control (grey) and the legume treatments (orange).

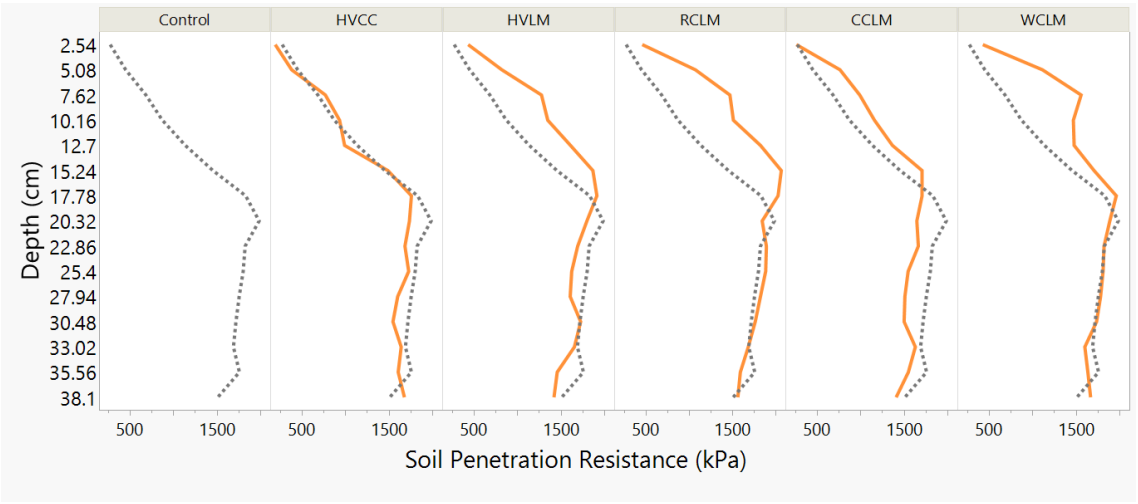


Figure 16 Soil penetration resistance response to treatments across the soil profile in the fall of 2025 in Knoxville, TN. Different colored lines represent the control (grey) and the legumes treatments (orange).

(Table 13). At the third layer, the control treatment presented the lowest soil resistance compared to the other treatments, while the WCLM, RCLM, and HVLM treatments demonstrated the highest soil resistance. Similar trends were observed at the fourth layer, where the control, HVCC, and CCLM treatments showed lower soil penetration resistance. This pattern persisted at the fifth layer, with the HVCC, control, and CCLM treatments continuing to display reduced soil penetration resistance

compared to the other treatments. Beyond the sixth layer of soil depth, no differences in penetration resistance were observed among the treatments. These observations indicate the inefficiency of the use of legume species, especially the RCLM and WCLM, as living mulches, to mitigate soil compaction problems in the soil profile.

Table 13 Soil penetration resistance (kPa) in the layers that presented differences among the treatments in 2025 in Knoxville, TN

Treatment [a]	Depth (cm) [b]		
	7.62	**10.16**	**12.7**
Control	683.3 c	873.4 b	1,137.7 cd
HVCC	769.9 bc	933.7 b	991.2 d
HVLM	1,278.4 ab	1,350.3 a	1,611.7 ab
RCLM	1,473.8 a	1,514.0 a	1,827.2 a
CCLM	991.2 abc	1,160.7 ab	1,367.5 bcd
WCLM	1,560.0 a	1,470.9 a	1,479.5 abc
P value	0.0230	0.0104	0.0062

[a] Treatments: HVCC (hairy vetch cover crop), HVLM (hairy vetch living mulch), RCLM (red clover living mulch), CCLM (crimson clover living mulch), and WCLM (white clover living mulch)

[b] Data were submitted to an ANOVA test, and means were separated according to Student's t-test (α=0.05); means in a column followed by the same letter were not significantly different.

4.3. Biological Indicators

4.3.1. Soil Respiration

In 2024 at the Spring Hill location, no differences in soil respiration were observed among the treatments during the V6 growth stage, indicating that the treatments applied at this stage did not have a measurable impact on soil microbial activity (Table 14). However, at the R1 growth stage, differences were identified among the treatments. The WCLM treatment exhibited higher soil respiration rates than both the other living mulch treatments and the control. This suggests that the WCLM treatment may have enhanced conditions for microbial activity, resulting in increased respiration rates. Additionally, when comparing soil respiration rates across the growth stages in 2024 (V6 and R1), no differences were detected.

Table 14 Soil respiration rate (CO_2, mg kg^{-1}) through different corn growth stages in 2024 and 2025 in Spring Hill, TN

	2024		2025	
Treatment [a]	**V6 [ns b]**	**R1 [b]**	**V6 [ns b]**	**R1 [ns b]**
Control	100.05	102.28 bc	123.10	55.27
HVCC	72.80	139.23 ab	125.95	59.95
HVLM	84.86	80.75 c	108.42	74.12
RCLM	89.07	104.10 bc	109.60	108.15
CCLM	100.97	118.35 bc	98.10	79.07
WCLM	128.43	170.63 a	105.10	67.00
P value	0.8069	0.0032	0.7385	0.1546

[a] Treatments: HVCC (hairy vetch cover crop), HVLM (hairy vetch living mulch), RCLM (red clover living mulch), CCLM (crimson clover living mulch), and WCLM (white clover living mulch)

[b] Means were submitted to an ANOVA test, and separated according to Student's t-test (α=0.05); means in a column within a year followed by the same letter or ns were not significantly different.

Unlike the previous year, in 2025 at Spring Hill, no differences in soil respiration were observed among the treatments at either the V6 or R1 corn growth stage (Table 14). However, when comparing soil respiration rates between the growth stages, differences were identified. Higher mean soil respiration rates were observed at the V6 stage than at the R1 stage. This observation suggests that soil microbial activity was more pronounced during the vegetative phase of both corn and legume species, potentially due to increased root exudation, nutrient cycling, and favorable environmental conditions that promote microbial processes. In contrast, the lower soil respiration rates at the R1 stage may reflect a change in plant resource allocation toward reproductive development, and the end of the influence of the legume species, resulting in reduced organic matter inputs and subsequently lowering microbial activity.

When comparing soil respiration data from Spring Hill in 2025, differences were observed between the two sampling times, V6 and R1 corn growth stages ($P < 0.0001$) (Figure 17). Specifically, the soil respiration rate recorded in V6 was higher than the

rate in R1. This suggests that the effects of the organic intercropping management on soil respiration were more pronounced in the early corn development stage than the reproductive stage.

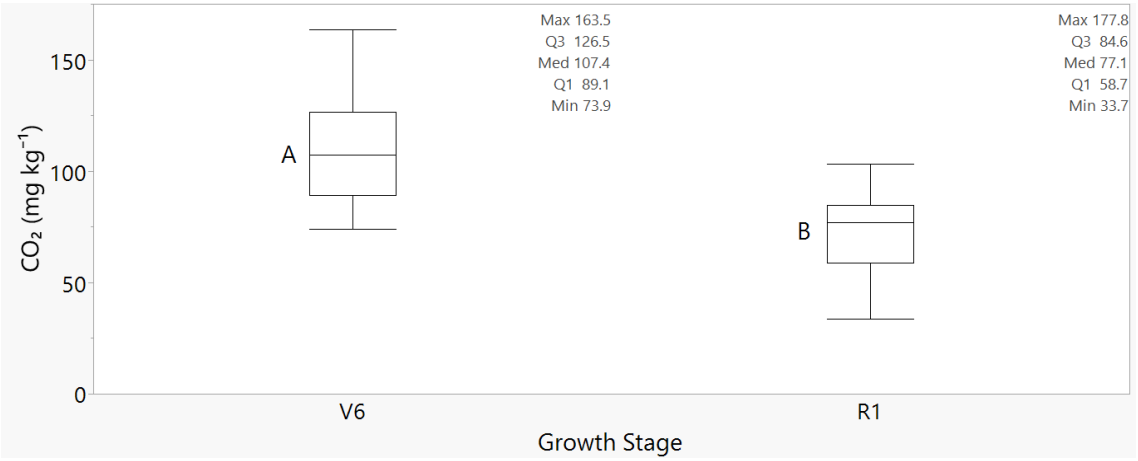


Figure 17 Comparison of soil respiration rates (mg kg^{-1}) across the corn growth stages in 2025 in Spring Hill, TN; means between growth stages were separated according to Student's t-test (α=0.05); means of different growth stages followed by the same letter were not significantly different.

When comparing soil respiration data from Spring Hill in 2024 and 2025, differences were observed between the two years (P = 0.048) (Figure 18). Specifically, the average soil respiration rate recorded in 2024 was higher than the rate in 2025. This suggests that the effects of the organic intercropping management on soil respiration were more pronounced in the first year of the system implementation.

At the Knoxville location in 2024, no differences in soil respiration were observed among the treatments during the V6 corn growth stage (Table 15). However, at the R1 corn growth stage, differences were detected among the treatments. Specifically, WCLM and RCLM exhibited higher soil respiration rates than the hairy vetch (CC and LM) and control treatments, suggesting that these legume species may have provided more favorable conditions for microbial activity during the reproductive phase of corn development. When comparing soil respiration rates across

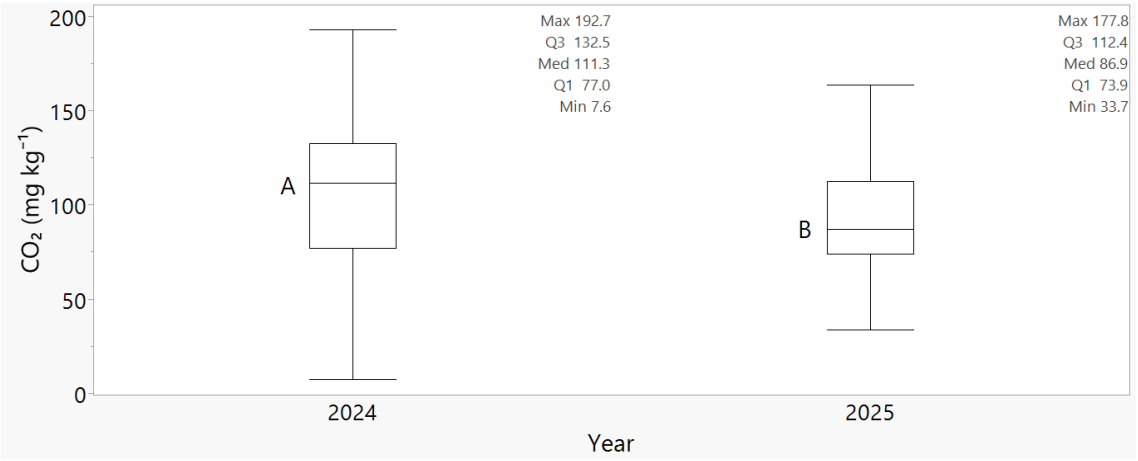


Figure 18 Comparison of soil respiration rates (mg kg^{-1}) across 2024 and 2025 in Spring Hill, TN; means between years were separated according to Student's t-test (α=0.05); means of different years followed by the same letter were not significantly different.

growth stages (V6 and R1) in 2024, no differences were detected, indicating that microbial activity remained relatively stable between the vegetative and reproductive stages. However, when analyzing soil respiration rates among the treatments using data from both growth stages (V6 and R1) from 2025, differences were found (Table 15). Among all treatments, the RCLM treatment presented the highest soil respiration rate, differing significantly from both the control and the hairy vetch (LM and CC) treatments. This finding highlights the greater potential of RCLM to enhance soil biological activity than the other legume species used in this experiment.

Table 15 Soil respiration rate (CO_2, mg kg^{-1}) through different corn growth stages in 2024 and 2025 in Knoxville, TN

	2024		2025	
Treatment [a]	**V6 [ns b]**	**R1 [b]**	**V6 [ns b]**	**R1 [ns b]**
Control	60.10	43.87 b	57.60	70.50
HVCC	36.55	44.95 b	43.50	62.37
HVLM	58.75	35.47 b	59.55	110.62
RCLM	61.97	77.12 a	48.52	63.67
CCLM	56.07	56.85 ab	49.95	81.32
WCLM	56.50	72.90 a	64.17	81.20
P value	0.1681	0.0051	0.6318	0.5848

[a] Treatments: HVCC (hairy vetch cover crop), HVLM (hairy vetch living mulch), RCLM (red clover living mulch), CCLM (crimson clover living mulch), and WCLM (white clover living mulch)
[b] Means were submitted to an ANOVA test, and separated according to Student's t-test (α=0.05); means in a column within a year followed by the same letter or ns were not significantly different.

At the Knoxville location in 2025, no differences in soil respiration rates were observed among the treatments during either the V6 or R1 growth stage, or when analyzing data combined across the two stages (Figure 19). This indicates that the treatments applied during this period did not result in measurable differential variations in microbial activity among the treatments. However, when comparing soil respiration rates across growth stages, differences were identified (*P* = 0.0086). Higher soil respiration rates were observed at the R1 stage than at the V6 stage, which is the opposite of what was observed in Spring Hill in 2025.

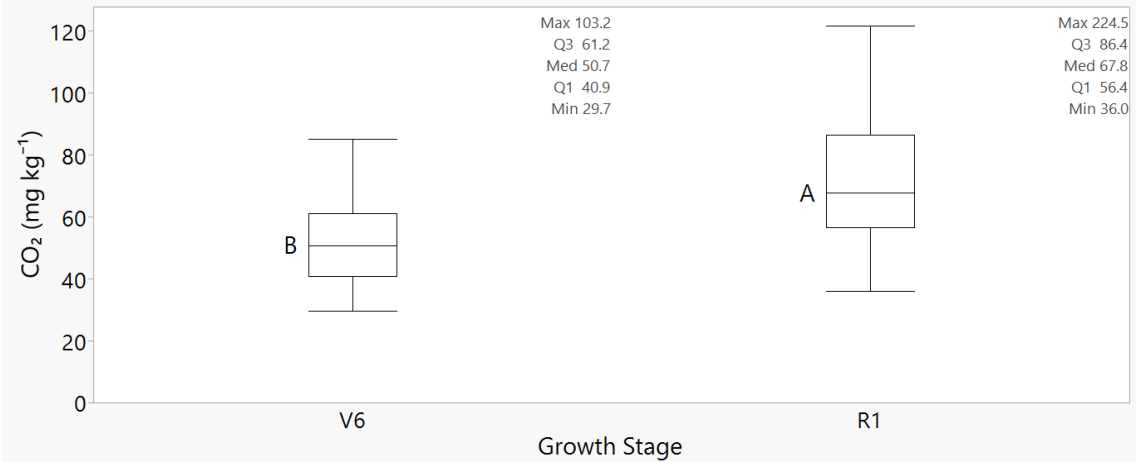


Figure 19 Comparison of soil respiration rates (mg kg^{-1}) across the corn growth stages in 2025 in Knoxville, TN; means between growth stages were separated according to Student's t-test (α=0.05); means of different growth stages followed by the same letter were not significantly different.

When comparing soil respiration data from Knoxville in 2024 and 2025, notable differences were observed between the two years (*P* = 0.0448) (Figure 20). Soil respiration rates in 2025 was higher than the rates recorded in 2024. This increase suggests that environmental conditions and crop performances (corn and legumes)

may have been more beneficial to promoting microbial activity over the short-term period of one year to another, emphasizing the importance of monitoring year-to-year variations to better understand how management practices interact and influence soil biological activity.

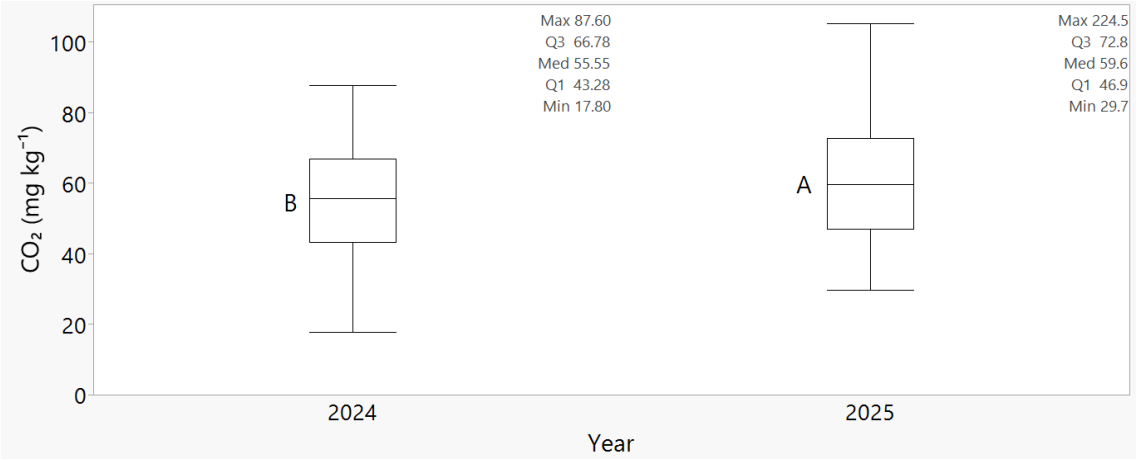


Figure 20 Comparison of soil respiration rates (mg kg^{-1}) across 2024 and 2025 in Knoxville, TN; means between years were separated according to Student's t-test (α=0.05); means of different years followed by the same letter were not significantly different.

4.3.2. Permanganate-oxidizable Carbon (POXC)

At the Spring Hill site in 2024, no differences in POXC levels were observed among the treatments during either of the growth stages (V6 and R1) (Table 16). This indicates that the applied management practices did not have a measurable differential impact on POXC levels at these specific stages of corn development. Similarly, in 2025, the same pattern persisted, without differences in POXC levels being detected among the treatments during either of the corn growth stages (Table 16).

When comparing POXC levels between 2024 and 2025 at the Spring Hill site, notable differences in mean POXC values were observed ($P < 0.0001$) (Figure 21). The treatments in 2025 exhibited higher mean POXC levels than those recorded in 2024. The increase in POXC levels observed in 2025 suggests that the legume intercropping

Table 16 POXC rate (mg kg^{-1}) through different corn growth stages in 2024 and 2025 in Spring Hill, TN

	2024		2025	
Treatment [a]	**V6 [ns b]**	**R1 [ns b]**	**V6 [ns b]**	**R1 [ns b]**
Control	489.00	497.00	563.00	550.00
HVCC	535.00	541.00	596.00	552.00
HVLM	490.00	536.00	604.00	540.00
RCLM	521.00	507.00	577.00	563.00
CCLM	536.00	506.00	578.00	576.00
WCLM	540.00	540.00	640.00	593.00
P value	0.7427	0.7246	0.2365	0.8703

[a] Treatments: HVCC (hairy vetch cover crop), HVLM (hairy vetch living mulch), RCLM (red clover living mulch), CCLM (crimson clover living mulch), and WCLM (white clover living mulch)

[b] Means in a column within a year followed by ns were not significantly different.

system contributed to improving soil health by promoting organic matter decomposition and carbon stabilization within the soil matrix, highlighting the potential benefit of integrating legume species into organic corn systems in enhancing soil carbon dynamics over time. Furthermore, the consistent increase in POXC level in 2025 over 2024 indicates that the benefits of this organic management may accumulate over time, reinforcing its value as a long-term tool for improving the soil carbon dynamics.

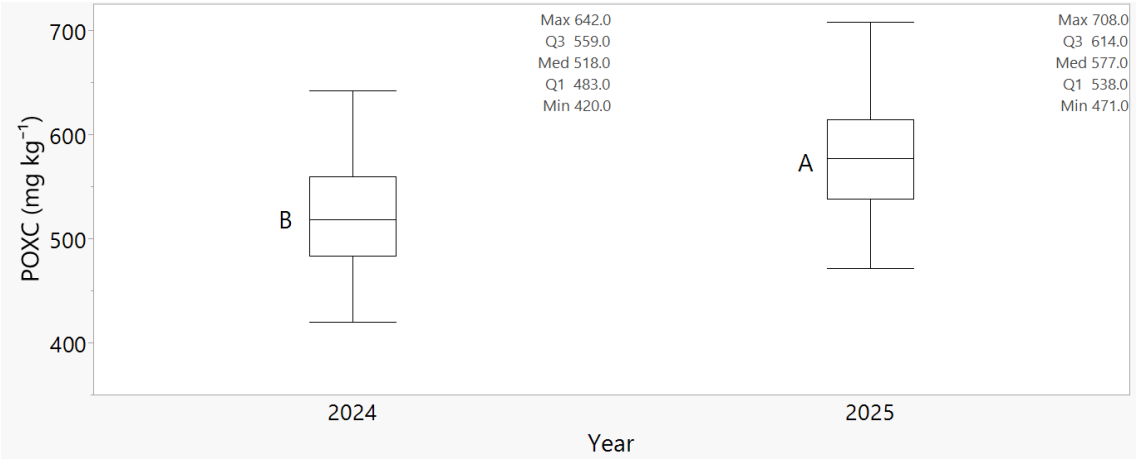


Figure 21 Comparison of POXC rates (mg kg^{-1}) across 2024 and 2025 in Spring Hill, TN; means between years were separated according to Student's t-test (α=0.05); means of different years followed by the same letter were not significantly different.

No differences in POXC levels were observed among the treatments in Knoxville during 2024 across both growth stages, V6 and R1 (Table 17). However, notable differences in average POXC levels were identified between the two-corn growth stages, with higher POXC levels recorded during the V6 stage than at the R1 stage. The higher POXC levels during the V6 stage may indicate increased biological activity and organic matter turnover early in the growing season, especially because the effect of the legume species is still alive and active. A similar pattern was observed in Knoxville in 2025, where no differences in POXC levels were detected among the treatments at either growth stage sampling, V6 or R1 (Table 17). This consistency in POXC levels among treatments suggests that the management practices applied during 2025 did not have a measurable differential impact on POXC levels. Unlike the observations from 2024, no differences in average POXC levels were noted between the V6 and R1 growth stages in 2025.

Table 17 POXC rate (mg kg^{-1}) through different corn growth stages in 2024 and 2025 in Knoxville, TN

	2024		2025	
Treatment [a]	**V6 [ns b]**	**R1 [ns b]**	**V6 [ns b]**	**R1 [ns b]**
Control	368.50	329.50	474.50	431.25
HVCC	380.00	341.00	458.75	424.75
HVLM	409.50	345.00	427.25	458.00
RCLM	352.75	375.00	465.00	404.50
CCLM	349.00	350.25	410.75	418.75
WCLM	399.25	311.74	444.75	443.00
P value	0.5779	0.4112	0.7023	0.9538

[a] Treatments: HVCC (hairy vetch cover crop), HVLM (hairy vetch living mulch), RCLM (red clover living mulch), CCLM (crimson clover living mulch), and WCLM (white clover living mulch)

[b] Means in a column within a year, followed by ns were not significantly different.

When comparing the average POXC levels between V6 and R1 corn growth stages in 2024 in Knoxville, notable differences were observed, with higher POXC levels recorded in V6 than in R1 (P = 0.0171) (Figure 22). This evidences the effectiveness of

organic corn management within a legume intercropping system in enhancing POXC levels in the early corn development stages. Despite this result in 2024, the same effect was not observed in 2025.

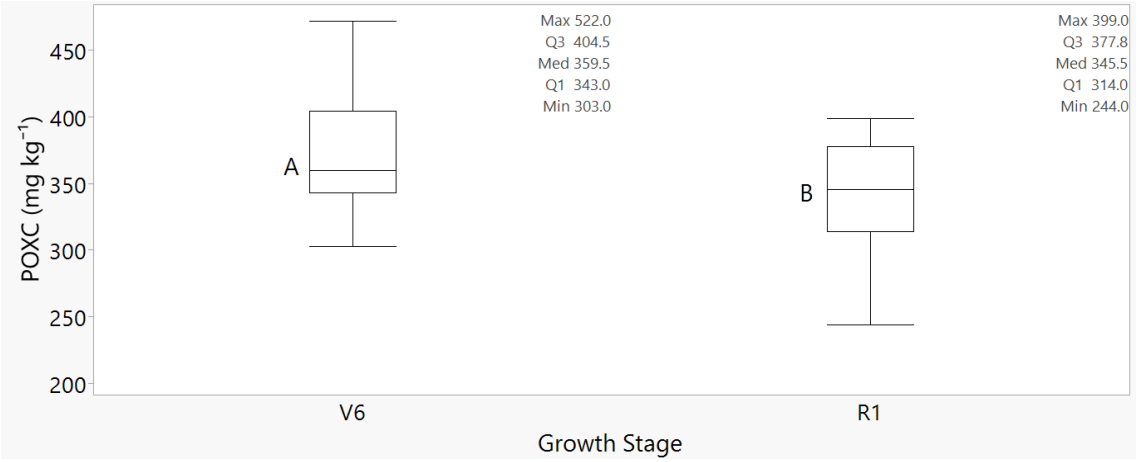


Figure 22 Comparison of POXC rates (mg kg^{-1}) across the corn growth stages in 2024 in Knoxville, TN; means between growth stages were separated according to Student's t-test (α=0.05); means of different growth stages followed by the same letter were not significantly different.

When comparing the average POXC levels between 2024 and 2025 in Knoxville, notable differences were observed, with higher POXC levels recorded in 2025 ($P < 0.0001$) (Figure 23). This highlights the effectiveness of organic corn management within a legume intercropping system in enhancing POXC levels over a relatively short period. The overall increase in POXC levels observed in 2025 over 2024 exhibited the potential cumulative benefits of integrating legumes into organic corn cropping systems in soil carbon dynamics. The same pattern was observed at the Spring Hill site, with the average POXC levels being higher in 2025 than in 2024.

4.3.3. Soil Organic Carbon (SOC) Stock

No differences in SOC stock were observed among the treatments during both 2024 and 2025 years (summer or fall) at the Spring Hill location (Table 18). This finding indicates that despite the treatments implemented, the SOC stock remained consistent across all treatments at all sampling times. The lack of differences highlights the

challenges of achieving changes in SOC through short-term interventions, particularly under organic intercropping systems.

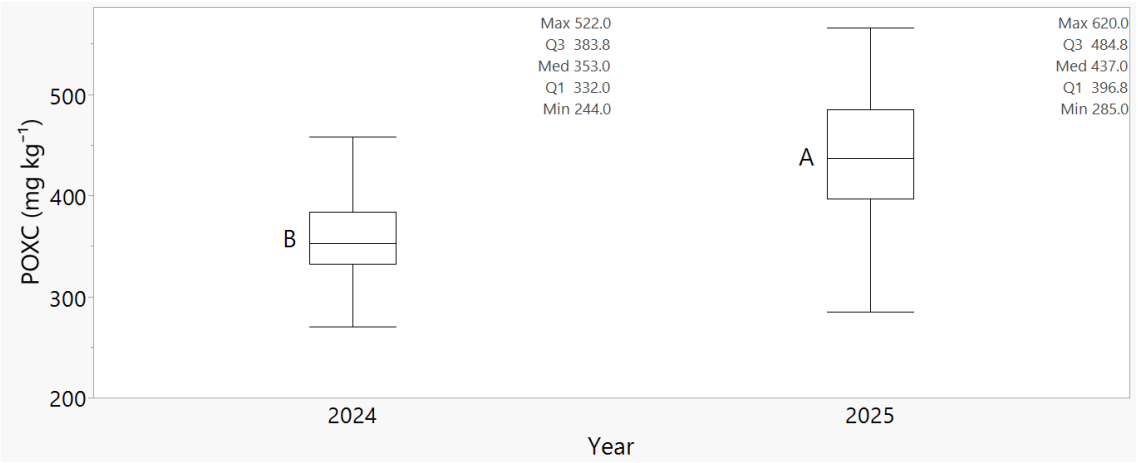


Figure 23 Comparison of POXC rates (mg kg^{-1}) across 2024 and 2025 in Knoxville, TN; means between years were separated according to Student's t-test (α=0.05); means of different years followed by the same letter were not significantly different.

Table 18 SOC stock (Mg ha^{-1}) across 2024 and 2025 in Spring Hill, TN

Treatment [a]	2024 Summer [ns b]	2025 summer [ns b]	2025 Fall [ns b]
Control	30.17	29.21	22.39
HVCC	33.94	31.04	33.69
HVLM	29.39	29.96	34.38
RCLM	31.17	30.09	27.99
CCLM	28.97	29.63	37.69
WCLM	34.51	31.73	30.14
P value	0.3739	0.3739	0.7336

[a] Treatments: HVCC (hairy vetch cover crop), HVLM (hairy vetch living mulch), RCLM (red clover living mulch), CCLM (crimson clover living mulch), and WCLM (white clover living mulch)

[b] Means within a column followed by ns were not significantly different.

When comparing the SOC stock treatment averages between 2024 and 2025 (summer and fall), no differences were detected (*P* = 0.9306), reinforcing the conclusion that the applied treatments did not have a measurable differential impact on SOC levels over the two-year period (Figure 24).

No differences in SOC stock were observed among the treatments during 2024 or 2025 at the Knoxville location (Table 19). During the summer of 2025, SOC stock measurements could not be calculated due to the soil sampling issues,

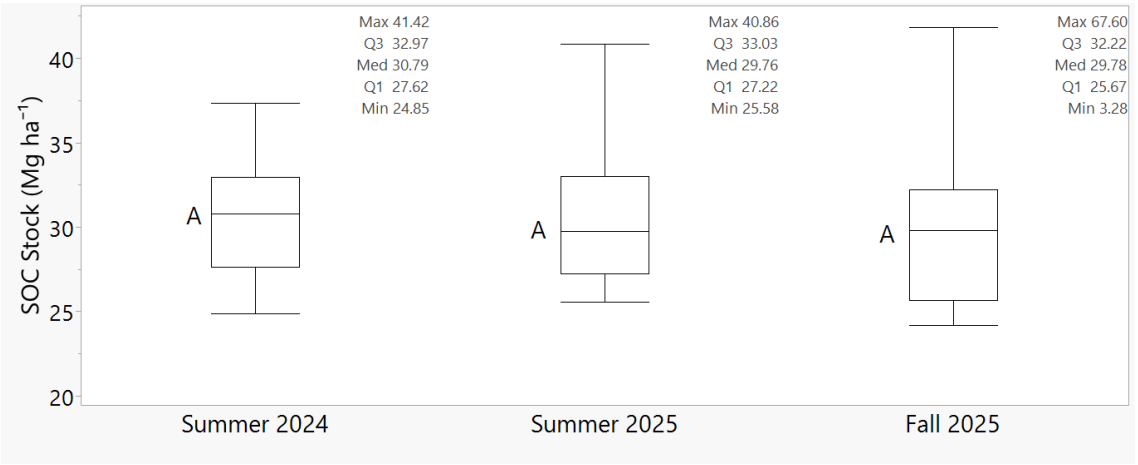


Figure 24 Comparison of SOC stock values (Mg ha^{-1}) across 2024 and 2025 sampling times in Spring Hill, TN; means among different times followed by the same letter were not significantly different.

affecting the bulk density values. This finding indicates that despite the treatments implemented, the SOC stock remained consistent across all treatments at both sampling times. Similar to the observations in Spring Hill, the lack of differences highlights the challenges of achieving changes in SOC through short-term interventions.

Table 19 SOC stock (Mg ha^{-1}) across 2024 and 2025 in Knoxville, TN

Treatment [a]	2024 Summer [ns b]	2025 Fall [ns b]
Control	27.41	25.35
HVCC	26.29	25.40
HVLM	27.40	24.64
RCLM	26.09	31.30
CCLM	26.28	26.49
WCLM	26.20	26.18
P value	0.9982	0.3022

[a] Treatments: HVCC (hairy vetch cover crop), HVLM (hairy vetch living mulch), RCLM (red clover living mulch), CCLM (crimson clover living mulch), and WCLM (white clover living mulch)

[b] Means within a column followed by ns were not significantly different.

Similar to the findings in Spring Hill, when comparing the SOC stock treatment averages between 2024 and 2025, no differences were detected at the Knoxville location, reinforcing the conclusion that the applied treatments did not have a measurable differential impact on SOC levels over the two-year period (P = 0.97)

(Figure 25). These consistent findings in SOC stock suggest that short-term management practices under organic intercropping systems may not be sufficient to drive significant changes in carbon sequestration or retention within the soil. It also emphasizes the importance of considering long-term approaches to enhance SOC storage, emphasizing the need for further research to explore how this specific organic intercropping management interacts with soil dynamics to optimize carbon sequestration over time.

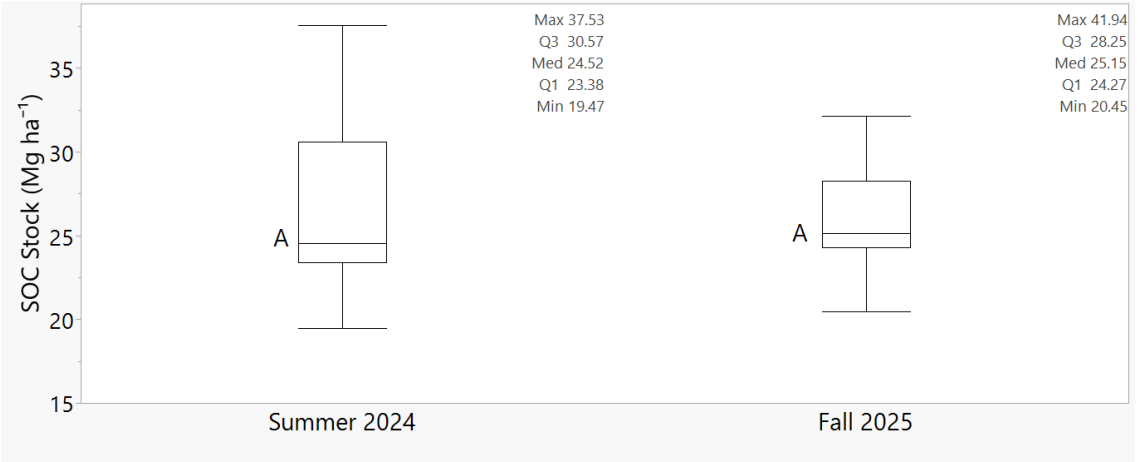


Figure 25 Comparison of SOC stock values (Mg ha^{-1}) across 2024 and 2025 sampling times in Knoxville, TN; means of different times followed by the same letter were not significantly different.

4.3.4. Earthworm Population

No differences in earthworm populations were observed among the treatments at the Spring Hill site in 2024, indicating that the applied management practices did not significantly influence earthworm presence during that year (Table 20). However, in 2025, at the same site, the WCLM treatment presented a higher earthworm population than the other treatments. When comparing data from Spring Hill across the two years, no differences in earthworm populations were detected among treatments overall.

Table 20 Earthworm population dynamics across the 2024 and 2025 growing seasons in Spring Hill, TN

Treatment [a]	2024 [ns b]	2025 [b]	Average [ns b]
Control	11.00	8.75 b	9.87
HVCC	5.75	9.25 b	7.50
HVLM	14.00	4.00 b	9.00
RCLM	8.25	12.00 b	10.12
CCLM	21.67	3.25 b	11.11
WCLM	12.25	35.50 a	23.87
P value	0.4799	0.0004	0.0604

[a] Treatments: HVCC (hairy vetch cover crop), HVLM (hairy vetch living mulch), RCLM (red clover living mulch), CCLM (crimson clover living mulch), and WCLM (white clover living mulch)

[b] Means were submitted to an ANOVA test, and separated according to Student's t-test (α=0.05); means in a column followed by the same letter or ns were not significantly different.

Similar to the findings in Spring Hill, no differences in earthworm populations were observed among the treatments in Knoxville in 2024, with no differences being detected in 2025 (Table 21). Additionally, when comparing data across the two years, no differences were detected among the treatments.

Table 21 Earthworm population dynamics across the 2024 and 2025 growing seasons in Knoxville, TN

Treatment [a]	2024 [ns b]	2025 [ns b]	Average [ns b]
Control	0.50	1.00	0.75
HVCC	0.25	0.00	0.12
HVLM	1.00	3.50	2.25
RCLM	0.25	2.25	1.25
CCLM	0.00	0.00	0.00
WCLM	0.25	0.75	0.50
P value	0.7754	0.1097	0.0648

[a] Treatments: HVCC (hairy vetch cover crop), HVLM (hairy vetch living mulch), RCLM (red clover living mulch), CCLM (crimson clover living mulch), and WCLM (white clover living mulch)

[b] Means in a column followed by ns were not significantly different.

4.4 Weather Conditions

In September 2023, when legumes were established in Spring Hill, precipitation was 45% below the 30-year average, while air temperature was 5% higher than the normal (Table 22). In October 2024, when RCLM and WCLM treatments were

replanted, a severe drought occurred, with only 0.2 mm of rainfall recorded—representing a nearly 99% reduction relative to the 30-year average. Overall, mean temperatures during the 2024 and 2025 growing seasons were higher than the 30-year averages (Table 22).

Table 22 Precipitation (mm) and air temperature (°C) from the 2023 to 2025 growing seasons, and 30-year average in Spring Hill, TN

Month	30-year avg.	2023	2024	2025
Precipitation (mm)				
January	116.3	-	127.5	87.1
February	129.6	-	126.2	104.3
March	136.6	-	155.9	120.6
April	123.6	-	100.8	131.8
May	129.6	-	212	144.2
June	113.2	-	65.5	182.6
July	112.5	-	195.8	50.5
August	92.8	-	18.7	18.7
September	112.7	60.9	168.9	192.7
October	104.7	22.8	0.2	-
November	101.9	76.2	86.1	-
December	147.4	73.6	129	-
Air temperature (°C)				
January	3.2	-	0.8	-.02
February	5.1	-	8.2	5.2
March	7.9	-	11.1	11.8
April	14.6	-	16.1	16.7
May	19.4	-	21.2	19.7
June	23.6	-	24.7	24.8
July	25.6	-	26.2	27.2
August	24.9	-	25.3	24.6
September	21.6	22.7	22.3	22.1
October	14	15.2	16.6	-
November	8.1	12.7	12.4	-
December	4.8	7.2	5.7	-

In September 2023, legumes were initially established in Knoxville, however, due to extremely low precipitation (90% below the 30-year average), they had to be replanted at the end of October (Table 23). In October 2024, when the RCLM and WCLM treatments were replanted, another severe drought occurred, with only 5.9

mm of rainfall recorded – representing a nearly 93% reduction relative to the 30-year average.

Table 23 Precipitation (mm) and air temperature (°C) from the 2023 to 2025 growing seasons, and 30-year average in Knoxville, TN

Month	30-year avg.	2023	2024	2025
Precipitation (mm)				
January	132	-	0.9	47.7
February	134.6	-	-	121.5
March	132	-	106.9	67.1
April	129.5	-	86.5	133.5
May	106.6	-	145.9	159.3
June	99	-	66.9	53.3
July	152.4	-	262.1	132.3
August	96.5	-	73.3	54.7
September	101.6	9.9	140.1	114.5
October	91.4	11.9	5.9	59.1
November	101.6	51.8	53	-
December	147.3	126.7	117.4	-
Air temperature (°C)				
January	3.8	-	-9.2	0
February	6.1	-	11.5	6.8
March	10.5	-	11.2	11.5
April	15.5	-	15.6	15.4
May	20	-	20	18.8
June	24.4	-	23.8	24
July	26.1	-	25.5	26.5
August	25.5	-	23.9	23.5
September	22.2	23.2	21.6	19
October	16.1	16.5	15.2	-
November	9.4	11.2	12.6	-
December	5.5	7.2	5.3	-

Overall, mean temperatures during the 2024 and 2025 growing seasons remained relatively similar to the 30-year average (Table 23).

4.5. Corn Yield

No differences in corn yield were observed among the treatments during the 2024 and 2025 growing seasons at the Spring Hill location (Table 24). This result indicates that the applied treatments did not significantly influence corn yield across the evaluated growing seasons.

Table 24 Corn yield (Mg ha^{-1}) across the 2024 and 2025 growing seasons in Spring Hill, TN

Year	Control	HVCC [a]	HVLM [a]	RCLM [a]	CCLM [a]	WCLM [a]	P value
2024 [ns b]	1.28	0.24	1.20	0.37	0.72	0.93	0.6867
2025 [ns b]	3.11	2.76	4.20	4.74	3.30	3.96	0.8223

[a] Treatments: HVCC (hairy vetch cover crop), HVLM (hairy vetch living mulch), RCLM (red clover living mulch), CCLM (crimson clover living mulch), and WCLM (white clover living mulch)

[b] Means were submitted to an ANOVA test, and separated according to Student's t-test (α=0.05); means in a row followed by ns were not significantly different.

However, when analyzing the average corn yield at Spring Hill across 2024 and 2025, differences were observed (Figure 26). The average yield in 2025 was 3.68 Mg ha^{-1}, nearly five times greater than the 0.74 Mg ha^{-1} recorded in 2024 (P <0 0.001). This pattern suggests that the benefits of organic intercropping management may be cumulative, contributing to improved yields over time. Nevertheless, environmental factors - particularly precipitation and temperature – likely played a major role in driving the year-to-year differences in corn yield (Table 22). Although total precipitation during the growing seasons (April to September) of 2024 (761.7 mm) and 2025 (720.5 mm) exceeded the 30-year average (684.4 mm), drought conditions in June and August of 2024 adversely affected corn yield (Tables 22 and 24).

In Knoxville, differences in corn yield were observed during the 2024 growing season, with the control treatment exhibiting the highest yield (2.29 Mg ha^{-1}) and outperforming the hairy vetch (HVCC and HVLM), RCLM, and CCLM treatments (Table 25). This result suggests that intercropping systems may be less productive under irregular precipitation or drought conditions, such as those experienced in Knoxville in 2024 (Table 23), likely due to increased competition for water among the cash crop and legume species. In 2025, no yield data were recorded because severe weed pressure overwhelmed both the legumes and the corn, making harvest and data collection impossible.

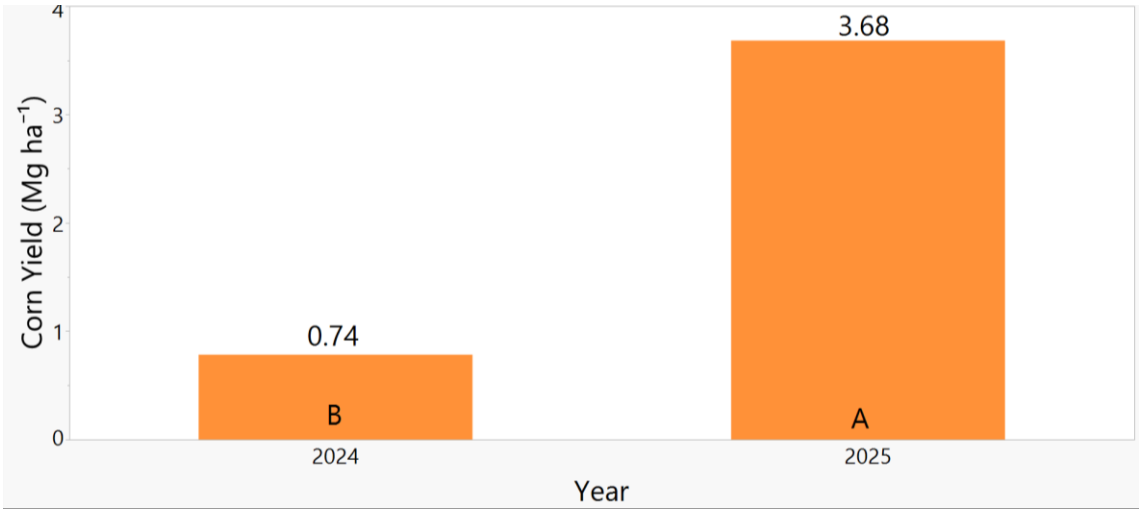


Figure 26 Average corn yield (Mg ha^{-1}) across the 2024 and 2025 growing seasons in Spring Hill, TN; means between years were separated according to Student's t-test (α=0.05); means of different years followed by the same letter were not significantly different.

Table 25 Corn yield (Mg ha^{-1}) across the 2024 growing season in Knoxville, TN

Year	Control	HVCC [a]	HVLM [a]	RCLM [a]	CCLM [a]	WCLM [a]	P value
2024 [b]	2.29 a	0.74 c	1.01 bc	1.22 bc	0.95 bc	1.73 ab	0.025

[a] Treatments: HVCC (hairy vetch cover crop), HVLM (hairy vetch living mulch), RCLM (red clover living mulch), CCLM (crimson clover living mulch), and WCLM (white clover living mulch)

[b] Means were submitted to an ANOVA test, and separated according to Student's t-test (α=0.05); means in a row followed by the same letter were not significantly different.

Chapter Five: Discussion

5.1. Chemical Indicators

5.1.1 Chemical Characterization

After two years of implementation, the organic intercropping with legumes as cover crops or living mulches had minimal effects on soil chemical properties at either Spring Hill or Knoxville location. In Spring Hill, soil pH, cation exchange capacity (CEC), and macro and micronutrient concentrations remained stable across the treatments, suggesting that a two-year implementation period did not substantially impact soil fertility compared to the control (Table 1). These findings are consistent with previous studies (Calderon et al., 2016; Culman et al., 2012), which suggest that short-term implementation of legume cover crops or living mulches may not immediately alter soil chemical properties. The uniformity in nutrient levels observed could be attributed to the soil's inherent fertility, existing organic matter content, and nutrient buffering capacity. In summary, although short-term changes in soil chemical properties were negligible, these results underscore the potential for long-term improvements in soil fertility and sustainability through the continued use of cover crops and living mulches in organic intercropping systems.

Similarly, in Knoxville, most soil chemical properties were unaffected, although slight variations in pH and copper availability were observed among the treatments (Table 2). Soil pH ranged from 5.57 to 6.12, with HVCC (6.12) treatment presenting the highest value, while HVLM (5.57) and WCLM (5.57) treatments exhibited slightly acidic conditions. The copper concentrations differed across the treatments, with RCLM (1.20 mg kg^{-1}) showing the lowest concentration and CCLM (1.67 mg kg^{-1}) the highest. This

variation suggests that root exudates of legume species and mulch management can influence micronutrient dynamics locally. These findings align with previous research (Calderon et al., 2016; Culman et al., 2012), which reported minimal short-term changes in soil chemical properties under cover cropping or living mulch systems. Overall, these results suggest that short-term adoption of legume-based intercropping systems does not substantially alter soil chemical properties, highlighting the importance of long-term management to achieve measurable improvements in soil fertility and nutrient dynamics.

5.1.2. Ammonium-N and Nitrate-N

At the Spring Hill site, both management and interannual variability influenced soil inorganic N dynamics, with treatment effects evident only during the V10 corn growth stage sampling in 2024. These results highlight the importance of considering temporal factors when assessing N availability in intercropping systems with cover crop and living mulch. Similar year-dependent responses have been reported in such systems, emphasizing the critical role of climatic conditions in N mineralization and availability (Drinkwater et al., 1998; Finney et al., 2016).

In Spring Hill during 2024, soil nitrate-N levels at the V10 corn growth stage significantly increased under the legume-based treatments, particularly those involving hairy vetch (Table 3). This increase aligns with the rapid decomposition and mineralization of crop residues with low C/N. Hairy vetch is recognized for its nitrogen fixation capacity and rapid residue turnover, which contribute to early-season soil nitrate-N availability (Ranells & Wagger, 1997; Teasdale et al., 2007). The enhanced nitrate-N availability during early vegetative growth suggests improved synchronization between N release and corn N demand, a key factor in optimizing N

use efficiency in corn production systems (Cassman et al., 2002). Despite differences in nitrate-N concentration at V10 in 2024, ammonium-N and total inorganic N were not significantly affected by the treatments. This indicates that the use of legumes primarily influenced the form of N rather than the total amount of extractable inorganic N**.** Similar patterns have been reported in other studies, where increased nitrification following residue mineralization changes soil N toward nitrate-dominated pool without necessarily increasing total inorganic N availability (Kaye & Hart, 1997; Quemada & Cabrera, 1995).

Post-harvest inorganic N concentrations in 2024 at the Spring Hill site showed no differences among the treatments. This convergence of soil N pools late in the season likely reflects cumulative crop N uptake, potential leaching losses, and microbial immobilization during residue decomposition (Dinnes et al., 2002; Varvel & Peterson, 1990). The absence of significant post-harvest differences indicates that increased early-season nitrate-N availability under legume treatments did not result in higher residual soil N, which is agronomically desirable. Similar findings have been reported in long-term studies, where legume treatments boosted early-season N supply without increasing post-harvest nitrate-N compared to unfertilized controls (Finney et al., 2017; Sainju et al., 2007). These findings reinforce the idea that well-managed legume cover crops can improve N availability to the cash crop while reducing environmental N losses.

In Spring Hill in 2025, no differences were observed in nitrate-N, ammonium-N, or total inorganic N, at either V10 or post-harvest stage (Table 4). The lack of treatment effects in 2025 contrasts with the early-season response noted in 2024 and highlights the influence of year-specific environmental conditions (soil moisture,

temperature, and residue decomposition rates) on N mineralization dynamics. Interannual variability in N responses is documented in biologically mediated N systems and is influenced by differences in precipitation, temperature, and soil moisture, which regulate microbial activity and residue decomposition (Booth et al., 2005; Dividson et al., 1990).

Across both years, in Spring Hill, inorganic N levels generally increased from V10 to post-harvest, particularly for nitrate-N, suggesting net mineralization throughout the growing season. Variability in N dynamics was observed in the living mulch treatments, which may result from competition between the mulch and the cash crop, as well as differences in residue placement and decomposition rate (Hartwig & Ammon, 2002). The lack of consistent treatment effects observed in 2025 highlights the importance of conducting long-term studies to evaluate the N benefits provided by the introduction of legumes in an intercropping system.

The results obtained from Spring Hill indicate that legumes (as cover crop or living mulch) can enhance early-season nitrate-N availability without increasing post-harvest soil inorganic N. This finding aligns with broader evidence suggesting that biologically fixed N from legumes can partially replace the use of synthetic N fertilizer while minimizing environmental risks (Drinkwater et al., 1998; Snapp et al., 2005). Long-term studies remain essential to understand the variability and agronomic benefits from introducing legumes in an organic intercropping system in soil N dynamics.

Analyzing data obtained in Knoxville during 2024, at the V10 corn growth stage, soil nitrate-N and ammonium-N concentrations were significantly affected by the treatments (Table 6). The HVLM treatment recorded the highest nitrate-N

concentration (9.67 mg kg^{-1}) and total inorganic N (16.17 mg kg^{-1}), which were significantly greater than the control and most other treatments. Similarly, both HVLM (6.50 mg kg^{-1}) and WCLM (6.65 mg kg^{-1}) exhibited higher ammonium-N concentrations than the control (3.75 mg kg^{-1}). These results indicate that living mulches may enhance early-season N availability. This finding aligns with previous studies, which support that legume residues with low C/N ratios can rapidly mineralize and increase soil inorganic N, particularly nitrate-N (Ranells & Wagger, 1997; Teasdale et al., 2007). At post-harvest, soil nitrate-N and ammonium-N concentrations did not show differences among the treatments. This convergence of soil N pools at the end of the season likely reflects cumulative crop N uptake and microbial immobilization during residue decomposition (Drinkwater et al., 1998; Sainju et al., 2007).

In 2025, at the V10 corn growth stage in Knoxville, nitrate-N concentrations were significantly influenced by the treatments, while ammonium-N and total inorganic N did not present differences (Table 7). The RCLM treatment had the highest early-season nitrate-N (4.35 mg kg^{-1}), followed closely by CCLM (4.20 mg kg^{-1}) and WCLM (3.75 mg kg^{-1}), while in contrast, the HVLM (2.05 mg kg^{-1}) exhibited the lowest value. This observation differs from the 2024 data, where HVLM was dominant in early-season nitrate-N, suggesting that interannual environmental conditions strongly impact the N contribution of different legume species (Dividson et al., 1990; Finney et al., 2016). This indicates that treatment effects in early 2025 primarily influenced nitrate-N rather than the total inorganic soil N pool, a phenomenon reported in other studies, where microbial nitrification rates and residue composition played a critical role in determining the form of mineral N available to the crop (Kaye & Hart, 1997; Ranells & Wagger, 1997). At post-harvest, nitrate-N and total inorganic N

concentrations were significantly affected by the treatments, with WCLM exhibiting the highest total N (28.85 mg kg^{-1}) and nitrate-N (27.25 mg kg^{-1}), while the control and HVCC treatments recorded the lowest values. Ammonium-N concentrations did not present differences among the treatments. The higher post-harvest nitrate-N levels under WCLM (27.25 mg kg^{-1}), HVLM (23.05 mg kg^{-1}), and RCLM (22.92 mg kg^{-1}) suggest that these living mulch species can provide residual soil N for subsequent crops, aligning with prior observations that legume residues with moderate C/N ratios can provide a sustained N supply while minimizing the risk of excessive immobilization (Drinkwater et al., 1998; Teasdale et al., 2007).

The difference in early and late-season N dynamics between 2024 and 2025 in Knoxville illustrates the significant role of year-specific environmental factors, such as soil moisture and temperature, in N mineralization and nitrification processes in legume-based systems (Booth et al., 2005). These results demonstrate the potential for legume living mulches to provide both early-season and residual N in organic intercropping corn systems. The presence of significant post-harvest nitrate-N under WCLM, CCLM, and RCLM suggests that N contribution from these treatments could partially replace the use of synthetic N fertilizers. However, annual variability highlights the importance of site and year-specific management (Hartwig & Ammon, 2002; Snapp et al., 2005). The absence of excessive ammonium-N indicates that these systems are unlikely to contribute to environmental N losses through volatilization, while still providing some residual N pool for subsequent crops.

5.1.3. Inorganic N Forms Across Soil Profile

In Spring Hill, nitrate-N and ammonium-N concentrations declined from 2024 to 2025 across the 0–15 cm and 15–30 cm soil layers, resulting in a reduction in total

available N (Table 5). This pattern suggests not only lower inorganic N availability in 2025, but also a potential shift in the balance between N mineralization, plant N uptake, and N losses. Reduced nitrate-N concentrations across these layers indicate either decreased net mineralization or increased synchronization between N release and crop N demand, limiting residual N in the soil profile. Additionally, lower nitrate-N at depth suggests reduced leaching, which may be associated with drier soil conditions or more efficient plant uptake in 2025. These temporal and depth-related trends are consistent with findings by Podlasek et al. (2021), who reported strong environmental control over N dynamics. At 30–60 cm, nitrate-N and total N were significantly lower in 2025, while ammonium-N remained unchanged, reinforcing the contrasting mobility of these N forms. The stability of ammonium-N at this depth likely reflects its adsorption to cation exchange sites, which buffers it against temporal fluctuations, whereas nitrate-N remains highly sensitive to transport processes such as leaching (Geisseler et al. (2024). Overall, the consistent decline in nitrate-N across depths points to interannual variability in N cycling processes, likely driven by differences in soil moisture regimes, temperature-dependent microbial activity, and crop N uptake intensity.

In Knoxville, mineral N dynamics also exhibited a pronounced year effect (Table 8), but with clearer evidence of surface-driven processes influencing the entire profile. In the 0–15 cm layer, significant reductions in both nitrate-N and ammonium-N in 2025 led to a marked decline in total available N, suggesting a reduction in net N mineralization or a more rapid turnover of mineralized N through plant uptake and microbial immobilization. Because this layer is the primary zone of organic matter inputs and microbial activity, year-to-year differences in residue decomposition

rates—likely governed by moisture and temperature—would strongly influence N availability (Brady et al., 2008; Havlin et al., 2016). The decline observed in 2025 may indicate less favorable conditions for microbial mineralization or greater crop demand depleting available N pools. At 15–30 cm, nitrate-N and total N remained lower in 2025, while ammonium-N again showed no significant variation, highlighting the role of nitrate-N mobility in transmitting surface-level changes deeper into the soil profile. This pattern suggests that reduced nitrate-N production or increased uptake in surface layers limited its downward movement. Similarly, at 30–60 cm, lower nitrate-N concentrations in 2025 further support the interpretation of reduced leaching fluxes or diminished surplus N in the system (Havlin et al., 2016; Nieder et al., 2018).

Collectively, these results demonstrate that interannual variability in soil mineral N is primarily driven by nitrate-N dynamics, reflecting its sensitivity to environmental conditions and biological demand. Greater N availability in 2024 suggests that climatic conditions that year likely promoted higher mineralization rates or reduced plant uptake relative to N supply, resulting in residual nitrate-N throughout the soil profile. In contrast, the 2025 conditions appear to have favored tighter N cycling, with reduced accumulation and movement of nitrate-N. These findings emphasize that, in organic systems, year-specific interactions among climate, microbial activity, and crop demand play a dominant role in regulating both the magnitude and vertical distribution of available N, ultimately influencing N use efficiency and potential N losses.

5.2. Physical Indicators

5.2.1. Bulk Density

During the summer of 2024 in Spring Hill, treatments incorporating legumes demonstrated positive effects on soil density (Table 9). Specifically, reductions in soil density were observed in the CCLM (1.109 g cm^{-3}), HVLM (1.102 g cm^{-3}), and RCLM (1.099 g cm^{-3}) treatments compared to HVCC (1.211 g cm^{-3}) and the control (1.185 g cm^{-3}). These results suggest that the introduction of legumes, primarily as living mulch, can effectively reduce soil density in the surface layer (<15 cm) during the initial months of the growing season. However, no differences in soil density among the treatments were detected at subsequent sampling times in the summer and fall of 2025.

When analyzing the average soil density values across different sampling times, a significant decrease was observed from summer 2024 (1.130 g cm^{-3}) to summer 2025 (1.021 g cm^{-3}), suggesting a potential short-term beneficial effect in reducing soil density. However, when comparing the values obtained in summer 2025 (1.021 g cm^{-3}) with those from fall 2025 (1.122 g cm^{-3}), an increase in soil density was noted at the end of the growing season. This increase may be attributed to the life cycle of the legumes, which were alive, and actively producing biomass and maintaining an active root system during the summer sampling period. By fall, these species had completed their life cycles, ceasing biomass production and root activity, thereby limiting their contribution to soil structure improvement and leading to a rise in soil density.

In Knoxville, no differences in soil density were observed among the treatments at the two sampling times, summer 2024 and fall 2025 (Table 10). During the summer of 2025, sampling problems occurred that made the use of the results obtained impossible. When analyzing the average soil density values from summer 2024 (1.259 g cm^{-3}) to fall 2025 (1.054 g cm^{-3}), a decrease of approximately 16% was noted. This

result aligns with findings from Spring Hill, suggesting that the integration of legumes with organic management practices can effectively reduce soil density in the surface layer (0-15 cm) over time. In addition, these differences are detectable within the initial years of system implementation, highlighting the potential benefits of such practices for improving soil physical properties.

Koudahe et al. (2022) observed that long-term accumulation of soil carbon by cover crops has the potential to reduce soil compaction by reducing soil bulk density while enhancing soil aggregation and porosity. However, as the benefits of cover crops often take time to manifest, it is necessary to evaluate their long-term effects on soil health and their contribution to soil physical properties, among diverse cropping systems and climates. Hartwig and Ammon (2002) granted to cover crops the ability to enhance soil structure and water retention capacity by promoting the formation of aggregates through the binding of soil particles by organic matter. Additionally, the decomposition of plant residues by soil microorganisms produces substances that further bind these aggregates into larger structures. Improving soil permeability and aeration, fostering biomass production, and improving root growth conditions.

Karlen et al. (1994) found no differences in soil bulk density resulting from corn stover rates and residue management. However, it was observed that variations in soil bulk density across different depths (0-50 cm), emphasizing the need for depth-specific evaluations to better understand soil physical properties. Dozier et al. (2017) found similar results after one cycle of cropping and cover crops in bulk density values, observing narrow ranges that did not differ by tillage method (conventional tillage, chisel till and no-till), or rotation (cash crops and cover crops). Nonetheless, recorded bulk density increased with soil depth, consistent with the mulching effect of residue

accumulation or tillage incorporation of residue in the surface. These studies demonstrate the influence of residue maintenance on the soil surface to reduce soil density, especially in the surface layer of soil profile.

5.2.2. Soil Moisture

Legume-based treatments, whether used as cover crops or living mulches, did not affect soil moisture content relative to the control in Spring Hill during 2024 or 2025 (Table 11). Despite the absence of treatment-specific effects, soil moisture varied considerably across corn growth stages (V6, V10, and R1). The results from both years indicate that the organic intercropping system has a greater ability to retain soil moisture during the vegetative growth stages (V6 and V10) than the reproductive stage (R1). In 2024, average soil moisture values were significantly higher at V6 (324.1 g kg^{-1}) than at V10 (212.6 g kg^{-1}) and R1 (213.5 g kg^{-1}), suggesting enhanced water retention during early vegetative corn development. Similarly, in 2025, the highest average soil moisture was recorded at V10 (401.4 g kg^{-1}), followed by V6 (380.7 g kg^{-1}), while R1 exhibited substantially lower values (206.7 g kg^{-1}). These differences among growth stages are likely driven by the combined corn physiological demands of water and seasonal precipitation patterns throughout the growing seasons (Table 22).

In Knoxville during 2024, at the V6 corn growth stage, HVLM (394.5 g kg^{-1}), CCLM (382.3 g kg^{-1}), and WCLM (379.9 g kg^{-1}) demonstrated significantly higher soil moisture levels than the control (312.1 g kg^{-1}). This improvement underscores the potential of living mulches to enhance soil conditions during early stages of corn development. The observed benefits may be attributed to continuous inputs of root exudates and organic substrates provided by the living mulches, being more pronounced under permanent vegetative cover than in systems with bare soil or

terminated cover crops, contributing to better soil moisture retention. During the V10 growth stage, the trend of improved soil moisture levels persisted, with WCLM (421.8 g kg^{-1}) and HVLM (389.9 g kg^{-1}) treatments showing significantly higher values than the control (334.3 g kg^{-1}) and HVCC (323.4 g kg^{-1}) treatments. The reduced soil moisture observed in the control and HVCC may be attributed to the absence of living vegetation, which restricts continuous inputs of organic residues. In contrast, treatments that maintain perennial or undersown vegetative cover, such as living mulches, provide ongoing contributions to soil organic matter and residue accumulation, enhancing water retention and overall soil health. At the R1 corn growth stage, the same pattern was observed, with WCLM (325.4 g kg^{-1}) exhibiting the highest soil moisture level. In contrast, the HVCC (236.3 g kg^{-1}) and control (227.8 g kg^{-1}) treatments showed significantly lower values. Intermediate responses were observed in CCLM (294.8 g kg^{-1}), HVLM (277.3 g kg^{-1}), and RCLM (276.7 g kg^{-1}), highlighting a gradient of effectiveness among the different living mulch treatments. Overall, the findings obtained in Knoxville in 2024 highlight that the use of living mulches offers more consistent and sustained benefits throughout the corn-growing season compared to systems relying solely on terminated cover crops or bare soil. By maintaining active soil coverage and living root systems, the living mulches continuously supply carbon and organic residues, which protect the soil, enhance its structure, and contribute to improved soil moisture retention.

Average soil moisture over the treatments in Knoxville during the 2024 growing season varied significantly across corn growth stages (Figure 13). The highest soil moisture was recorded at V10 stage (370.8 g kg^{-1}), closely followed by the V6 stage (362.4 g kg^{-1}), with no difference between these two vegetative stages. In contrast, soil

moisture decreased sharply during the R1 stage (273.0 g kg^{-1}), which was significantly lower than the two vegetative stages. This decline in soil moisture during the reproductive stage likely reflects increased crop water demand coupled with seasonal climatic factors, such as elevated temperatures and reduced precipitation later in the growing season, and the fact that the effect of the legumes ceases after their life cycles are completed (Table 23).

When analyzing the soil moisture data collected in Knoxville during the 2025 growing season, soil moisture content varied significantly among the treatments and corn growth stages (Table 12). At the V6 corn growth stage, all living mulch treatments demonstrated higher soil moisture levels than the HVCC (392.7 g kg^{-1}) and control (385.9 g kg^{-1}) treatments. Among the living mulch treatments, HVLM (464.5 g kg^{-1}), CCLM (452.4 g kg^{-1}), WCLM (450.2 g kg^{-1}), and RCLM (435.6 g kg^{-1}) were statistically equal, highlighting their ability to enhance soil water retention during the early growing season. At the V10 stage, differences among the treatments became more pronounced, with HVLM (292.3 g kg^{-1}) maintaining the highest soil moisture level, followed by RCLM (277.5 g kg^{-1}) and WCLM (276.3 g kg^{-1}), without differences between them. In contrast, the control (251.1 g kg^{-1}) and HVCC (233.2 g kg^{-1}) treatments showed the lowest moisture values, indicating that living mulch systems are particularly effective at preserving soil moisture during the peak vegetative corn growth stage, when crop water demand is high. By the R1 growth stage, soil moisture levels declined across all treatments, however, living mulch treatments, especially those featuring WCLM (164.3 g kg^{-1}), HVLM (158.5 g kg^{-1}), and RCLM (153.7 g kg^{-1}), retained significantly greater soil moisture than CCLM (125.7 g kg^{-1}), the control (111.6 g kg^{-1}), and HVCC (87.0 g kg^{-1}). This sustained advantage suggests that living mulches

can mitigate soil moisture depletion later in the growing season, thereby supporting crop development during critical reproductive phases. Overall, these results demonstrate that living mulches consistently improved soil moisture retention throughout corn growth stages, with the extent of this improvement varying according to the legumes.

Average soil moisture over the treatments in Knoxville during the 2025 growing season varied significantly across corn growth stages (Figure 14). The highest soil moisture was recorded at the V6 stage (430.2 g kg^{-1}), followed by a substantial decline at V10 (264.7 g kg^{-1}) and a further reduction at the R1 stage (133.4 g kg^{-1}). This progressive decrease in soil moisture reflects the increasing water demand from the corn crop as it transitions through its growth stages, compounded by seasonal environmental factors, such as elevated temperatures and reduced precipitation later in the growing season (Table 23). Overall, these results demonstrate a clear temporal pattern of soil moisture depletion as corn development advances, and the use of legumes as living mulches showed their potential to mitigate water stress.

5.2.3. Soil Penetration Resistance

Legume-based treatments, whether used as cover crops or living mulches, did not significantly affect soil penetration resistance in Spring Hill at the end of the experiment in fall 2025 (Figure 15). All measured values remained within the optimal range for crop production described by Hamblin (1986), well below the thresholds considered restrictive for root growth (2,000–4,000 kPa), indicating that soil physical conditions remained favorable for root development. Similarly, in Knoxville, no overall treatment effects were detected across the soil profile (0–38 cm); however, localized differences were observed in the 7.62–12.7 cm layer, where living mulch treatments

showed higher penetration resistance compared to HVCC and the control (Table 13). Despite these differences, all values still fell within the non-restrictive limits, suggesting no adverse impact on root growth or soil functionality.

These results indicate that although living mulch systems may slightly modify soil strength in specific layers, such changes are not sufficient to impair root penetration or crop productivity. Instead, they highlight the spatial variability of soil physical responses to management, emphasizing the importance of evaluating penetration resistance at deeper layers. Overall, both sites demonstrate that integrating legumes into organic intercropping systems does not negatively affect soil structural quality.

Lampurlanés and Cantero-Martínez (2003) observed variations in soil penetration resistance among tillage practices, with no-till exhibiting higher values than minimum tillage. Despite the increased penetration resistance, no-till management demonstrated a positive effect on soil conditions, as evidenced by greater root density than the other tillage systems. This finding highlights the beneficial impact of no-till practices on root development and overall soil health. These results are relevant to the present study, where HVCC and the control treatments were terminated using a cultivator prior to corn planting, while the living mulch treatments employed strip-till methods. The use of strip-till in living mulch treatments may contribute to improved soil structure and root growth, aligning with the positive effects observed under no-till management in the research by Lampurlanés and Cantero-Martínez (2003).

5.3. Biological Indicators

5.3.1. Soil Respiration

Soil respiration dynamics varied across locations, years, and corn growth stages, with limited and inconsistent treatment effects (Tables 14 and 15). In both Spring Hill and Knoxville during 2024, no differences among the treatments were observed at the V6 stage; however, at R1, certain living mulch treatments—particularly WCLM and RCLM—exhibited higher respiration rates than the control. These increases suggest enhanced microbial activity and greater availability of labile carbon under specific legume-based systems during the reproductive stage. Despite these stage-specific responses, treatment effects were not consistent across years, as no differences were observed in 2025 at either site, indicating strong interannual variability.

Temporal patterns further highlight the influence of environmental conditions and crop development on soil respiration (Tables 24 and 25). In Spring Hill, respiration decreased from 2024 to 2025 (Figure 18) and was lower at R1 than V6 in 2025 (Figure 17), suggesting reduced microbial activity or substrate availability, and potentially greater stabilization of soil organic carbon. In contrast, Knoxville exhibited an increase in respiration at R1 relative to V6 in 2025 (Figure 19), as well as a slight overall increase between years (Figure 20), reflecting site-specific conditions that may have promoted microbial activity and carbon turnover. These contrasting trends reinforce the importance of local environmental drivers in regulating soil biological processes.

Seasonal fluctuations observed in this study align with previous research linking soil respiration to crop phenology. Ding (2017) and Bhandari et al. (2025) reported that respiration typically increases during vegetative growth, peaks at maximum biomass

accumulation, and declines during reproductive stages, reflecting shifts in root activity and carbon inputs. However, the variability observed between V6 and R1 stages in this study suggests that these patterns are not always consistent and may depend on interactions with environmental factors.

Linn and Doran (1984) identified a strong relationship between microbial respiration and soil moisture content, noting peak activity at 60% water-filled pore space. In contrast, Ding et al. (2017) did not find a significant correlation between CO_2 flux and water-filled pore space, but suggested that soil moisture may indirectly influence respiration by altering soil temperature – a finding consistent with that by Wildung et al. (1975) emphasizing the interdependence of moisture and temperature in regulating microbial activity.

Ding et al. (2017) further observed a positive relationship between CO_2 flux and soil temperature, with seasonal patterns more closely aligned with temperature than moisture fluctuations. Soil temperatures ranged from 19°C to 34°C, but only 18% to 25% of seasonal variation in CO_2 fluxes could be attributed solely to temperature changes – indicating additional factors such as substrate availability or microbial community composition played critical roles. Interestingly, Bhandari et al. (2025) contradicted these findings by reporting no significant correlation between soil respiration and either temperature or moisture levels.

Overall, these results indicate that soil respiration in organic intercropping systems is driven more by temporal and environmental variability than by short-term management practices. While certain legume-based treatments may enhance microbial activity under specific conditions, their effects are not consistent across sites or years. These findings highlight the need for long-term studies to better capture

cumulative management effects and to disentangle the relative contributions of biotic and abiotic factors regulating soil respiration. Understanding these dynamics is essential for optimizing carbon cycling and improving the sustainability of agroecosystems.

5.3.2. Permanganate-oxidizable Carbon (POXC)

Treatments involving legumes, whether used as cover crops or living mulches, did not influence POXC levels across sampling stages, years, or locations, indicating a limited short-term effect of the treatments on this labile carbon fraction (Tables 16 and 17). Although a stage-specific difference was observed in Knoxville in 2024, with higher POXC at V6 than at R1, this pattern was not consistent across years or sites, suggesting that crop developmental stage effects on POXC are variable and likely influenced by environmental conditions and plant–soil interactions rather than treatments alone. In contrast, when averaged across the treatments, POXC levels increased from 2024 to 2025 at both locations, indicating that temporal factors and cumulative management effects may play a more important role than the individual treatments. This aligns with the understanding that POXC, as a labile and biologically active fraction of soil organic carbon, is highly sensitive to recent carbon inputs and environmental conditions. As highlighted by Culman et al. (2012), POXC responds more rapidly to management changes than more stable SOC fractions, making it a useful indicator of short-term shifts in soil carbon dynamics.

Previous studies help contextualize the variability observed in the present results. Thapa (2024) reported an increase in POXC over a two-year conventional corn–cover crop–cotton–cover crop rotation, particularly in the top 5 cm of the soil profile, highlighting the effectiveness of this indicator for monitoring short-term changes

under different soil management practices. Ginkaes (2020) demonstrated that POXC levels could vary significantly with sampling timing, spatial location, and year, with reductions linked to tillage and increased decomposition of labile organic matter. Similarly, Dutter et al. (2025) reported no consistent effect of cereal rye cover crops on POXC, even after long-term implementation, emphasizing the inherent variability of this fraction. In contrast, other studies have shown that positive effects on POXC may emerge under specific conditions and longer durations. For example, Hill et al. (2021) reported the highest POXC levels under organic systems combining cover crops and manure, highlighting the importance of sustained and diverse carbon inputs.

Taken together, these findings suggest that while legume-based intercropping systems have the potential to enhance labile carbon pools, their effects on POXC are not immediate and depend on factors such as duration of implementation, residue inputs, management intensity, and environmental conditions. The increases observed between years in this study point to the sensitivity of POXC to temporal dynamics, reinforcing its value as an early indicator of soil carbon changes, while also underscoring the need for long-term studies to capture consistent treatment effects.

5.3.3. Soil Organic Carbon (SOC) Stock

The SOC stock did not differ among the treatments at either location or sampling time (Tables 18 and 19). These results indicate that the short-term introduction of legumes in an organic intercropping system had a negligible effect on SOC accumulation under the conditions of this study. This limited response is not unexpected, as changes in SOC typically occur gradually and are strongly influenced by time, residue inputs, and environmental conditions. This finding aligns with that by Thapa (2024), who reported that after a two-year conventional corn–cover crop–

cotton–cover crop rotation under different tillage systems, TOC was not sensitive to soil management practices.

In contrast, longer-term studies have demonstrated that organic management practices can substantially enhance SOC. For example, Littrell (2020) reported greater SOC concentrations under systems incorporating cover crops and manure than conventional management, highlighting the importance of consistent carbon inputs and the role of more stabilized organic materials, such as manure, in promoting SOC accumulation. Similarly, Pimentel et al. (2024) observed that maintaining crop residues, such as sugarcane straw, over multiple years improved soil aggregation and SOC storage, emphasizing the critical role of residue retention and long-term management in building soil carbon, particularly in highly weathered soils. At a broader scale, Jian et al. (2020) demonstrated through a meta-analysis that cover cropping can increase SOC by 15.5% in surface soil layer, although these benefits are generally observed over medium- to long-term periods and are less pronounced in deeper soil layers. Together, these findings help explain the absence of SOC changes in the present study, where the relatively short duration and potentially limited carbon inputs from the legume treatments were insufficient to produce measurable differences.

Overall, while legumes and cover crops have clear potential to enhance SOC, their effects are cumulative and context-dependent. The results of this study reinforce the importance of long-term adoption and sustained residue inputs, to achieve meaningful increases in SOC stocks in organic intercropping systems.

5.3.4. Earthworm Population

Legume-based treatments, whether used as cover crops or living mulches, did not affect earthworm populations in 2024 at either site, and in 2025, a significant increase was observed only for the WCLM treatment in Spring Hill. When averaged across years, no differences were detected among the treatments at either location, indicating that short-term implementation of legumes in organic intercropping systems has limited and inconsistent effects on earthworm abundance.

These findings are consistent with previous studies showing that changes in earthworm populations often require longer periods or are influenced by multiple interacting factors. For instance, Roarty et al. (2017) reported no significant differences in earthworm populations among various winter cover crop treatments over a three-year rotation, despite numerical increases in some species such as pea and oat. Their results also highlighted that cover crop species differ in their ability to support earthworms, with Brassicaceae species showing lower populations, and that higher biomass production does not necessarily translate into greater earthworm abundance, emphasizing the role of residue quality.

Similarly, Baldivieso-Freitas et al. (2018) found that common organic management practices, including reduced tillage and green manure use, did not consistently increase earthworm populations, although organic fertilization with manure played a key role in supporting earthworm activity. This suggests that food quality and nutrient inputs may be more important drivers of earthworm populations than management practices alone. A broader perspective is provided by Blanco-Canqui (2022) who reviewed multiple studies and found that while cover crops often enhance earthworm populations, responses are highly variable and depend on factors such as

residue quality (particularly low C/N ratio), duration of adoption, reduced soil disturbance, and favorable climatic conditions, such as higher precipitation and temperature.

Taken together, these studies help explain the limited response observed in the present experiment. The short duration of legume implementation, combined with site-specific environmental conditions and potential differences in residue quality, likely constrained measurable increases in earthworm populations. Therefore, although cover crops and living mulches have the potential to improve soil biological activity, their effects on earthworms are gradual and context-dependent, reinforcing the importance of long-term management and favorable environmental conditions to promote meaningful changes in soil fauna.

Chapter Six: Conclusion

Overall, the incorporation of legumes as cover crops and living mulches in organic intercropping systems produced limited short-term effects on soil chemical properties, soil organic carbon stocks, and biological indicators across both sites. Soil chemical attributes remained largely unchanged, likely due to the inherent soil fertility, buffering capacity, and the relatively short duration of the study. Similarly, SOC stocks and earthworm populations did not respond significantly to the treatments, reinforcing that measurable changes in these indicators typically require long-term management.

In contrast, legume-based systems influenced soil nitrogen dynamics, particularly by enhancing early-season nitrate-N availability, with some evidence of residual nitrogen contributions depending on legume species and environmental conditions. However, these effects were highly variable across years and sites, emphasizing the strong influence of climatic factors and interannual variability on nitrogen mineralization and availability.

Short-term improvements in soil physical properties were observed, including reductions in bulk density and increased soil moisture under living mulch systems, particularly during corn vegetative growth stages. These effects, however, were not consistently maintained over time or across locations. Soil penetration resistance remained within optimal ranges, indicating no negative impacts on root growth. Soil respiration and POXC responses were also inconsistent across the treatments but showed sensitivity to temporal and environmental factors, with POXC increasing over

time regardless of treatment, suggesting a cumulative effect of organic management practices.

Collectively, these findings indicate that legume-based cover crops and living mulches can enhance specific soil functions—particularly nitrogen cycling, soil moisture retention, and short-term soil structure—without detrimental effects on soil physical quality. However, the variability observed across sites and years highlights the importance of environmental conditions in regulating these responses. Long-term studies are essential to better quantify the sustainability, agronomic efficiency, and soil health benefits of legume integration in organic intercropping systems.

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Vita

Rodrigo Henrique Aberti Garrido was born in Sorocaba, São Paulo, Brazil, and was raised with his family in Jaguariúna, São Paulo. He earned his Bachelor's degree in Agronomic Engineering from the University of São Paulo in 2021. During his undergraduate studies, he developed an interest in plant and soil sciences, participating in projects related to crop production and soil health management.

In 2024, he began his Master's degree in Plant Sciences at the University of Tennessee-Knoxville, where he worked under the supervision of Dr. Frank Yin. His research focused on soil health indicators, with an emphasis on organic intercropping systems.

After completing his master's degree, he intends to return to his professional career, focusing on research and development in the agricultural industry.