Influence of Cover Crops and Fertility Management on Soil Health and Soil Microbial Community

by

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ABSTRACT

The southeastern United States has a long history of soil degradation due to intensive agriculture, and conservation agriculture practices aim to improve soil productivity. Conservation management practices include nutrient management, crop rotation, maintaining a soil cover, and conservation tillage. In order to measure the effect that management has on the soil, soil health indicators are used. Soil health indicators include analyses that measure the biological organisms in the soil, the soil chemical composition and nutrient content, and the physical structure of the soil. The objectives of this study were to evaluate the short and long-term effects of cover crops and soil fertility management on soil health indicators on Southeastern soil types.

At the historic Cullars Rotation (est. 1911) in Auburn, Alabama, eight fertility and cover crop treatments were selected to evaluate their impact on soil health and the soil microbial community after 110 years of management. The treatments were complete fertility, no nitrogen (N), no winter legume cover crop, no N or cover crop, no phosphorous (P), no potassium (K), no lime, and no amendments or cover crop. Soil samples were taken from these treatments three times throughout the growing season from the 0-10 cm depth. Soil properties evaluated were soil moisture (θ_m), pH, soil texture, cation exchange capacity (CEC), plant available soil nutrient content with Mehlich-I extraction, microbial biomass carbon (MBC), soil respiration (R_s), soil organic carbon (SOC) and N, permanganate oxidizable carbon (POXC), autoclaved citrate extractable (ACE) protein, aggregate stability, and total bacteria gene copies with quantitative polymerase chain reaction (qPCR) analysis. The ‘complete fertility’ treatment was higher in SOC and POXC than all other treatments across all sampling times due to increased plant biomass production and therefore higher C inputs into the soil. Conversely, the ‘no amendment’

treatment preformed lowest in many of the soil health indicators measured including SOC, POXC, and ACE protein. Soil organic C and POXC did not show large responses to cover cropping, despite many reported responses of these indicators to conservation management in previous studies. Under N limited conditions, cover crops showed a response in ACE protein levels, but not when commercial N was applied in the rotation. No other soil health indicators showed a cover crop effect when inorganic N fertilizer was not applied. Aggregate stability was not correlated with any other soil health indicator nor was it affected by any treatment, and it may not be a viable soil health indicator for this soil type. Microbial biomass C, R_s, POXC, ACE protein, SOC, and total bacteria were all positively correlated to each other. This would indicate that these are effective soil health indicators in many cases and that each of these soil properties are related to one another. However, soil health indicators were not always reflective of soil fertility. For example, the 'no lime' treatment contained equivalent SOC and POXC as the 'complete' fertility treatment at some sampling dates due to reductions in microbial population. The 'complete' treatment had 2-3 times greater total bacteria and MBC than the low pH treatments (i.e., 'no lime' and 'no amendments', respectively), and this smaller microbial population reduced the amount of SOC being decomposed. When used to evaluate very low fertility treatments, some soil health indicators may be misleading, and a variety of indicators should be used to understand the complex soil dynamics that contribute to soil health and productivity.

In order to determine the effect of cover crop monocultures and mixtures on soil health, cover crop experiments were established in the Coastal Plain and Tennessee Valley regions of Alabama and four years of data were collected at each location. Cover crops were incorporated into cotton (*Gossypium hirsutum*) and legume cash crop rotations, and the treatments included

monocultures and two- and three-way mixtures of cereal rye (*Secale cereale*), crimson clover (*Trifolium incarnatum*) and forage radish (*Raphanus sativus*). Cash crop yield, cover crop biomass, SOC, POXC, aggregate stability, and soil strength ($AUC_{C.I.}$) were evaluated. Cover crop biomass was variable from year to year and was dependent on planting date and weather conditions. In the Coastal Plain, treatments containing clover had on average 44% higher above ground biomass than those that did not have clover. At the northern Tennessee Valley location, the rye treatments performed slightly better than clover, but both rye and clover had much higher biomass than radish. In one site-year, a two-species mixture produced more biomass than both of its monoculture constituents. In the top 5 cm of soil, all cover crops with the exception of the radish monoculture increased SOC by 23% compared to the winter fallow treatment. In the 5-10 cm depths, rye-radish and rye-clover mixes increased SOC by 17% compared to the fallow. Similarly, some cover crop treatments were able to increase POXC compared to the fallow control. In the Coastal Plain, cover crop treatments had little effect on SOC and POXC due to the coarse and low organic matter soil type. In Tennessee Valley, the soil is finer-textured and can retain more soil organic matter than the coarser-textured Coastal Plain soil. Soil organic C and POXC were both highly correlated, and both of these indicators may be useful for determining the effects of cover cropping in some soil types. Aggregate stability did not show many meaningful differences at either location. Soil strength was highly variable with season, but it was affected four out of the eight site-years of this study. Treatments containing rye or clover decreased soil strength in the Tennessee Valley by 19% after four years of cover crop utilization. Differences in soil strength were also observed in the Coastal Plain, but they were inconsistent. In the Tennessee Valley, the rye monoculture and each 2-species mixture were able to increase cotton yield 25% compared to the no cover crop control. Conversely, there were no cover crop

treatments that were able to increase cash crop yield in the Coastal Plain. Utilization of cover crops shows the potential to improve soil health and reverse the effects of soil degradation depending on the soil type and the cover crops used.

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LIST OF ABBREVIATIONS

θ_m	Soil Moisture
ACE	Autoclaved Citrate Extractable
$AUC_{C.I.}$	Area Under the Curve for Cone Index
C	Carbon
CEC	Cation Exchange Capacity
CFI	Chloroform Fumigation Incubation
GWC	Gravimetric Water Content
K	Potassium
MBC	Microbial Biomass Carbon
N	Nitrogen
P	Phosphorous
POXC	Permanganate Oxidizable Carbon
qPCR	Quantitative Polymerase Chain Reaction
R_s	Soil Respiration
SOC	Soil Organic Carbon
SOM	Soil Organic Matter
TB	Total Bacteria
TN	Total Nitrogen
TVREC	Tennessee Valley Research and Extension Center
WHC	Water Holding Capacity
WREC	Wiregrass Research and Extension Center
WSA	Water Stable Aggregates

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I. LITERATURE REVIEW

INTRODUCTION

Land degradation has become an increasingly pressing threat to agriculture, especially in the southeast United States. Soils in the Southeast were formed under forests with high rainfall and are now acidic and low in fertility (Mylavarapu et al., 2014). This, combined with a history of intensive land use, has led to degraded soils. Additionally, increased heat stress, rainfall extremes, and increasing atmospheric carbon (C) concentrations threaten the future of agriculture in the Southeast (Ingram et al., 2013). Cropland in this region has been decreasing since 1985 due to urban sprawl, increasing pressure on the remaining cropland to produce more per acre while reducing its environmental impact (Ingram et al., 2013). There is a need to identify the long-term impacts that agricultural management has on soil health in this region (Shaw et al., 2006).

In order to restore the soil's health while maintaining production and profitability, producers use conservation agriculture practices like reduced tillage, cover crop utilization, crop rotation, and proper nutrient management. These practices aim to reduce erosion and runoff, as well as enhance the overall biodiversity of the system to promote biological processes in the ecosystem like nutrient cycling and disease suppression. Ultimately, the goal of conservation agriculture is to improve crop production in a sustainable way (FAO, 2021). More specific goals of conservation agriculture include C sequestration, prevention of soil loss, and reduction of nutrient pollution. Conservation agriculture practices have been shown to preserve and improve deteriorating soil health and can help combat the many threats facing the future of Southeastern agriculture.

Cover crops are plant species that are planted in the off season of the cash crop. Typically, in the southeast U.S., cover crops are planted in the winter and are used as a soil cover after the cash crop is harvested. They are then terminated shortly before planting of the next cash crop. Different species of plants used as cover crops can bring various benefits to the production system. There are many agronomic influences that cover crops can have on the production system. The potential benefits of cover crops include weed suppression, C sequestration, moisture conservation, improved soil physical properties, and nutrient scavenging (Blanco-Canqui, et al., 2011; Acuña & Villamil, 2014; Reese et al., 2014; Abdollahi & Munkholm, 2014; Rankoth et al., 2019; Chalise et al., 2019; Restuccia et al., 2020; Pittman et al., 2020). The soil health benefits that cover crops can provide, along with weed suppression, increased soil moisture, and nutrient scavenging combine to create a more productive and sustainability-focused crop system.

Many benefits of conservation agriculture are well known, but its impact on the soil microbial community is more difficult to determine. The majority of soil microorganisms are still unidentified, and their functions and interactions with different agricultural systems are yet to be fully understood. Therefore, further research into soil microbial communities may lead to a better understanding of how microorganisms can help combat soil degradation and ultimately achieve the goals of conservation agriculture (Singh & Gupta, 2018).

Changes in soil health can be difficult to measure over a short period of time because many soil health parameters take time to improve or degrade. Long-term experiments provide unique opportunities to measure a variety of soil characteristics that have undergone more than a century of the same treatments. Few agricultural experiments have been in place as long as those found in Table 1.1, and many discoveries have been made at these sites. Due to the historical

nature of these experiments, many have flaws in their experimental design. However, the long duration of these experiments makes them a useful tool in studying sustainability and soil degradation over time. In Auburn, Alabama, there are two long-term (>100 year) experiments that have both been part of many significant research findings (Entry et al., 1996; Mitchell et al., 2008; Chavez et al., 2014; Zhao et al., 2015; Santana-Pereira et al., 2020).

SOIL HEALTH INDICATORS

Soil is vital resource to global agriculture, and its functionality can be measured through the use of soil health indicators. Soil health is "the continued capacity of soil to function as a vital living ecosystem that sustains plants, animals, and humans" (Scott, 2019). Soil health indicators are often grouped into biological, chemical, and physical properties of the soil, which can create a comprehensive understanding of the soil's health when evaluated together.

There are many types of soil analyses that can be used to measure soil health. However, not all are useful as a way to understand the impact of agricultural practices. Some soil health indicators are more dynamic than others and exhibit faster or larger-scale responses to management (Cardoso et al., 2013). An effective indicator of soil health should be responsive to management and be applicable to a variety of soil types (Norris et al., 2020). Other important considerations include ease of interpretation, adaptability on a large scale, cost effectiveness, reproducibility, and how the indicator is affected by management (Scott, 2019).

Soil Microbial Properties

The majority of the biological diversity in agroecosystems lies in the soil microorganisms (Roger-Estrade et al., 2010; Brussaard et al., 2007). Microbial diversity is essential to soil health and quality (Garveva et al., 2004), and soil biodiversity can stabilize the ecosystem in the presence of stress or a disturbance such as drought, fire, or pest pressure (Brussaard et al., 2007). For example, some plant pathogens have specific antagonists, and if the microbial community remains diverse there is a greater chance that an antigen will be present to fight against certain pathogens (Brussaard et al., 2007). Shifts in the microbial community can be seen with a change in land use, plant growth stage, management practices, and potentially with climate change, and some of these shifts could result in a loss of diversity (Inceoglu et al., 2011; Roger-Estrade et al., 2010; Wagg et al., 2014; Dubey et al., 2019). If soil biodiversity is lost, the ecosystem services it provides will be lost with it (Wagg et al., 2014).

Soil Microbial Biomass

Depending on the quantity and composition of the organisms, the soil biota has the potential to greatly improve the capacity of a soil to enhance a sustainable agricultural system. The biological component of soil consists of both macro and microorganisms. Soil macro-organisms include nematodes, arthropods, and earthworms. While these organisms are essential to the ecosystem, recent literature has primarily focused on the biomass of soil microorganisms in evaluations of overall soil health. Measuring the biomass of soil microorganisms can indicate the health of the soil due to the large impact that they have on plant growth. Soil microorganisms perform a variety of organism-specific functions and provide ecosystem services including

nutrient cycling, disease suppression, detoxification of harmful chemicals, improvement to the soil structure, and assistance with plant nutrient uptake (Lehman et al., 2015a).

One of the key roles of soil microorganisms is the cycling of nutrients in the soil. Microorganisms can convert non-available mineral forms of phosphorous (P) to plant-available hydrogen phosphate ions ($H_2PO_4^-$ and HPO_4^{2-}) and organic forms of nitrogen (N) to plant-available nitrate and ammonium (NO_3^- and NH_4^+). The rate at which the microorganisms are able to perform these functions is dependent on nutrient availability as well as environmental factors, such as temperature and moisture (Liski et al., 2003; Xu & Yuan, 2017). Cropping systems that rely on cover crops or crop litter to provide plant-available nutrients utilize the soil biota's ability to decompose crop residues and release plant available nutrients (Liski et al., 2003).

In order to cycle essential plant nutrients, microorganisms need access to C. Soil microorganisms use different forms of C as their energy source. Autotrophs that are able to utilize carbon dioxide (CO_2) in the atmosphere are largely responsible for atmospheric C sequestration, and heterotrophs that need organic C as their energy source are responsible for a large part of biomass C decomposition (Gougoulias et al., 2014). The majority of soil microbes are heterotrophs, and some form symbiotic relationships with plants. For example, arbuscular mycorrhizal fungi receive their C from their plant hosts as they assist in the uptake of soil nutrients (Bolan, 1991).

The size of the microbial biomass determines how quickly these vital ecosystem functions can be performed (Bohme et al., 2005). A common method used to determine the quantity of soil microbial biomass is chloroform fumigation incubation (CFI). In this method, soil samples are collected, fumigated with chloroform, and incubated. The biomass is determined by quantifying the CO_2 released post-fumigation. A limitation of CFI is its limited capacity to

accurately represent acidic soils (Jenkinson et al., 2004). There has also been much debate over the subtraction of a control when using this method (Franzluebbers et al., 1999). In some cases, the subtraction of the control led to a negative result because the procedure assumes that there are fewer dead organisms in the control soil post-fumigation (Alef, 1993). However, others argue that if the limitations are accepted and the soil is pre-incubated and wetted prior to fumigation that the method will still be valid (Martens, 1995). Despite its limitations, CFI was found to be more closely related to soil organic C than the alternative method, chloroform fumigation extraction (Franzluebbers et al., 1999).

Many factors influence the size of the microbial population, including inherent soil properties. It has been found that a low soil pH can decrease the microbial population, further emphasizing the need to amend the soil with agricultural limestone in the primarily acidic Southeast region (Hiltbold et al., 1985). A low microbial biomass along with other pH limitations like aluminum toxicity make it difficult for plants to thrive in an acidic soil. In the long-term study at the Cullars rotation in Auburn, Alabama, it was found that liming of the soil for pH resulted in a ten-fold increase in microbial growth (Zhao et al., 2015). Additionally, organic matter and soil texture, which both vary according to soil type, determine the capacity of the soil to sustain microbial life (Girvan et al., 2003; Williams et al., 2013; Nunes et al., 2020). Since many soil microorganisms depend on organic matter for energy, and organic matter is inherently low in Southeast soils, it is important that organic matter is built up and conserved.

Changes in the size of soil microbial biomass carbon (MBC) can be found with alterations to land management such as tillage, irrigation, cover cropping, and fertilization strategy (Balesdent et al., 2000; Hamel et al., 2006). Many studies have shown that conventional tillage practices lead to a decrease in MBC due to the decrease in organic matter, increased soil

density, and other changes in soil properties that result from tillage (Feng et al., 2003; Shaw et al., 2006; Nunes et al., 2020). Microbial biomass is a dynamic property that has a strong temporal fluctuation (Hamel et al., 2006). Thus, in order to accurately determine the effect of land management on the microbial community, microbial biomass should be measured at multiple points in time. For example, in a cover crop experiment using legumes and cereal crops, the presence of a cover crop was shown to increase MBC in the first year of the experiment but not in the second year due to inconsistent weather conditions from year to year (Mendes et al., 1999). In addition to time, depth has also been shown to have an effect on MBC. It has been found that MBC can be slightly higher in the 10-20 cm soil depths with cover crop use compared to treatments without cover crops, but the upper 10 cm of soil was variable (Abdollahi & Munkholm, 2014).

Soil Microbial Communities

In total, there can be up to 10 billion microorganisms in a gram of soil (Torsvik and Ovreas, 2002). In agricultural experiments, total bacterial gene copies have been reported around $10^8 – 10^9$ per gram of soil and total fungal gene copies are reported around $10^7 – 10^8$ per gram of soil (Andronov et al., 2012; Zhang et al., 2015; Song et al., 2015). The soil microbial population as a whole has many benefits, but bacteria and fungi have more specific ecosystem roles. For example, both bacteria and fungi are able to sequester C in the soil; but fungi have a higher C utilization efficiency, meaning that a soil with a higher fungi to bacteria ratio would be better able to build soil organic carbon (SOC) (Jastrow et al., 2007). Litter decomposition is also affected by soil microbial community structure, as only fungi have been found to be able to initiate the decomposition of lignin, a primary component of plants (Jastrow et al., 2007). There

are many more differences between microbial communities in addition to these examples, and a further understanding of interactions between soil microorganisms and plants under a variety of conditions could assist in the manipulation and management of soil biota in agricultural systems (Jansson & Hofmockel, 2018).

In order to get more detailed information on the communities of microorganisms present in the soil, DNA analysis can be performed using real-time or quantitative polymerase chain reaction (qPCR) analysis. This method allows for the targeting of specific sequences and can be performed rapidly (Lehman et al., 2015a). However, qPCR can only target known DNA sequences, and the majority of soil microorganisms are currently uncultured and unrepresented in DNA libraries used for identification (Mazzola, 2004; Lehman et al., 2015a; Choi et al., 2017; Jansson & Hofmockel, 2018). Real-time PCR analysis of total 18S and 16S gene copies has been found to correlate with other biological soil health indicators such as ester-linked fatty acid methyl ester (EL-FAME) under land management changes, and qPCR is a potentially viable indicator of soil health (Pérez-Guzmán et al., 2020).

Managing soil pH is one of the best ways to increase microbial diversity in an agricultural system, and the highest amount of microbial diversity is found in neutral to alkaline soils (pH 6-8) (Fierer & Jackson, 2006; Chaparro et al., 2012). Bacteria are strongly affected by changes in pH, whereas fungi can tolerate a wider pH range (Rousk et al., 2010). This finding is backed by a study conducted in the Southeast by Lauber et al. (2008) which showed pH was a key driver of variation in the bacterial community, while changes in the fungal community were related closest to soil fertility, namely soil P. Liming practices to manage soil pH increased bacterial abundance and decreased fungal abundance, leading to a decrease in the fungal to bacterial ratio in a study by Holland et al. (2018). Since fungi are more tolerant of low soil pH,

their dominance will increase with decreasing pH (Strickland & Rousk, 2010). However, increased fertilizer inputs and nutrient availability have been found to decrease fungal to bacterial dominance (Strickland & Rousk, 2010). In addition, Jangid et al. (2008) found that the use of organic fertilizer amendments increased diversity of soil bacterial communities when compared to inorganic fertilizers.

In addition to soil fertility management, soil conservation management practices can also influence the composition of the soil microbial community. Crop rotations implemented over a long period of time have been found to increase richness and diversity in the soil microbiome (Venter et al., 2016). Although it can be difficult to quantify, tillage events can also dramatically change the soil microbiome composition (Roger-Estrade et al., 2010). Adoption of no-tillage management can increase both bacterial and fungi communities proportionately (Strickland & Rousk, 2010). A Southeastern study conducted across several management strategies including cover cropping, crop rotation, and fertilizer rate found that crop management practices and fertilizer source caused a difference in soil health indicators such as pH, inorganic N, SOC, and soil organic carbon to nitrogen (C:N) ratio which explained approximately 55% of the total of the variation in bacterial and fungal communities (Chen et al., 2018).

Environmental factors can also play a part in microbial community composition. An analysis by Castro et al., (2010) on a southern Ultisol found that fungal abundance increased with increasing atmospheric temperature, and bacterial abundance increased with increased temperature and CO_2 levels, meaning that climate change may play a part in shaping microbial communities as time goes on.

Soil Chemical Properties

Soil chemical properties such as soil organic matter, nutrient content, pH, and cation exchange capacity are typically effective indicators of crop performance and are related to the nutrient content and availability of the soil (Norris et al., 2020). Chemical analyses are common baseline soil health tests and are used to make fertilizer and lime recommendations to improve plant growth responses. However, it is important to keep in mind that all aspects of soil health work together to create a sustainable and productive soil. These chemical indicators used in conjunction with other types of soil health indicators can help create a better understanding of soil health.

Soil pH

The ideal pH range for most crops is between 6 and 7. However, most soils in the Southeast have an inherently lower pH and require amendments to raise the pH (Sikora, 2014; Mylavarapu et al., 2014). The high annual precipitation in this region causes leaching of base cations leading to highly acid soils (Mylavarapu et al., 2014). The release of organic acids and nitrification from ammonium-based fertilizers can also increase the acidity of the soil (Fageria & Baligar, 2008). Low pH leads to the saturation of the soil cation exchange capacity with hydrogen and aluminum ions, which limits the availability of other plant essential nutrients like N and P and increases the toxicity of aluminum and manganese (Fageria & Baligar, 2008; Sikora & Kissel, 2014; Halvin et al., 2014). Soil acidity can be one of the most yield-limiting factors in crop production (Fageria & Baligar, 2008).

Soil pH is measured with a calibrated electrode that measures the hydrogen ion concentration in solution. Soil is combined with water or a weak salt solution to bring the ions in the soil into solution so they can be measured. Soil pH measurement, along with the buffer pH measurement, allows for an approximation of how much agricultural limestone will need to be added to the soil to bring the pH up to a productive level.

The application of agricultural limestone, or lime, has been used to increase the pH of the soil for many years. Lime is most often composed of calcium carbonate ($CaCO_3$) and increases the pH by neutralizing hydrogen ions in the solution. Cotton is mildly tolerant of acidity and historical experiments conducted in the 1930s and 40s found that liming the soil could increase cotton yield (Adams, 1958). Corn is more tolerant of acidity and will typically be limited by a soil nutrient deficiency before pH, but liming was still found to increase corn yield indirectly in these historical experiments (Adams, 1958). Wheat and many legume crops are sensitive to pH, therefore a small yield increase may be observed with liming (Adams, 1958; Cope, 1984).

Soil Available Nutrients and Fertility

Plant-available nutrients in the soil must be evaluated to determine the level of crop productivity and this can help determine if fertilizer will be needed. Nitrogen, P, and potassium (K) are the most common limiting plant essential nutrients.

Nitrogen is used by plants to create proteins, nucleic acids, and is also a key part of the structure of chlorophyll (Marschner, 1995; Halvin et al., 2014). Plants with adequate N will appear dark green due to the high rate of photosynthetic activity (Halvin et al., 2014). Nitrogen is highly mobile within the plant, and if N becomes deficient, the leaves will turn chlorotic

beginning in the lower leaves (Halvin et al., 2014). For grasses, N deficiency symptoms will begin at the tip on the leaf and travel down through the midrib, but broadleaf plants will show an overall yellowing of the leaf (Halvin et al., 2014). Eventually the leaves can turn necrotic and die (Halvin et al., 2014). Historic fertilizer trials in the southeast U.S. reported corn has a very high response rate to N in the early to mid-1900s, and that soybean does not respond to N if properly inoculated (Rouse, 1968). Long-term crop rotation experiments conducted circa 1984 across Alabama concluded that N fertilizer was able to increase the yield of corn, cotton, and wheat, but not soybean (Cope, 1984).

The primary function of P in plants is energy storage (Halvin et al., 2014). Phosphorous is a prominent structural element and is used for synthesis of nucleic acids, adenosine triphosphate (ATP), phospholipids, and some sugars (Marschner, 1995). Photosynthesis is heavily dependent on the concentration of P in the chloroplasts (Marschner, 1995). Plants with insufficient P will appear stunted with a dark green color (Halvin et al., 2014). Some plants will appear purple, starting at the leaf margins and spreading toward the center of the leaves on the lower leaves first and can ultimately turn necrotic (Halvin et al., 2014). Later in the growing season, P is transferred to the reproductive tissue, so a P deficiency can result in poor seed development and maturity (Halvin et al., 2014). It has been shown that adequate soil P can increase cotton yield by about 50% in Alabama (Rouse, 1968). Soybean and corn have shown a very similar yield response to P fertilization (Rouse, 1968). Long-term fertility experiments across Alabama found a 10% average yield increase across all crops with P fertilization when compared to treatments that did not receive any P fertilizer (Cope, 1984).

In the plant, K remains in its cationic form (Halvin et al., 2014). Potassium helps maintain plant-water relations such as osmotic potential, turgor pressure of cells, nutrient

transport, and stomatal movement (Marschner, 1995). Potassium is also responsible for the activation of many essential plant enzymes and the synthesis of proteins (Marschner, 1995). Potassium is highly mobile in the plant and is largely related to crop quality (Halvin et al., 2014). A deficiency in K will result in chlorotic leaf edges leading to necrosis, typically in older leaves first, but these symptoms can occur in younger leaves in crops which mature quickly (Halvin et al., 2014). In small grains, a K deficiency can lead to lodging or stalk breakage (Halvin et al., 2014). Potassium is a key nutrient for cotton production. Experiments conducted circa 1968 found that K fertilizer rate determined cotton yield (Rouse, 1968). Corn yield was not as responsive to K as cotton, and soybean yield had an intermediate response (Rouse, 1968).

For acidic soils with low activity clays, the Mehlich-I extraction method is used to evaluate the nutrient content of soils (Mylavarapu et al., 2014). This method is capable of determining the majority of the plant essential macronutrients with the exception of N and sulfur (Maguire and Heckendorn, 2011). Nitrogen content is determined with either combustion analysis or with extraction and colorimetric analysis.

Soil nutrient content is commonly adjusted with fertilizer to achieve individual yield goals. Fertilizer can be inorganic and synthetically produced, or it can come from organic sources like animal manure. Practices to reduce nutrient loss like cover cropping or minimizing tillage help keep soil nutrients in place so that they can be used by a future crop, and these practices also help prevent environmental harm (Meisinger et al., 1991; DeLaune & Sij, 2012).

Cover crops can provide nutrient scavenging to a crop production system. Some deep-rooted cover crops are able to utilize leachable nutrients like N and keep them near the soil surface for the subsequent cash crop (Hirsh et al., 2021). These cover crops can also prevent runoff that may contain N which can become an environmental contaminant (Haruna &

Nkongolo, 2020). Additionally, legume cover crops can supply organic N to the following crop, although it takes time for the N in the cover crop biomass to become plant available (Sarrantonio & Gallandt, 2008). For a nutrient like P that is not leachable, cover crops can help keep P sediments from eroding away by keeping the soil covered (Haruna & Nkongolo, 2020).

Soil Organic Matter

Soil organic matter (SOM) is the organic fraction of the soil exclusive of undecayed plant and animal residues (SSSA, 2008). The organic matter found in the soil has a larger sink of organic C than both the atmosphere and above-ground vegetation combined (Lehmann & Kleber, 2015). Ultisols cover a large area of the Earth, and they contain a large C pool (Lal, 2004). Plants sequester C by removing CO_2 from the atmosphere and converting it to plant tissue through photosynthesis (Lal, 2004). The plant tissue then becomes SOM once the plant dies and is decomposed by microorganisms in the soil. Soil organic matter is able to provide C sequestration to the global ecosystem, but it also plays a key role in plant growth. Soil organic matter typically has a high cation exchange capacity (CEC).

Soil organic matter aids in nutrient retention, increases water holding capacity and soil water retention, supports the physical structure of the soil, and influences many other soil properties (Doran et al., 1996; Rawls et al., 2003). Additionally, the more SOM that a soil contains, the larger the microbial community that can be sustained (Bai et al., 2020). Soil organic matter is typically regarded as a baseline or primary soil health indicator, and significant shifts in this indicator can be measured in 3 to 5 years (Lehman et al., 2015b; Stott, 2019). The most common method for measuring SOM content of a soil is loss on ignition. The SOM is measured

with a series of increasing heating temperatures that remove the weight of the SOM by igniting it and converting it to gas. Soil organic matter can also be estimated by measuring SOC with combustion analysis and measuring the CO_2 produced from the reaction.

A long history of agricultural management practices detrimental to soil health have caused a depletion of SOM in the southeast United States (Nash et al., 2018). In the Ultisols of the southeast United States, SOM is typically low due to the highly weathered nature of these soils, and an organic matter content of about 2% is considered high for this region (Doran et al., 1996). In order to restore these depleted organic C sinks in the soil, soil management strategies need to change (Nash et al., 2018). Not only can conservation management benefit the overall health of the soil, but it can also sequester more of the C from the Earth's atmosphere.

Management choices such as frequent tillage can have detrimental effects on SOM. Depending on the management history and properties of the soil, a tillage event can cause a large decrease in SOM, therefore damaging the soil structure and decreasing the benefits that organic matter can bring to the agroecosystem (Balesdent et al., 2000; Feng et al., 2003; Rottler et al., 2019). A meta-analysis by Nunes et al. (2020) of 302 studies found that in response to no tillage management, Ultisols show the largest positive effects on SOC when compared to other common agricultural soil orders. Nutrient management practices, i.e., fertilizer application and type, can impact the SOC storage across a variety of climates (Waqas et al., 2020). For example, N fertilizer application has shown to facilitate SOC sequestration (Zang et al., 2016). Additionally, cover crops have the potential to increase SOC storage (Valkama et al., 2020; Jian et al., 2020). A study conducted in the southeastern U.S. found that no tillage combined with cover cropping was able to increase SOC compared to intensive tillage practices without a cover crop (Singh et al., 2020).

Permanganate Oxidizable Carbon

Permanganate oxidizable carbon (POXC) is a common soil health indicator that represents active or easily degradable soil C that can be useful for representing long-term C sequestration potential (Hurisso et al., 2016). Active C refers to organic carbon fractions such as MBC and carbohydrates that can be readily broken down by soil microorganisms (Weil et al., 2003). Permanganate oxidizable C is measured by adding soil to a potassium permanganate solution and using ultraviolet spectroscopy to determine how much of the soil carbon was oxidized. This method of carbon analysis is commonly used to measure the "active" fraction of SOC. However, it has been found that this method captures slightly more C than what is readily available to microorganisms (Romero et al., 2018). Permanganate oxidizable C has been found to positively correlate with other common soil health indicators such as SOC and MBC (Weil et al., 2003; Culman et al. 2012; Morrow et al., 2016; Wang et al., 2017).

As an indicator of soil C, POXC is one of the indicators most sensitive to management and can detect differences in as little as 1 to 3 years in Southeastern soil types (Culman et al., 2012; Stott, 2019). Changes in POXC can be directly related to management, and an increase in POXC can lead to an increase in soil function and health (Stott, 2019). Research conducted in the Southeast found that POXC was most sensitive to the amount of residue left in the field and was less sensitive to tillage treatments after thirty-nine years (Singh et al., 2020). A study analyzing multiple long-term studies (20-40 years) from this region also found that no-tillage management was able to increase the POXC in the shallower depths (Jagadamma et al., 2019). Cover crop treatments can also increase POXC in the U.S. Coastal Plain in the top 30 cm of soil (Wang et al., 2017).

Soil Protein

Soil protein is largely composed of organic N in the soil, and autoclaved citrate extractable (ACE) protein analysis represents a broad range of proteins in the soil (Hurisso et al., 2018). Autoclaved citrate extractable protein analysis can be beneficial to soil health testing because it indicates the fraction of N that is readily available to the soil microbial population (Hurisso et al., 2018). Management practices that increase the C:N ratio of soil, such as cover cropping, can result in lower levels of ACE protein. This analysis is able to reflect the quality of the organic matter, not just the amount (Moebius-Clune et al., 2017).

Using a bicinchoninic acid assay, a wide range of organic ACE proteins can be extracted and quantified (Geisseler et al., 2019). This protein represents the organic N that can be mineralized by soil microorganisms, and it positively correlates with other N indicators such as N mineralization (Moebius-Clune et al., 2017; Geisseler et al., 2019).

According to a study by Cappellazzi and Morgan (2021) analyzing soils from across North America, ACE protein shows significant responses to decreased tillage, cover cropping, and residue retention. However, ACE protein is a relatively new soil health indicator, and more research needs to be conducted to determine the effect of conservation management practices on ACE protein content specifically in the Southeast.

Soil Physical Properties

Another important aspect of soil health is the soil physical properties, which includes structure, texture, stability, and density. These properties can influence soil health to a large extent and will ultimately have long-term effects on the production system. The stability of the

soil structure over time decreases erosion potential and can maintain soil aeration (Colombi et al., 2018). Soil structure and penetration resistance can influence water availability to plant roots as well as the ability of roots to elongate (Colombi et al., 2018). A lower bulk density and therefore higher soil aeration will allow for better root growth and plant water and nutrient uptake (Fageria & Stone, 2006; Colombi et al., 2018).

Soil Aggregate Stability

Aggregate stability is one of many indicators of the soil's physical structure and its ability to sustain life. There are two types of soil aggregates, macroaggregates (> 250 μm) and microaggregates (53-250 μm), and each are influenced by different factors (Stott, 2019). Macroaggregate stability can be positively influenced by management practices such as no tillage and crop rotation as well as the exudates produced by soil biota (Miller & Jastrow, 2000; Moebius et al., 2007). One of the biggest soil biota contributors is mycorrhizal fungi, which produce proteins that bind soil particles together (Miller & Jastrow, 2000; Rilling et al., 2002). However, in soils with a high clay content there is very little response to mycorrhizal fungi in the soil structure, so aggregate stability focuses on microaggregates which are primarily formed by the mineral structure (Miller & Jastrow, 2000; Stott, 2019). Soils in the southeast United States which have a high concentration of iron and aluminum oxides rely on these oxides as the aggregate forming agent. The interaction between the oxides and SOM determines the aggregate stability (Huang et al. 2010; Zhao et al., 2017). The combination of clays, oxides, SOC, soil biota, carbonates, through cohesive forces creates cemented aggregates that influence soil water, erosion, nutrient content, root growth, and ultimately crop performance (Bronick & Lal, 2005).

These aggregates, along with the pore space between them and soil water make up the soil structure (Kooistra & Noordwijk, 1995). The arrangement of different sizes of aggregates along with their relative pore space allows for different types of microorganisms to have access to the SOC source held within the aggregates (Carter & Stewart, 1995). This phenomenon not only protects the organic matter, but it also provides the microorganisms with protection from larger organisms (Ladd et al., 1993).

Aggregate stability is commonly measured with the wet sieve method in which the soil is brought to field moisture and dunked in water multiple times. The soil is then dunked into a dispersal solution to correct for the sand fraction. This process mimics soil saturation and the ability of the soil to maintain its structure.

When soil aggregates are disturbed such as during rainfall events, aggregates can disperse and a surface seal can form, which will decrease the infiltration rate (Radcliffe et al., 1991; Stott, 2019). A destruction of soil aggregates can lead to crust formation, erosion, and a decrease in water holding capacity (Stott, 2019). One way to prevent the destruction of soil aggregates is to keep the soil covered (Tang et al., 2011). Conservation practices such as leaving crop residue on the soil can increase the formation of macroaggregates and the percentage of water stable aggregates (WSA) when compared to uncovered soil or more conventional management strategies (Levi, 2007; Cochran, 2010; Tang et al., 2011). In the Southeast, no tillage treatments were also found to increase WSA when compared to tilled treatments after thirty-nine years (Singh et al., 2020).

Penetration Resistance

Soil compaction plays a large part in the physical health of a soil and has a large economic impact on global agriculture (Soane & van Ouwerkerk, 1994). A compacted soil can adversely affect plant growth by restricting germination, restricting root growth, and reducing water uptake (Soane & van Ouwerkerk, 1994).

One way that soil compaction can be evaluated is by measuring the penetration resistance, which is directly related to both traffic intensity and compaction level (Usowicz & Lipiec, 2009). Increased penetration resistance due to traffic negatively correlates with crop yield (Nelson et al, 1975; de Moraes et al., 2020). A higher penetration resistance will result in a shallower root system and will ultimately lead to a cycle of increasing compaction in the topsoil due to the drying out of the soil occupied by the roots (Colombi et al., 2018).

Soil compaction can be caused by traffic, tillage, and climate (Soane & van Ouwerkerk, 1994; Feng et al., 2003). Increased soil moisture content decreases the weight bearing capacity of the soil, and soil has an increased chance of compaction if there were to be a tillage or traffic event on the soil during high moisture (Kondo & Dias Junior, 1999).

Management practices such as reduced traffic, decreased subsoil cultivation, and increased organic matter inputs help the soil retain its structure and reduce compaction (Hamza & Anderson, 2005). Research conducted in the southeast United States has shown that a conservation tillage system can have the highest potential for increasing crop yield while decreasing penetration resistance and bulk density (Nouri et al., 2019). A study conducted in the Tennessee Valley region found that the conventional tillage systems had the greatest compaction compared to reduced tillage systems (Schwab et al., 2002). Other research in the Southeast found

that incorporating cotton into soybean and corn rotations can increase penetration resistance because cotton is a low residue crop, and the reduced residue inputs over a period of fifteen years reduced the soil stability (Nouri et al., 2019).

RESEARCH OBJECTIVES

The restoration of soil health in the southeast U. S. is essential to the future of crop production. Many conservation practices have been proposed to improve soil health. However, in the short-term these practices do not always result in measurable differences in soil health. If conservation practices such as cover cropping can prove to be economically sustainable in the long-term, this could increase adoption of these practices.

Cover crop adoption has been increasing as conservation efforts have become more widespread, but it can be difficult for producers to justify due to time and cost restraints. As with any management practice, cover crop utilization will look different for every operation which can make it difficult to determine if the cost of implementing a cover will justify the outcome. The positive effects of cover crops are not always apparent every growing season due to climate and management differences, and many soil properties take time to change. When assessing the benefits of conservation management practices such as cover crops, it is important to evaluate the soil health over a long period of time.

In order to measure soil health, suitable indicators must be identified. Soil health is difficult to quantify, but by utilizing a variety of soil analyses the productivity and sustainability of soils can be estimated. Many soil health indicators are used around the world to improve understanding of the soil, but not all indicators will reflect changes in management across every

soil type and production system. Emerging soil health indicators and their relationships to soil fertility and conservation management as well as their correlation to other soil health indicators needs to be determined in the southeast U.S.

The objectives of this study were (1) to determine the effect of long-term (>100 years) cover cropping and fertility management on soil health indicators, the soil microbiome, and crop yield in the Southeast, and (2) determine the effect of shorter-term (i.e., 4 years) cover cropping on soil health indicators and crop yield in the Southeast.

TABLE 1.1. Long-term (>100 year) experiments from around the world.

Experiment	Location	Year Est.	Nature of Study
Broadbalk Experiment	Hertfordshire, UK	1843	Wheat fertility experiment
Morrow Plots	Urbana-Champaign, Illinois, USA	1876	Crop rotation and fertility experiment
Sanborn Field	Columbia, Missouri, USA	1888	Crop rotation and manure experiment
Magruder Plot	Stillwater, Oklahoma, USA	1892	Wheat fertility experiment
Old Rotation	Auburn University, Alabama, USA	1896	Cotton rotation and fertility experiment
Cullars Rotation	Auburn University, Alabama, USA	1911	Crop rotation and fertility experiment
Permanent Topdressing Experiment	Rutherglen Centre, Department of Primary Industries, Rutherglen, Victoria, Australia	1912	Pasture fertility experiment

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II. LONG-TERM FERTILITY AND COVER CROP EXPERIMENT: IMPACT ON SOIL HEALTH AND SOIL MICROBIOME

INTRODUCTION

At the turn of the 20th century, cotton had become the primary crop for most of the southeast United States due to the suitable climate and the traditional knowledge of cotton farming (Fite, 2009). In 1911, 12% of the land in Alabama was used to plant cotton and average lint yields at the time were approximately 240 kg ha^{-1} (USDA NASS, n.d.). One hundred and ten years later, the cotton acreage has dropped to 0.16% of the state's land but at the same time, yield has increased to an average of 889 kg ha^{-1} (USDA NASS, n.d.).

In the early 20th century, producers were beginning to understand that poorly managed and degraded land would not be sustainable. Conservation practices such as crop rotation were not common practice among Southeastern farmers as most could not afford to implement them (Fite, 2009). In 1911, the Alabama State Legislature passed an appropriation known as the "local-experiment" law that allowed the Alabama Agricultural Experiment Station to conduct on-farm soil fertility research throughout the state (USDA, 1916). Expansion of research allowed for scientists to provide practical information to farmers (Fite, 2009).

The Cullars Rotation, also known as the Alvis field, was started in 1911 by J. A. Cullars and John P. Alvis. Mr. Cullars and Mr. Alvis allowed Professor George F. Atkinson of Agricultural and Mechanical College of Alabama, now Auburn University, to conduct many cotton research projects on their farm. The fertility and crop rotation experiment, now known as the Cullars Rotation, is the only one of these experiments that is still conducted today. Under the "local-experiment" law, the land was bought by the Alabama Polytechnic Institute, now Auburn

University, in 1938 from the children of John P. Alvis. In 2003, the Cullars Rotation was placed on the National Register of Historical Places by the National Park Service. The experiment has now been running for 110 years and is the oldest continuous soil fertility experiment in the southern United States.

The southeast United States has a history of intensive land use dating back to before the Cullars Rotation began, and this experiment may allow for an understanding of how this intensive use of land is affecting soil health. Soil health is a complex assessment consisting of biological, physical, and chemical properties that all contribute to the overall productivity of the soil. Many dynamic soil health properties are indicative of the soil's ability to produce but evaluating only a few of these properties can lead to a false interpretation of a soil's production capacity. A complete understanding of the soil's health can be obtained by measuring a number of different soil properties such as the microbial community, the soil nutrient content and chemical composition, and the physical state of the soil. Additionally, conservation practices such as cover cropping and reduced tillage aim to improve the land degradation seen in this region, and effective soil health analysis can evaluate the extent to which these practices are able to restore the soil.

The soil microbial community can be measured through direct measurements such as microbial biomass carbon (MBC) or total bacterial counts, or it can be estimated by indirect evaluations such as soil respiration (R_s). Soil microorganisms, especially bacteria, are sensitive to soil amendments that alter soil pH (Rousk et al., 2010). Total microbial biomass has been found to decrease in the Southeast with increasing acidity (Hiltbold et al., 1985; Zhao et al., 2015). In addition to soil management, increasing atmospheric temperature and carbon dioxide (CO_2)

concentration can increase the soil microbial population in Southeastern soil types (Castro et al., 2010).

Soil chemical properties are commonly altered with soil amendments, but rarely are these soil amendments withheld for over 100 years on Southeastern cropland. Soil pH and nitrogen (N), phosphorous (P), and potassium (K) concentrations are typically maintained at certain levels, but the Cullars Rotation contains treatments which exclude each of these soil amendments. In addition to overall crop productivity, these chemical properties also affect other aspects of soil health. Soil organic carbon (SOC) is a commonly used soil health indicator, and nutrient management can impact SOC storage (Waqas et al., 2020). Reduced carbon (C) inputs due to reduced crop productivity can lead to a decrease in SOC. In the Southeast, Singh et al. (2020) found that conservation practices of cover cropping and reducing tillage were able to increase SOC. Permanganate oxidizable carbon (POXC), a fraction of SOC, can estimate the "active" fraction of carbon in the soil. This soil health indicator can be sensitive to crop residue left on the soil in the Southeast and also influenced by nutrient management or cover cropping (Singh et al., 2020). On a soil type similar to this region, Wang et al. (2017) found that cover crops were able to increase POXC in the soil. An emerging soil health indicator, autoclaved citrate extractable (ACE) protein, estimates the quality of soil organic matter, and can be sensitive to tillage, cover cropping, and residue retention (Cappellazzi & Morgan, 2021). However, ACE protein has not been extensively studied on Southeastern soil types and more information is needed to determine how it relates to overall soil health.

The physical structure of the soil, namely aggregate stability, can influence soil nutrient and water retention, root growth, and is a key aspect of soil health. Studies performed on Southeastern soil types found that conservation practices such as retention of residue was able to

improve soil aggregate stability (Levi, 2007; Cochran, 2010). Typically, management practices that increase soil organic matter will increase aggregate stability. However, organic matter is not the only component of soil aggregates, so not all soil types will show differences in aggregate stability due to management differences (Bronick & Lal, 2005).

Many of these soil health indicators have been studied in short-term experiments. The Cullars Rotation allows us to analyze how different soil properties interact to create sustainable crop production following fertility and cover crop management after a long period of use. The objective of this study is to evaluate a variety of soil health indicators along with the soil microbial community in order to determine the impact of over 100 years of soil fertility and cover crop treatments on the soil.

MATERIALS AND METHODS

EXPERIMENTAL DESIGN

The Cullars Rotation is located in Auburn, Alabama (32° 35' 15.8064" N lat; 85° 28' 56.4312" W long) and was established circa 1911 on a Marvyn loamy sand (fine-loamy, kaolinitic, thermic Typic Kanhapludult). The experiment consists of three blocks (east, middle, and west) each in a different stage of a cotton-corn-wheat-soybean rotation. The Cullars Rotation initially contained 11 fertility treatments, but in 1914 three additional treatments were added to study the effect of a leguminous cover crop. The three blocks are now divided into 14 treatments and each plot is 6.1 x 30.2 m (Figure 2.1). There is a 1 m alley between treatments and a 6 m alley between blocks. The dates of major field operations for this study are found in Table 2.1. Although the field contains 14 treatments, the following eight were used from each block for this

experiment: no N fertilizer applied (plot A), no N fertilizer applied or legume cover crop planted (plot B), no soil amendments and no legume cover crop (plot C), no legume cover crop with N applied (plot 1), no P fertilizer applied (plot 2), complete fertilization (plot 3), no K fertilizer applied (plot 6), and no agricultural limestone applied (plot 8; more detailed information about the treatments is found in Table 2.2). Initially, the Cullars Rotation was managed with conventional tillage; but after 1997 the field was converted to a conservation tillage system with annual in-row subsoiling.

SOIL AND PLANT SAMPLING

Soil samples were collected on April 27, 2020, July 7, 2020, and October 6, 2020, from each plot using a 2.5-cm diameter soil probe for the 0-10 cm depth. Approximately 15 cores were taken from each plot and composited in a bucket. Between sampling each plot, the probes and corresponding buckets were surface-sterilized with 70% ethanol to prevent cross contamination between samples. During the sampling events, collected soil samples were kept in a cooler until they could be processed. Immediately after sampling, each sample was sieved to 4 mm for analysis. A portion of each sample was kept at 4°C degrees for microbial analysis, a second portion was stored at -80°C for DNA analysis, and a third portion was air-dried at room temperature for chemical and physical analysis. A subset of the air-dried soil was finely ground with a coffee grinder for total carbon analysis by dry combustion.

Cover crop biomass was sampled prior to termination by collecting aboveground plant material from two 0.25-m^2 randomly-selected areas in each plot. The biomass was dried for at least 48 h at 60°C and weighed to obtain dry weight. Cash crop yields were hand harvested from

the two center rows of each plot. Cotton samples were ginned and yield is reported on a lint-yield basis.

ANALYSIS

Soil Microbial Analysis

Gravimetric water content (GWC) was determined at each soil sampling period. A subset of each soil was weighed into pre-weighed metal tins and dried at 105°C for 48 h, then the soil was weighed again to determine the GWC. To determine water holding capacity (WHC), a 125 mL Erlenmeyer flask containing a funnel lined with a No. 42 filter paper was weighed, and 20 g of field moist soil was placed inside the funnels. Next, 25 g of water was poured into each funnel, which were covered with foil and let stand overnight (approximately 12 h). The next morning, water remaining in the flasks was weighed. Gravimetric water content and WHC capacity were determined in order to perform microbial biomass carbon analysis.

Microbial biomass carbon and soil respiration were determined with chloroform fumigation incubation (CFI) according to Howarth and Paul (1994). Samples that were kept at field moisture and 4°C were weighed to 25 g on a dry-weight basis and placed into 150 mL beakers. Water was added to the samples to bring them up to 50% of their water holding capacity. The beakers were placed into mason jars with approximately 1.5 mL of water in the jar to prevent soil drying. The jars were closed, and all samples were incubated for 5 d at approximately 22°C. After this period, samples were placed into a desiccator, and a beaker of 40 mL ethanol-free chloroform with boiling chips was placed in the center. Once arranged, the desiccator lid was closed, and a vacuum hose was attached. A vacuum was drawn until the

chloroform started to boil, and the vacuum pump remained on for an additional 30 s. This step was repeated twice, and on the second time, the chloroform was boiled for 2 min. Once these steps were completed, the desiccator was sealed and kept in the dark for 24 h. After this incubation period, the desiccators were opened, and the beaker containing chloroform was removed. Residual chloroform was removed with the vacuum pump six times for 3 min. The beakers of soil were placed back into their mason jars, along with a vial containing 5 mL of sodium hydroxide (NaOH) to absorb CO_2. Samples were then incubated for 10 d at room temperature (22°C ± 1°C). After this second incubation period, the NaOH solution was removed from the jars and precipitated with 2 mL of 1.5 M barium chloride ($BaCl_2$) and then titrated with 0.25 M hydrochloric acid (HCl). Microbial biomass carbon was calculated according to Equation 2.1 (Howarth & Paul, 1994).

EQUATION 2.1:

$$Biomass\ C\ \left(\frac{ug}{g\ soil}\right)$$

$$= [(blank\ HCl\ \mu L - unknown\ HCl\ \mu L) * (0.25\ M\ HCl * 6)\ \div dry\ soil\ wt.(g)]/0.41$$

DNA extraction was performed using a DNeasy® Powersoil Pro Kit (Qaigen, Hilden, Germany). The extracted DNA was analyzed using quantitative PCR (qPCR) to determine quantities of fungi and bacteria populations. qPCR reaction mixtures consisted of 7.5 µl of SsoAdvanced Universal SYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA), 0.5 µl of

20 mg/mL bovine serum albumin (BSA), 0.5 µl of each 10 µM primer, 5 µl of soil DNA sample diluted to 1 ng μl^{-1}, and PCR grade H_2O to a final reaction volume of 15 µl.

16S primers used were Eub338 and Eub518 (Fierer et al., 2005) with an annealing temperature of 60°C. Thermocycling conditions were 95°C for 3 min, followed by 35 cycles of 95°C for 15 s and annealing temperature for 30 s, followed by a melting curve of 95°C for 15 s, 60°C for 30 s, and 95°C for 15 s. Samples were analyzed using a StepOne™ Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA). A 10:1 dilution series of bacterial plasmid (*Enterococcus faecalis*) was used as the standard curve for quantitation.

Soil Chemical Analysis

Soil pH was determined according to Sikora and Kissel (2014). A 20-g sample of air-dried, sieved soil was added to a 50 mL plastic tube and 20 mL of water was added to each sample. Samples were shaken for approximately 5 min and allowed to sit for 1 h. The pH was then read using a sympHony™ benchtop meter (VWR International, Radnor, PA).

Autoclaved citrate extractable protein was determined according to the modified procedure by Schindelbeck et al. (2016). Three grams of air-dried soil was placed into plastic 50-mL tubes and extracted with 20 mM sodium citrate, pH 7.0. Samples were shaken at 180 rpm for 5 min and autoclaved at 121°C and 15 psi. The suspension was clarified by centrifuging at 10,000 g for 3 min and the supernatant was used for protein analysis by bicinchoninic acid assay. Using a 96-well plate, samples were mixed with a working reagent composed of a copper sulfate solution and a clear reagent mixture. Standards were prepared using bovine serum albumin. Samples were incubated at 37°C for 60 min. Finally, samples were read using a µQuant

microplate reader (Biotek, Winooski, VT) at 562 nm. The ACE protein content was determined according to Equation 2.2, where *a*, *b*, *c* are derived from quadratic regression analysis of the standards (Schindelbeck et al., 2016).

EQUATION 2.2:

$$mg\ ACE\ g^{-1}\ soil = \left(\frac{-b \pm \sqrt{b^2 - 4ac}}{2a}\right) * 8\ \frac{mL\ solution}{mL\ soil} \div 1000\ \frac{\mu l}{mg}$$

Mehlich-I extractable nutrients were analyzed according to Hue and Evans (1979). A 5-g sample of air-dried, sieved soil was mixed with 20 mL of Mehlich-I solution (0.05 N HCl + 0.025 N H_2SO_4). Samples were shaken at a rate of 180 oscillations min^{-1} for 5 min. After shaking, samples were filtered with No. 1 filter paper and analyzed with an ICP-MS to determine extractable concentrations of P, K, Ca, and Mg. Cation exchange capacity (CEC) was determined with the ammonium acetate method (pH 7) using an auto-extractor according to Sumner and Miller (1996).

Permanganate oxidizable C was determined according to Weil et al. (2003). A 2.5-g sample of sieved, air-dried soil was placed into a 50 mL centrifuge tube, and 18 mL of deionized water and 2.0 mL of 0.2 M potassium permanganate ($KMnO_4$) stock solution were added to each tube. Samples were hand shaken for 2 s and placed on a shaker at 240 oscillations min^{-1} for 2 min. Samples were then placed into a dark area for 10 min to allow the soil to settle. After this time, 0.5 mL of sample was placed into a new 50 mL centrifuge tube along with 49.5 mL of deionized water. Samples were inverted to mix, and analyzed on a μQuant microplate reader

(Biotek, Winooski, VT) at 550 nm. The absorbance measured from the unknown samples were compared to a set of standards with known concentrations of $KMnO_4$ solution. The POXC levels were determined with Equation 2.3 where *abs* is the measured absorbance, *a* is the intercept of the standard curve, and *b* is the slope of the standard curve (Weil et al., 2003).

EQUATION 2.3:

$$mg\ POXC\ kg^{-1} = \left[0.02\ M - \left(a + (b * abs)\right)\right] * 9000\ \frac{mg\ C}{mol} * 0.02\ \frac{L\ solution}{kg\ soil}$$

Soil organic carbon (SOC) was determined with combustion analysis. Soils were air-dried and ground to a fine powder with a coffee grinder. A $CHNS/S_{IR}$ elemental analyzer was used (Elementar, Lagensebold, Germany).

Soil Physical Analysis

Water stable aggregates (WSA) were determined with the wet sieve method described by Kemper and Rosnenau (1986). A portion of the air-dried soil from each sample was sieved to 1-2 mm to isolate aggregates. Four grams of soil was then weighed and placed into 240 mesh mm^{-1} sieves. Samples were wetted slowly to a moisture level near field capacity using a humidifier. The humidified samples were immersed in deionized water using a Wet Sieving Apparatus (Eijkelkamp Soil & Water, Giesbeek, The Netherlands) at a rate of 35 times min^{-1} for three minutes into pre-weighed metal containers. After this 3-minute period, new pre-weighed containers were placed beneath the sieves that contained a diluted sodium hexametaphosphate

($Na(PO_3)_6$) and NaOH dispersal solution. Samples were then lowered into this solution at a rate of 35 times min^{-1} until all aggregated soil had been dispersed. Each metal container was dried at 105°C until all water had evaporated. The metal containers were weighed again, and the weight of the soil collected, corrected for the dispersal solution, and used to determine the percentage of WSA (Equation 2.4)(Kemper & Rosnenau, 1968).

EQUATION 2.4:

$$WSA\% = [soil\ wt.after\ sieving\ (g) - wieght\ of\ dispersal\ solution]/[4\ g - sand\ fraction\ (g)] * 100$$

Particle size analysis was determined according to the Gee and Bauder (1986) pipet method. Ten grams of an air-dried soil sample was placed into a 250 mL centrifuge bottle with 50 mL of water and 10 mL of dispersing agent (Na_2CO_3 and $Na_6(PO_3)_6$). Samples were shaken for 16 h. After shaking, samples were sieved to 53 μm to remove the sand fraction. The sand was washed with acetone and dried at 100°C until completely dry. The remaining fraction was placed in a 1000 mL cylinder with distilled water. The suspension was mixed for 5 min and let stand for 5 h. After this time, 25 mL of the suspension was pipetted out and placed into a 50 mL beaker containing 25 mL of water. This suspension was then dried at 100°C for approximately 12 h. Both fractions were weighed to determine percent sand and clay.

Data Analysis

Data were analyzed with SAS® v. 9.4 using the PROC GLIMMIX procedure. Mean separations were determined using Tukey's significant difference (HSD) test ($\alpha = 0.05$). Treatment, sampling date, and the interactions between variables were analyzed as fixed effects. Randomized experimental design was not done due to the historical design of the experiment, so block could not be used for a random statement. Due to the length of the experiment and number of crop rotations each block has undergone, each block was treated as replication of the treatments despite each block being in a different crop each year. Data that did not achieve normality were log or square root transformed to achieve normality.

RESULTS

Soil Characterization

Values for soil pH were not able to meet assumptions for normality, but pH was correlated with many other normally-distributed soil health indicators measured indicating that this analysis is robust. Soil pH varied according to treatment ($P<0.0001$). As expected, the two treatments that had not received lime for the duration of the experiment had the lowest pH values (Table 2.3). The 'no lime' treatment had the lowest soil pH of 4.51. The 'no amendment' treatment had a pH of 5.06. The lower pH in the 'no lime' treatment is likely due to the fact that ammonium-based fertilizers, including those used in this experiment, are acidifying (Bloom et al., 2005). For Alabama soil types, lime is recommended for cotton, corn, and soybean crops if the soil pH is lower than 6.0 (Mitchell & Huluka, 2012). All other treatments have a soil pH

above 6.0 since lime is applied to all treatments except the ‘no lime’ and ‘no amendment’ treatments when soil pH tests lower than 6.0.

Mehlich-I extractable nutrient concentrations also varied according to treatment. As expected, calcium (Ca) and magnesium (Mg) concentrations were lowest in the two treatments that have never received lime, and ‘no amendments’ was lower in Ca than the ‘no lime’ treatment (Table 2.3). The highest numerical Ca value was found in the ‘no K’ treatment, which may be due to reduced competition on cation exchange sites in the soil when soil K is limited. Values for Mehlich-I extractable K did not meet assumptions for normality, but due to the large sample size the analysis is still considered to be robust despite the lack of normality. Potassium concentrations were lower than all other treatments in the ‘no K’ and ‘no amendment’ treatments with the exception of ‘no lime’ (Table 2.3). Despite receiving K fertilizer, the ‘no lime’ treatment had lower Mehlich-I extractable K than the ‘complete’ and ‘no N/+legume,’ which both received K fertilizer. The retention of K by the soil may be lower in the ‘no lime’ treatment because the cation exchange sites are dominated by aluminum and hydrogen ions in low pH soils. The ‘no K’ treatment had higher P than every other treatment except ‘no lime’ (Table 2.3). The results show that P concentrations were lowest in treatments that did not receive any P fertilizer, and highest in plots with very low fertility but still received P fertilizer. This is likely because the reduced plant growth decreased overall nutrient uptake, leaving the majority of P fertilizer in the soil. Since P is not readily leachable, it can remain in the soil and build up if not taken up by plants. All treatments except the ‘no P’ and ‘no amendment’ treatments had Mehlich-I extractable P concentrations above the critical soil test level for cotton, corn, and soybean production in Alabama soil types (Mitchell & Huluka, 2012).

Across all treatments, the soil texture was similar (Table 2.3). Soils of the Cullars Rotation are sand dominated (81-85%) Coastal Plain soils with little clay (2.5%). Fertility treatments are not expected to change soil texture, and soil types across treatments are still comparable in this long-term experiment. However, CEC can be changed with management. Southeastern soils have inherently low CEC, and it is affected by pH, clay type, and organic matter. All treatments had the same percentage of clay content, so pH and organic matter were the driving factors that affected treatments for this experiment. The 'no amendment' treatment had very low pH and organic matter and had less than half of the CEC of all other treatments (Table 2.3). The 'no lime' treatment also had a low soil pH and organic matter content, but its CEC was equivalent to that of the 'complete' treatment. Despite its high CEC, the 'no lime' soil would likely not be able to retain as many plant essential nutrients as the 'complete' treatment because the 'no lime' exchange sites are likely dominated with aluminum and hydrogen ions due to its low soil pH.

Microbial Biomass Carbon

Microbial biomass C was significantly affected by treatment and sampling date (Table 2.4). The 'no lime' and 'no amendment' treatments had lower MBC than all other treatments (Table 2.5). These low soil pH treatments had about 30% less MBC than all other treatments. Due to reduced fertility and therefore reduced carbon inputs into the soil, there was a reduced energy source for heterotrophic microorganisms. In addition, Buchanan and King (1991) found that in systems with limited chemical fertilization MBC is related to C biomass inputs. All other treatments had similar quantities of MBC despite varying levels of fertility and soil organic matter. Research by Zhao et al. (2015) found that soil pH is the primary driver of the microbial

community in the Cullars Rotation, and both of the treatments with lower MBC had very low soil pH (<5.0) which may indicate that low soil pH can hinder bacterial growth. A meta-analysis of 41 studies by Kallenbach and Grandy (2011) found that organic inputs into the soil increased the MBC by an average of 36%.

The first sampling date (April 27, 2020) had higher MBC compared to the mid- and late-season sampling times (data not shown). Microbial biomass carbon can have a major spring peak with a decline in summer due to the decrease in the liable C pool during the growing season (Buchanan & King, 1991). At the end of the growing season, crop residue enters back into the soil and is able to be utilized by microorganisms. This may have led to the elevated levels of MBC in the spring season. However, weather conditions and moisture levels at the time of sampling may also have contributed to these differences (Hamel et al., 2006).

Microbial biomass C was significantly and positively correlated with many other soil health indicators measured including R_s, SOC, POXC, and ACE protein (Table 2.6). The correlation between MBC and R_s can be attributed to very similar laboratory measurement procedures. This correlation has been observed in other Southeastern studies for the top 5 cm of soil (Parajuli et al., 2021). Soil organic matter is both critical to the microbial community and partially composed of MBC.A strong correlation between SOC and MBC was observed in the current study. Diaz-Ravina et al. (1988) and Parajuli et al. (2021) also found that MBC and SOC correlated on similar soil types. Permanganate oxidizable C estimates the biologically labile C, meaning that it can be used by the microbial community (Weil et al., 2003). Correlations between MBC and POXC have also been found across a wide variety of soil types (Weil et al., 2003; Geraei et al., 2016; Bongiorno et al., 2019; Parajuli et al., 2021). There is limited research on the relationship between MBC and ACE protein. Since ACE protein is a measure of N that is

available to soil microorganisms, higher amounts of N can determine the rate at which microorganisms are able to metabolize carbon. Microbial biomass C was also correlated to soil moisture content.

Microbial biomass C was correlated to soil pH and seems to be more closely related to soil pH than soil fertility (Table 2.6). Despite low crop yields in 'no K', 'no P', and 'no N/-legume' treatments, the total microbial population was as large as that of the highest yielding 'complete' treatment. Soil amendments applied to these treatments were enough to support the same size population. Cover cropping, which is typically regarded as a conservation practice that improves soil health, did not show an effect on total microbial biomass. The only factor that caused a difference in the total microbial community after over 100 years of fertility treatments was soil pH.

Soil Respiration

Soil respiration was affected by treatment and sampling date (Table 2.4). The low fertility and low soil pH treatments (i.e., 'no lime', 'no amendment') had lower R_s values than all other treatments, but no other treatment effects were observed (Table 2.5). Both R_s and MBC were analyzed with the same laboratory procedure, and therefore have similar results. The 'no lime' and 'no amendment' treatments reduced the size of the microbial community, and therefore reduced levels of respiration. Respiration was approximately 50% lower on the third sampling date compared to the first two dates (data not shown). Other studies have found that soil respiration can taper off toward the end of the season, with periods of high respiration corresponding with rainfall and periods of maximum crop growth (Rochette et al., 1991). Crop

residue quantity and quality are the primary source of variability in R_s across the same soil types (Franzluebbers et al., 1995).

Soil respiration was correlated to other soil health indicators including POXC, ACE protein, and SOC, but the correlations with these soil health indicators were not as strong as they were to MBC (Table 2.6). Parajuli et al. (2021) also found a correlation between R_s and POXC and SOC at the 5-15 cm depth on a Southeastern soil. The relationship between R_s and ACE protein has not been studied on Southeastern soil types, but two Canadian studies have found a positive correlation between the two soil health indicators (Mann et al., 2019; Marshall et al., 2021).

Microbial Community Analysis

The quantity of total bacteria was affected by treatment and sampling date (Table 2.4). Across all sampling dates, the 'no lime' and 'no amendment' treatments had lower concentrations of bacteria than all other treatments (Table 2.5). Although these low soil pH treatments (<5.06) had fewer bacteria, they were still within the same order of magnitude (10^8 gene copies per gram of soil), and they were far from being baren. In an experiment across a strip gradient of soil pH values, Rousk et al. (2010) found a similar decrease in bacteria communities in soils with a pH less than 5 using the phospholipid fatty acid method of analysis. Additionally, the lack of fertilizer in the 'no amendment' treatment may have resulted in reduced bacteria counts. In a Swedish study using qPCR, Wessen et al. (2010) found that an unfertilized control can have lower amounts of bacteria than some fertilized treatments. However, in the same study, a treatment subjected to an acidifying fertilizer without correction for soil pH had a lower

bacteria count than the unfertilized control. A second study by Shen et al. (2010) found that after 23 years of N fertilization with no correction for soil pH, treatments with no fertilizer had higher total bacteria counts than those that did receive N. In both of these studies, the soil pH of the fertilized treatment was much lower than the unfertilized control, which is similar to the 'no lime' treatment for this experiment. However, bacteria counts were equivalent to the 'no amendment' treatment despite having a lower soil pH in this study. Both of these low soil pH treatments are well below the threshold for optimal bacterial growth, so differences in pH below that point may not create distinguishable differences in population size. In addition to the two low soil pH treatments, the 'complete' treatment also contained more total bacteria than the 'no K' treatment, but 'no K' was not different from any other higher fertility treatment (Table 2.5). Similar to other soil microbial indicators, the legume cover crop did not show an effect on bacterial populations.

Total bacteria counts were correlated with MBC, R_s, POXC, ACE protein, total nitrogen (TN), and SOC (Table 2.6). These correlations indicate that these soil health indicators are strongly related to the soil bacterial community, with the exception of WSA which did not have a significant relationship with bacteria. The majority of the bacteria in soil uses organic carbon as an energy source, and therefore an increase in SOC or POXC would allow for a larger bacteria population. Additionally, MBC and R_s are direct measures of the microbial population, and a large portion of that population is bacteria. Although total bacteria analysis is not widely used as a soil health indicator, its correlation to other common indicators show that it could be a useful tool for soil evaluation in the future. In addition to soil health indicators, total bacteria was also correlated to soil moisture and soil pH.

This study shows that even in soil that plants are unable to grow, soil bacteria are still abundant, they are just not at peak volume. It is clear that soil pH is the driving factor of the soil microbial community size, but the total bacterial population was also reduced in the 'no K' treatment. This indicates that these soil nutrients essential to plants may also play a role in shaping the size of the bacteria population.

Soil Organic Carbon

Treatment, sampling date, and the interaction between the two influenced SOC (Table 2.4). At the first sampling date, the 'complete fertility' treatment had 71% higher SOC than the 'no amendment' treatment, and 31-49% higher SOC than the 'no lime', 'N/-legume', 'no K', and 'no P' treatments (Figure 2.2). The recently terminated cover crop may have contributed to the increased SOC levels by increasing carbon inputs into the soil, creating a bank of C right before the cropping season. Kong et al. (2005) found that winter legume cover cropping was able to increase SOC in the long term (10-years). Limited fertility may have reduced plant biomass inputs into the 'no lime', 'no P', 'no K', and 'no amendment' treatments, resulting in lower SOC. Additionally, the higher MBC at the first sampling date may have increased the total SOC content.

At the second sampling date there were fewer differences in SOC, but the 'no amendment' plot had 53-63% lower SOC than the 'complete fertility', 'N/-legume', 'no N/+legume', and 'no K' treatments (Figure 2.2). Similar results were seen in the final date, where the 'no amendment' treatment was 50-58% lower in SOC than the 'complete fertility' and 'no P' treatments. The 'no P', 'N/-legume', 'no K', and 'no lime' treatments were not different

from the ‘complete’ at the second and third sampling dates. Soil organic C was not correlated with P, but it was moderately correlated with extractable K, Ca, and Mg content (Table 2.6). This indicates that P may play a smaller role in SOC than other soil amendments as Margenot et al. (2015) also found. At the second and third sampling times, the ‘no lime’ treatment did not differ from the ‘complete’. Although there was low yield in this treatment, some plants can grow enough to return a small amount of biomass into the soil. The SOC concentration in the ‘no lime’ treatment may be due to the smaller microbial community in the ‘no lime’ treatment that is unable to convert SOC that is in the soil. Although MBC makes up a small fraction of SOC, the reduced microbial biomass in low fertility treatments may be somewhat reflected in SOC concentrations.

Across all sampling dates the ‘complete’ treatment was higher in SOC than all other treatments (Table 2.5). The ‘no amendment’ treatment was lower in SOC than any other treatments and was almost three times lower in SOC than the ‘complete’ (Table 2.5). The long-term Morrow Plot experiment in Illinois found a larger decrease in SOC over time in the no soil amendment treatment compared to treatments that were fertilized (Nafziger & Dunker, 2011).The incorporation of a winter legume cover crop into the rotation may have increased the SOC at the first sampling date (Table 2.5; Figure 2.2). A study by Wuest (2014) also found increased SOC concentrations in the months just before planting, and lower SOC concentrations during the growing season in the top 7 cm of soil. For the most part, SOC levels relate to crop yields typically seen in each of these treatments (Table 2.7). Nafziger and Dunker (2011) also found differences in SOC to correlate to crop yield. Soil organic C content of a soil can aid in nutrient and water retention and therefore help in crop growth. Additionally, increased crop growth will lead to increased C inputs returning to the soil. Soil organic C was strongly

correlated with most other soil health indicators measured, and these relationships are shown in Figure 2.3. It is commonly thought that increased N fertilizer can increase soil C stocks, but the 'N/ -legume' and 'no N/ -legume' treatments did not differ in SOC at any sampling date, indicating that without a legume cover crop N fertilizer does not increase SOC in this soil type. Similar results were also seen at the Morrow Plots, where Khan et al. (2007) found that N fertilizer caused a decrease in SOC over time.

Soil organic carbon is typically regarded as the primary soil health indicator. However, results show this indicator may not always be useful by itself. Other indicators, like soil pH, are necessary in order to fully understand the health of a soil. These results show that maintaining soil fertility is important for building SOC in Coastal Plain soils, because improved fertility resulted in larger C biomass additions to the soil. Other soil fertility parameters, like soil pH, appear to be more useful in predicting crop production in this experiment, although SOC was correlated to soil pH (Table 2.6). SOC was one of the few selected soil health indicators that was responsive to legume cover cropping, although this difference was not apparent at every sampling time.

Permanganate Oxidizable Carbon

Permanganate oxidizable C was influenced by treatment and also had a treatment by sampling date interaction (Table 2.4). There was not a sampling date effect on POXC. Across every sampling date, the 'complete' treatment had 25% or more POXC than every other treatment (Table 2.5). The 'no amendment' treatment had the lowest POXC concentration, which was10 times lower than the 'complete'.

On the first sampling date, every treatment but ‘no P’ contained higher POXC than the ‘no amendment’ control (Figure 2.4). The ‘no P’ treatment was 61% lower in POXC than the ‘complete fertility’ treatment but was not different from any other treatment. At the second sampling date, the ‘complete fertility’ treatment was higher than the ‘no P’, ‘no N/-legume’, and ‘no amendment’ treatments. The ‘no amendment’ treatment also performed lower than every other treatment at this sampling date and at the third. On the third sampling date, the ‘complete fertility’ treatment was higher in POXC than the ‘no K’ and ‘no amendment’ treatments. There is minimal research on the links between P and K and POXC, but a deficiency in N, P, or K can stunt plant growth, and therefore decrease plant biomass C available to the soil after the growing season is over (Halvin et al., 2014). Changes in treatment differences in POXC at different sampling dates may be due to fluctuation in plant biomass decomposition and microbial biomass throughout the growing season.

Levels of POXC in the ‘no N/-legume’ treatment consistently decreased over the cropping season, whereas most of the other treatments either increased or peaked at the second sampling date. The lower biomass inputs and limited supply of N over the growing season may have led to a lower active C pool. The ‘complete’ treatment remained numerically higher than every other treatment throughout the growing season (Figure 2.4). Similarly, the ‘no amendment’ treatment was the lowest at every sampling date. This is consistent with findings by Zhang et al. (2020) who found that chemical fertilizer increased POXC levels in a long term (10-year) experiment. Although the ‘no lime’ treatment had very low fertility and low carbon biomass inputs, its POXC levels were not different from complete fertility at any single sampling date but was lower across all sampling dates (Table 2.5). This may be due to the low microbial biomass not being able to utilize POXC in the soil since soil microbes are less active at a low soil pH, and

POXC is built up instead of metabolized by microorganisms. Vazquez et al. (2019) also found that POXC was not affected by liming.

Permanganate oxidizable C was significantly correlated with SOC (Table 2.6), and this was also observed by Culman et al. (2012), Lucas and Weil (2012), Bongiorno et al. (2019), Parajuli et al. (2021), and Huang et al. (2021). Across a variety of climates, Bongiorno et al. (2019) found that POXC was significantly and strongly correlated to other physical, biological, and chemical soil measurements including SOC compared to other C fractions. Permanganate oxidizable C was also correlated to soil moisture and pH.

Weil et al. (2003) found POXC was responsive to SOC building practices (Weil et al., 2003), but at the Cullars Rotation POXC was not different between cover crop treatments after over 20 years of conservation tillage at any sampling time when other management factors were held consistent. This may indicate POXC does not respond to conservation practices that aim to improve soil C on Coastal Plain soils. Additionally, the low fertility of the 'no lime' treatment was not clearly reflected in POXC, which also leads to the conclusion that POXC is not reflective of all crop management practices despite POXC being highly correlated to most other soil health indicators.

Autoclaved Citrate Extractable Protein

Treatment affected ACE protein, but sampling date did not (Table 2.4). Despite having lower levels of MBC, SOC, and POXC than the 'complete' treatment, the 'no lime' treatment had ACE protein levels equivalent to the 'complete fertility' treatment and were both 67% higher than the control (Table 2.5). As expected, the 'no amendment' treatment had the lowest ACE

protein. This treatment had similar fertility to the ‘no lime’ treatment and performed similarly to ‘no lime’ in other soil health indicators. However, the ‘no lime’ treatment had much higher ACE protein content than the ‘no amendment’ treatment across all sampling times. This is similar to the SOC results at each sampling date (Figure 2.2). Not only was ‘no lime’ higher than ‘no amendment’, but this treatment also had higher ACE protein levels than ‘no N/-legume’, ‘N/-legume’, ‘no P’, and ‘no K’. The ‘no lime’ treatment received all other fertilizers and had fewer organic inputs, but due to the low soil pH, the microbial community was not able to breakdown the ACE protein or SOC in the soil. This may also partially explain why the ‘no lime’ treatment had a CEC similar to the ‘complete’. Soil organic C can contribute up to 30% of total soil CEC at neutral soil pH levels, but in acidic conditions the CEC of SOC is lower (Solly et al., 2020).

The ‘no N/-legume’ treatment was lower in ACE protein than the ‘no N/+legume’ treatment indicating that the addition of a winter legume with limited N can increase N available to microorganisms after over 20 years of cover cropping with conservation tillage (Table 2.5). ACE protein was correlated with TN, as ACE protein represents a pool of soil N (Table 2.6; Geissler et al., 2019). These results confirm that ACE protein can be used as an indicator of N that also responds to conservation management.

Autoclaved citrate extractable protein was also strongly correlated with POXC indicating that these C and N fractions are very closely related (Table 2.6). This relationship along with ACE protein’s correlation to other soil health indicators indicates that it is useful as a soil health indicator, but the ACE protein of the ‘no lime’ treatment was not aligned with soil fertility. Again, other soil factors and soil health analyses are necessary in order to determine the full effect that soil fertility treatments have on the soil health of this system. Most other soil health

indicators measured were correlated with soil pH, but ACE protein was not (Table 2.6). This indicates that in this low pH system, ACE protein may not be an effective indicator of soil health.

Aggregate Stability

Aggregate stability was not significantly affected by sampling date or treatment (Table 2.4). Despite the long duration of this experiment, fertility and cover crop treatments did not seem to significantly affect WSA on this soil type. Organic matter is inherently low in Southeastern soils (1-3%) which may have led to lower WSA overall, making it difficult to detect differences between treatments. However, numerically, higher fertility treatments corresponded to higher WSA values, whereas lower fertility treatments corresponded to lower WSA values (Table 2.5). Other researchers have found that cover crop did not affect WSA (Snapp & Surapur, 2018), while others found positive effects of cover crops across a variety of production systems (Lui et al., 2005; Sapkota et al., 2012; Steele et al., 2012; Locke et al., 2013). A meta-analysis by Blanco-Canqui and Ruis (2020) found that cover crops increased WSA in about 50% of studies analyzed.

Aggregate stability was not correlated with any other soil parameter measured (Table 2.6). On this sandy, Coastal Plain Ultisol, clays and oxides are the key binding agent of soil aggregates, and therefore organic matter may not have a large effect on WSA (Huang et al., 2010).

CONCLUSION

A number of biological, chemical, and physical soil health indicators were measured at the Cullars Rotation. After over 100 years of soil fertility treatments, soil texture did not change, but CEC, soil pH, SOC, POXC, MBC, ACE protein, R_s and total bacteria counts changed dramatically. The treatment with complete fertility tended to improve soil health based on selected soil health indicators measured, and the treatment that had not received any soil amendments tended to degrade soil health.

The soil microbial community showed a decrease in size with a decrease in soil pH. For the most part, lower fertility treatments led to a decrease in carbon inputs and therefore were lower in SOC and POXC than the complete fertility treatment. However, the treatment which did not receive lime had levels of SOC, POXC, ACE protein, and CEC comparable to the higher fertility treatments. This may be due to the smaller microbial community's limited capacity to break down organic matter. The treatment which had not received any soil amendments had a very low CEC, but the treatment which had not received lime had a CEC equivalent to all other amended treatments due to the high SOC content of this treatment. This indicates that these soil health analyses may not always reflect soil health and may not be able to predict crop productivity levels. Despite this, there were high correlations between most soil health indicators with the exception of aggregate stability. However, as results have shown, these indicators used individually do not provide a full understanding of the soil's health. When used to evaluate very low fertility treatments, some soil health indicators may be misleading, and a variety of indicators should be used to understand complex soil dynamics that contribute to soil health and productivity.

TABLE 2.1. Dates of field operations at the Cullars Rotation in the 2020 cropping season.

Crop	Block	Planting Date	Harvest Date
Legume Cover Crop	East	24 October 2019	6 April 2020
Wheat	Middle	19 November 2019	1 June 2020
Corn	East	16 April 2020	8 September 2020
Cotton	West	4 May 2020	1 October 2020
Soybean	Middle	3 June 2020	23 November 2020

TABLE 2.2. Description of treatments at the Cullars Rotation.

Plot	Treatment description	Winter legume	Fertility amendments					Year est.
			Nitrogen	Phosphorous	Potassium	Sulfur	Lime	
A	No N/ +legume	✓		✓	✓	✓	✓	1914
B	No N/ -legume			✓	✓	✓	✓	1914
C	No amendments	✓						1914
1	N/ -legume		✓	✓	✓	✓	✓	1911
2	No P	✓	✓		✓	✓	✓	1911
3	Complete	✓	✓	✓	✓	✓	✓	1911
6	No K	✓	✓	✓		✓	✓	1911
8	No lime	✓	✓	✓	✓	✓		1911

TABLE 2.3. Soil characterization data by treatment at the Cullars Rotation including soil pH, Mehlich-I extractable Ca, Mg, K, and P, cation exchange capacity (CEC), and soil texture at 0-10 cm.

						Soil Texture			
Treatment	**pH_{H2O}**	**Ca**	**Mg**	**K**	**P**	**Sand**	**Silt**	**Clay**	**CEC**
		mg kg^{-1} soil				%			cmol (+) kg^{-1} soil
Complete	6.73 ab*	681 a	78.5 ab	48.5 a	55.4 c	82.5	15.0	2.5	4.5 a
No N / +legume	6.41 b	521 ab	47.8 b	50.0 a	75.1 b	81.7	15.8	2.5	4.4 a
No N / -legume	6.83 a	584 a	58.0 ab	44.4 ab	74.9 b	82.5	15.0	2.5	3.8 a
N / -legume	6.70 ab	529 ab	65.4 ab	44.2 ab	49.2 c	82.5	15.0	2.5	3.9 a
No P	6.96 a	389 b	90.4 a	42.4 ab	7.19 d	83.3	14.2	2.5	3.6 a
No K	6.70 ab	740 a	86.8 a	20.3 cd	122 a	83.3	14.2	2.5	3.6 a
No Lime	4.51 d	60.6 c	5.53 c	33.4 bc	94.0 ab	84.2	13.3	2.5	4.3 a
No Amendments	5.06 c	27.0 d	6.89 c	18.3 d	8.69 d	85.0	12.5	2.5	2.3 b

*Values followed by the same letter within a column are not significantly different according to Tukey's HSD at $\alpha = 0.05$.

TABLE 2.4. Summary of analysis of variance (ANOVA) for selected soil health indicators including permanganate oxidizable carbon (POXC), water stable aggregates (WSA), soil organic carbon (SOC), autoclaved citrate extractable (ACE) protein, soil respiration (R_s), and microbial biomass carbon (MBC) in response to treatment, sampling date, and their interaction at the Cullars Rotation at 0-10 cm.

	ANOVA (Pr > F)							
Source of Variance	**df**	**ACE Protein**	**MBC**	**R_s**	**WSA**	**SOC**	**POXC**	**Total Bacteria**
Treatment (T)	7	<0.0001	<0.0001	<0.0001	0.1243	<0.0001	<0.0001	<0.0001
Date (D)	1	0.8409	0.0003	0.0001	0.8921	<0.0001	0.3656	0.0014
T x D	7	0.3352	0.2248	0.2495	0.0537	0.0149	0.0414	0.9853

Significant effects are determined at Tukey's HSD at $\alpha = 0.05$.

TABLE 2.5. Effect of fertility treatments and winter legume across all sampling dates on autoclaved citrate extractable (ACE) protein, microbial biomass carbon (MBC), soil respiration (R_s), water stable aggregates (WSA), soil organic carbon (SOC), permanganate oxidizable carbon (POXC), and total bacteria gene copies at the Cullars Rotation in 2020 at 0-10 cm.

Treatment	ACE Protein	MBC	R_s	WSA	SOC	POXC	Total Bacteria
	mg g^{-1} soil	mg C g^{-1} soil	µg CO_2-C g^{-1} soil	%	g kg^{-1} soil	mg kg^{-1} soil	GC g^{-1} soil
Complete	4.80 a*	295 a	104 a	87.28	9.58 a	406 a	7.32 x 10^8 a
No N / +legume	4.28 ab	252 a	85.6 a	85.69	7.64 b	307 b	6.49 x 10^8 ab
No N / -legume	3.38 cd	230 a	69.7 a	86.74	6.56 b	254 b	5.95 x 10^8 ab
N / -legume	3.90 bc	240 a	90.2 a	83.98	7.49 b	302 b	6.44 x 10^8 ab
No P	3.16 d	225 a	78.1 a	85.07	6.47 b	241 b	4.52 x 10^8 ab
No K	3.34 cd	243 a	77.2 a	89.48	6.52 b	257 b	4.27 x 10^8 b
No Lime	4.66 a	86.1 b	14.1 b	86.70	6.81 b	257 b	2.33 x 10^8 c
No Amendment	1.57 e	70.6 b	13.1 b	84.84	3.37 c	50.8 c	1.69 x 10^8 c

*Values followed by the same letter are not significantly different within a column according to Tukey's HSD at α= 0.05.

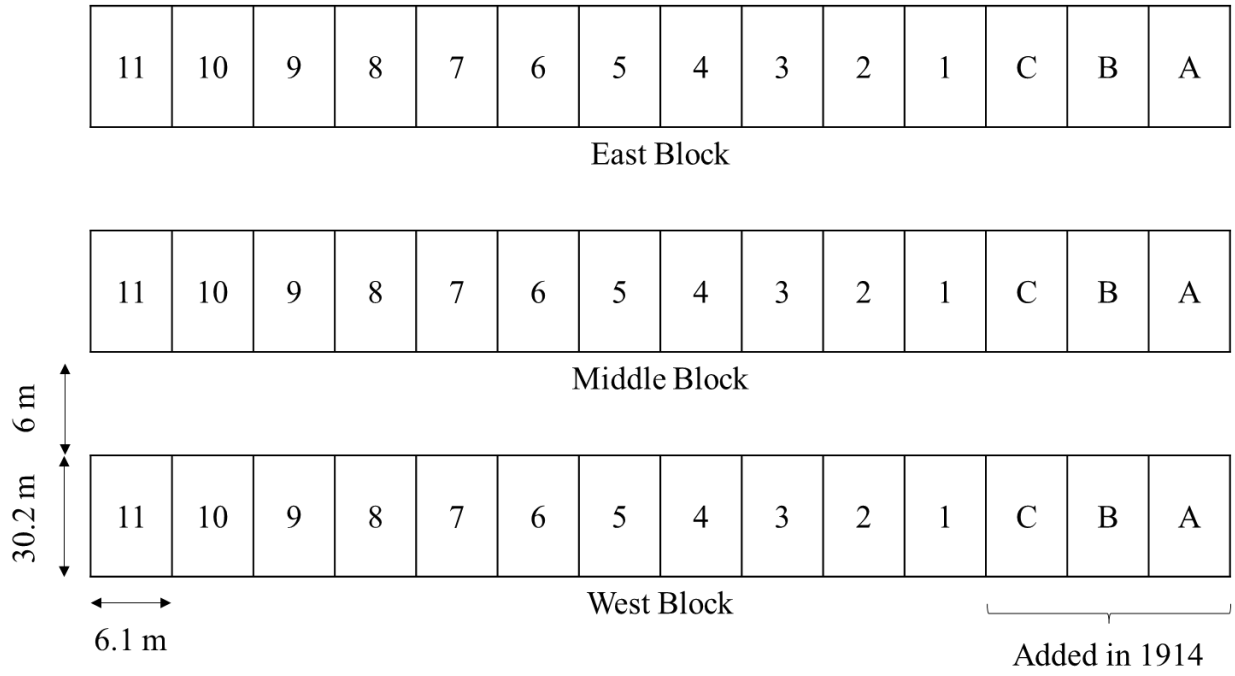
TABLE 2.6. Summary of analysis of Pearson Correlation Coefficients of soil moisture (θ_m), soil pH, microbial biomass carbon (MBC), soil respiration (R_s), permanganate oxidizable carbon (POXC), autoclaved citrate extractable protein (ACE), water stable aggregates (WSA), Mehlich-I extractable Ca, K, Mg, and P, total nitrogen (TN), soil organic carbon (SOC), and total bacteria gene copies (TB) across all sampling dates at 0-10 cm using 72 observations (N). "ns" indicates Pearson's Correlation Coefficients are not significant at $\alpha = 0.05$.

Pearson Correlation Coefficients, n = 72														
Prob > |r| under H0: Rho=0														
Variables	**θ_m**	**pH**	**MBC**	**R_s**	**POXC**	**ACE**	**WSA**	**Ca**	**K**	**Mg**	**P**	**TN**	**SOC**	**TB**
θ_m	1	0.5467	0.5002	0.6007	0.7384	0.4820	ns	0.6257	0.4683	0.5287	ns	0.6137	0.5686	0.4301
		<.0001	<.0001	<.0001	<.0001	<.0001		<.0001	<.0001	<.0001		<.0001	<.0001	0.0002
pH		1	0.7017	0.5090	0.4374	ns	ns	0.8308	0.3421	0.8430	ns	0.3386	0.3680	0.4837
			<.0001	<.0001	0.0001			<.0001	0.0033	<.0001		0.0036	0.0015	<.0001
MBC			1	0.5300	0.5778	0.4049	ns	0.6891	0.3070	0.6236	ns	0.5793	0.6856	0.6651
				<.0001	<.0001	0.0004		<.0001	0.0087	<.0001		<.0001	<.0001	<.0001
R_s				1	0.4813	0.2645	ns	0.4772	0.2831	0.4447	ns	0.4020	0.4661	0.4630
					<.0001	0.0248		<.0001	0.0160	<.0001		0.0005	<.0001	<.0001
POXC					1	0.8279	ns	0.6290	0.5831	0.5037	0.3279	0.7414	0.7980	0.5664
						<.0001		<.0001	<.0001	<.0001	0.0049	<.0001	<.0001	<.0001
ACE						1	ns	0.3216	0.4969	ns	0.4086	0.6691	0.7946	0.4646
								0.0059	<.0001		0.0004	<.0001	<.0001	<.0001
WSA							1	ns	ns	ns	ns	ns	ns	ns
Ca								1	0.2649	0.8184	0.4017	0.4301	0.4920	0.4943
									0.0245	<.0001	0.0005	0.0002	<.0001	<.0001
K									1	0.3002	ns	0.4717	0.4832	0.4409
										0.0104		<.0001	<.0001	<.0001
Mg										1	ns	0.4033	0.4125	0.3758
												0.0004	0.0003	0.0011
P											1	ns	ns	ns
TN												1	0.8504	0.5390
													<.0001	<.0001
SOC													1	0.6612
														<.0001

TABLE 2.7. Ten-year average yields of cotton, corn, soybean, wheat, and winter legume by treatment at the Cullars Rotation from 2011-2020.

	Crop Yield				
Treatment	**Cotton Lint**	**Corn**	**Soybean**	**Wheat**	**Winter Legume**
	kg ha^{-1}				
Complete	1193	7996	2500	2777	3186
No N/ +legume	981	6033	2717	1059	2604
No N/ -legume	925	2526	2717	911	-
N/ -legume	1168	7692	2477	2726	-
No P	632	3037	1026	1358	1141
No K	33	1347	1007	2106	1536
No Lime	61	1195	191	192	222
No Amendments	43	398	51	38	-

FIGURE 2.1. Plot layout of the Cullars Rotation.



Treatment Key	
A*	No N/ +legume
B*	No N/ -legume
C*	No amendments
1*	N/ -legume
2*	No P
3*	Complete
4	4/3 K
5	Rock Phosphate
6*	No K
7	2/3 K
8*	No lime
9	No sulfur
10	Complete + micronutrients
11	1/3 K

*Treatment used in current study.

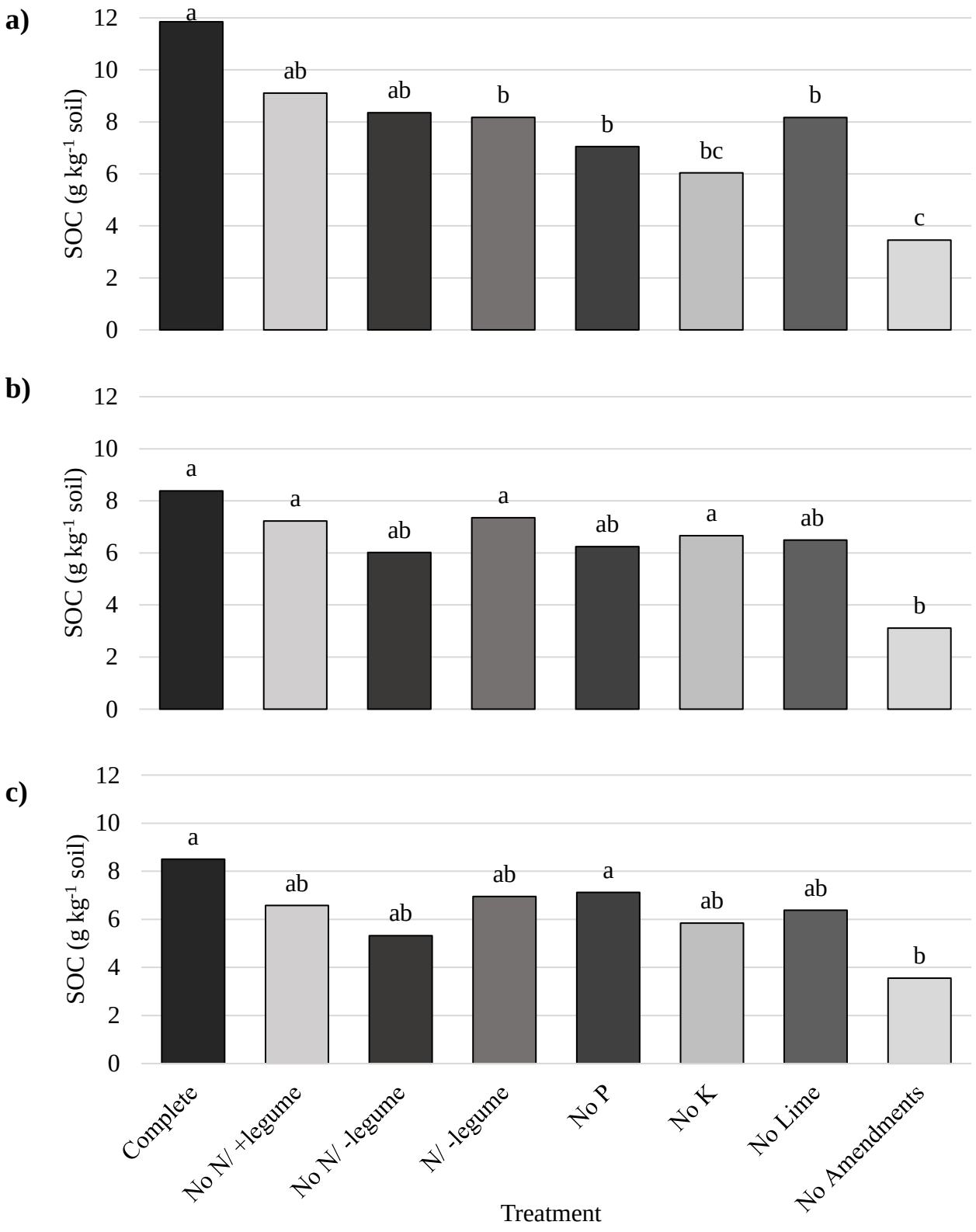


FIGURE 2.2. Effect of treatment by sampling date on soil organic carbon (SOC)(P=0.0149) at the Cullars Rotation at 0-10 cm on a) April 27, 2020, b) July 7, 2020, and c) October 6, 2020.

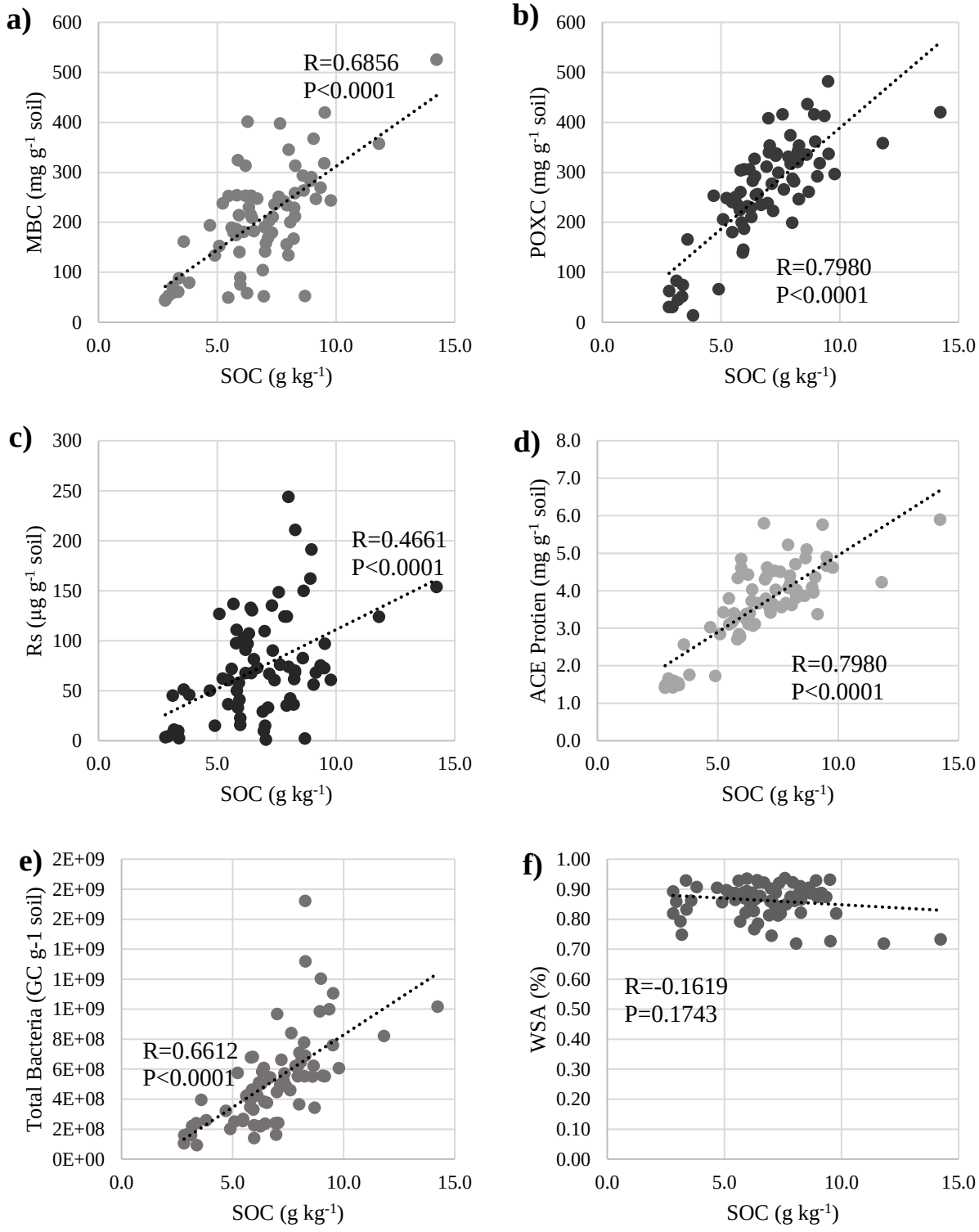


FIGURE 2.3. Correlations between soil organic carbon (SOC) and a) microbial biomass carbon (MBC), b) permanganate oxidizable carbon (POXC), c) soil respiration (R_s), d) autoclaved citrate extractable (ACE) protein, e) total bacteria, and f) water stable aggregates (WSA) and their Pearson's correlation coefficients at the Cullars Rotation at 0-10 cm.

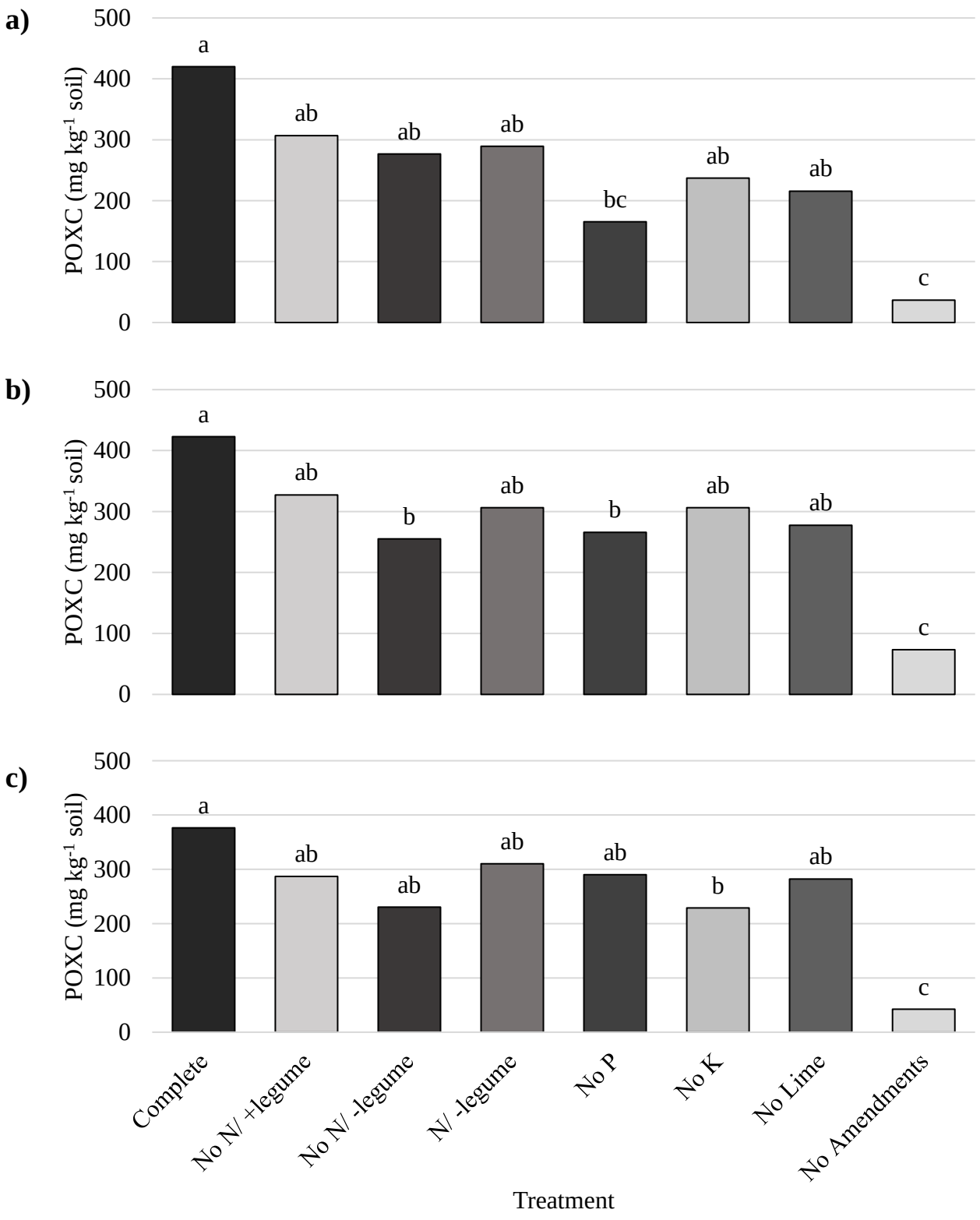


FIGURE 2.4. Effect of treatment by sampling date on permanganate oxidizable carbon (POXC) (P=0.0414) at the Cullars Rotation at 0-10 cm on a) April 27, 2020, b) July 7, 2020, and c) October 6, 2020.

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III. COVER CROP MONOCULTURES AND MIXTURES IMPACT SOIL HEALTH INDICATORS

INTRODUCTION

In the southeast United States, there is an increasing need for the adoption of conservation agriculture practices. Until the development of modern herbicides, almost all agricultural land in the United States was tilled frequently for weed control and seed bed preparation (Triplett & Dick, 2008). Constant tillage along with the wet, humid Southeastern climate has led to highly degraded soils in this region (Mylavarapu et al., 2014). In order to restore degraded soils, conservation practices such as cover cropping, conservation tillage, and crop rotations have been developed to restore soil health and increase crop yield potential. Cover crops can aid in the restoration of degraded soil by decreasing erosion, sequestering carbon, and increasing stability of soil aggregates (Bruce et al., 1995; Franzluebbers, 2005; Causarano et al., 2006). The practice of cover cropping has become more prevalent as government incentive programs in the U.S. have been created to promote the adoption of cover crops (Wallander et al., 2021).

Specific benefits provided to the soil by cover crops are closely tied to the species of cover crop(s) planted. Legumes such as crimson clover (*Trifolium incarnatum*) can provide supplemental N to crops while maintaining crop yield (Wittwer & van der Heijden, 2020). Overall, leguminous cover crops can add significant amounts of carbon and nitrogen to the soil as well as increase microbial activity (De Notaris et al., 2020; Kocira et al, 2020). Cereal rye (*Secale cereal*) is a common high-biomass cover crop in the southeast U.S. that has the potential to sequester carbon from the atmosphere and can decrease greenhouse gas emissions when

combined with no till management (Balkcom et al., 2013; Fiorini et al., 2020; Gong et al., 2021). Brassica cover crops have increased in popularity in recent years. However, there is a lack of understanding of the effect brassicas have on soil physical properties and soil health in the Southeast. Chen and Weil (2020) found that forage radish (*Raphanus raphanistrum*) had higher root penetration in compacted soils than rye. However, it is unclear whether Brassica cover crops decrease soil compaction on Southeastern soil types compared to other cover crops.

Many studies have examined the effects of winter cover crops on soil health indicators. The most popular soil health indicator is soil organic matter, often measured as soil organic carbon (SOC). However, response of SOC to cover crops can be variable. Ding et al. (2006) found a cover crop mixture of rye and a legume was able to increase SOC concentrations by 32% in the top 25 cm of soil compared to fallow treatments in a fine sandy loam soil after 15 years. Similarly, Mazzoncini et al. (2011) found after 15 years of legume cover crops in a loam soil, SOC concentration increased by an average of 9% in the top 30 cm of soil compared to the winter fallow control. An 8-year Canadian study by Chahal and Van Eerd (2018) found that a mixture of rye and a brassica had approximately 6-12% greater SOC than brassica alone and the fallow control in the top 15 cm of soil.

Not all cover crop research has shown positive effects of cover crops on SOC, especially in Ultisols of the southeast U.S. In Tennessee, Chu et al. (2017) found in a short-term (4-year) cover crop experiment that neither cover crop mixtures nor monocultures of small grains, legumes, or brassicas increased SOC in the top 15 cm. Multiple long-term (>20 years) experiments conducted in the Southeast (AL, TN) have found that small grain and legume cover crops do not always increase SOC in the top 15 or 30 cm (Motta et al., 2007; Jagadamma et al.,

2019). This can be attributed to Southeastern soil types and warm climate which favor rapid organic matter decomposition (Davidson & Janssens, 2006).

One soil health indicator is often not enough to fully understand the impact that management strategies have on the health of the soil. For example, the total SOC pool might not increase as quickly as certain carbon (C) fractions. Wang et al. (2017) found that forage radish cover was not able to increase SOC on a Coastal Plain soil after 1 year at any depth up to 105 cm. However, in this same study, forage radish did increase permanganate oxidizable carbon (POXC), after 1 year in the top 15 cm as well as the 90-105 cm depth (Wang et al., 2017). Conversely, a study by Pokhrel et al. (2021) on a Southeastern Alfisol found that cover crop monocultures including rye, legumes, and brassicas did not increase SOC or POXC concentrations in the top 10 cm of soil in the short-term (3 years). Another study on a Southeastern Alfisol by Singh et al. (2020) found that small grain cover crops increased POXC concentrations in the top 15 cm of soil compared to the winter fallow control after 39 years. These results indicate that it may take a long period (>3 years) of cover cropping for POXC to build up in Southeastern soils. In the southwest U.S., Ghimire et al. (2019) found that a six species mixture containing small grains, legumes, and brassicas increased POXC at cover crop termination in the top 15 cm when compared to brassica alone or a two-species mixture of a legume and brassica after 2 years.

In addition to soil carbon fractions, cover crops can also improve soil physical properties. A short-term (3-year) study by Adeli et al. (2019) in the Southeast found that a small grain winter cover crop decreased penetration resistance and increased aggregate stability on a low-organic matter Alfisol. Nouri et al. (2019) found that monoculture legume and small grain cover crops decreased penetration resistance compared to a no cover crop control in a 34-year

continuous cotton system in the Southeast. However, cover crops did not affect aggregate stability in this same study. Little research has been done on how brassica cover crops affect soil physical properties in the Southeast. However, a study in Idaho found that radish cover crops increased aggregate stability after 2 years (Lehrsch & Gallian, 2010). Additionally, a greenhouse study by Hudek et al. (2021) found some species of brassica cover crops increased aggregate stability and that all brassicas tested decreased penetration resistance compared to no cover crop in the top 25 cm of soil.

Many studies have examined the impact of cover crops on soil properties in the Southeast. However, the diversity of soil types, cash crop rotations, and cover crop species necessitates additional research on the impact of cover crops on soil health. In addition to soil health, cover crop systems must be feasible for farmers in terms of cost, planting date, and termination timing. The objective of this study was to evaluate the use of different cover crop species and mixes and the impact they have on soil health and crop yield in the short-term after continual use in a conservation tillage system.

MATERIALS AND METHODS

EXPERIMENTAL DESIGN

Two cover crop trialswere established in Belle Mina, AL at the Tennessee Valley Research and Extension Center (TVREC) and in Headland, AL at the Wiregrass Research and Extension Center (WREC) in 2017 to evaluate different mixes of winter cover crops incorporated into cotton-legume cash crop rotations. The soil at the TVREC location is classified as a Dewey silt loam (fine, kaolinitic, thermic Typic Paleudult), and the WREC location is a

Lucy loamy sand (loamy, kaolinitic, thermic Arenic Kandiudult). Prior to establishment of the experiment, the WREC location had been conventionally tilled and was converted to conservation tillage for the duration of this experiment. The TVREC location had been under no-till management for >20 years and remained no-till for this experiment.

This experiment includes 8 treatments replicated 4 times in a randomized complete block design. The eight treatments include winter fallow, 'Wrens Abruzzi' cereal rye, 'Dixie' crimson clover, and 'Sodbuster' Daikon radish monocultures, along with mixture treatments for all possible combinations of the three cover crop species. Cover crop seeding rates are found in Table 3.1. The plots are 7.3 by 10.7 m at WREC and 8.2 by 10.7 m at TVREC (Figure 3.1). All cover crops were drilled in 19 cm rows with a Great Plains 1205NT drill at WREC and a Great Plains 3P606NT drill at TVREC. Nitrogen fertilizer was applied to all treatments at 34 kg ha^{-1} at the time of cover crop planting. Cover crops were terminated with glyphosate.

Cash crops were planted approximately two to three weeks after cover crop termination. Dates of major field operations at both locations are found in Table 3.2. In 2018 and 2020, 'Deltapine 1646' cotton was planted at both locations. In 2019 and 2021, 'FloRun 331' peanut was grown at WREC and 'Asgrow 54XFO' soybean at TVREC. At WREC, row spacing was 91 cm for both crops. At TVREC, rows were spaced 102 cm apart for cotton and 76 cm for soybean. During the cash crop season, fields were irrigated with a center pivot system, but cover crops did not receive any irrigation.

SOIL AND PLANT SAMPLING

Soil samples were taken each year, 2 to 3 weeks after cover crop termination, at 0-5 cm, 5-10 cm, 10-15 cm, and 15-30 cm. Due to difficulty sampling in high clay soils, 15-30 cm samples were not taken at TVREC. Soil was collected using bucket augurs and approximately 10 subsamples from each plot were composited for each sample.

Cover crop biomass was sampled prior to termination by collecting aboveground plant material from two 0.25-m^2 randomly selected areas in each plot. Biomass was dried for at least 48 h at 60°C and weighed to obtain dry weight biomass.

ANALYSIS

Soil Analysis

Water stable aggregates (WSA) were determined with the wet sieve method described by Kemper and Rosnenau (1986). A portion of the air-dried soil from each sample was sieved to 1-2 mm to isolate aggregates. Four grams of soil was weighed and placed into 24 mesh cm^{-1} sieves. Samples were wetted slowly to a moisture level near field capacity using a humidifier. Humidified samples were immersed in deionized water using a wet sieving apparatus (Eijkelkamp Soil & Water, Giesbeek, The Netherlands). at a rate of 35 times min^{-1} for three minutes into pre-weighed metal containers. After this 3-minute period, new pre-weighed containers were placed beneath the sieves that contained a diluted sodium hexametaphosphate ($Na(PO_3)_6$) and sodium hydroxide (NaOH) dispersal solution. Samples were lowered into this solution at a rate of 35 times min^{-1} until all aggregated soil was dispersed. Each metal container

was dried at 105°C until all water had evaporated. The metal containers were weighed again, and the weight of the soil collected, corrected for the dispersal solution, was used to determine the percentage of WSA (Equation 3.1; Kemper & Rosnenau, 1968).

EQUATION 3.1:

$$WSA\% = [soil\ wt.after\ sieving\ (g) - wieght\ of\ dispersal\ solution]/[4\ g - sand\ fraction\ (g)] * 100$$

Soil strength analysis was used to measure penetration resistance according to Balkcom et al. (2016). Penetration resistance was measured during the cash crop season with a tractor-mounted hydraulic penetrometer with five probes. Readings were taken three times in each plot and the results were averaged. The middle probe was centered over the cash crop row, two were spaced 22.5 and 45 cm from the middle in the trafficked row to one side, and two were 22.5 and 45 cm from the middle in the non-trafficked row to the opposite side. Readings were taken from each probe at 25 Hz up to a depth of 50 cm, and the data were averaged for every 5 cm. Using the analysis method described by Balkcom et al. (2016), the area under the curve for the cone index ($AUC_{C.I.}$) was calculated following Equation 3.2 where *i* is the position of the row, CI_i is the average cone index value by row position, d_i is the distance between row positions, and k is the number of row positions. At the time of penetrometer sampling, soil moisture samples were taken from 0-15 cm and 15-30 cm depths with push probes. The soil collected was weighed and dried at 105°C for 48 h. The soil was then weighed again to determine GWC.

EQUATION 3.2:

$$AUC_{C.I.} = \sum_{i=1}^{k-1} \frac{\left[CI_{(i+1)} + CI_i\right] d_i}{2}$$

Permanganate oxidizable C was determined according to Weil et al. (2003). A 2.5-g of sieved, dried soil sample was placed into a 50 mL centrifuge tube, and 18 mL of deionized water and 2.0 mL of 0.2 M potassium permanganate ($KMnO_4$) stock solution were added to each tube. The samples were hand shaken for 2 s and then placed on a shaker at 240 oscillations min^{-1} for 2 min. The samples were then placed into a dark area for 10 min to allow the soil to settle. After this time, 0.5 mL of sample were placed into a new 50 mL centrifuge tube along with 49.5 mL of deionized water. The samples were inverted to mix, and analyzed on a Biotek μQuant plate reader at 550 nm. The absorbance measured from the unknown samples were compared to a set of standards with known concentrations of $KMnO_4$ stock solution. The POXC levels were determined with Equation 3.3 where *abs* is the measured absorbance, *a* is the intercept of the standard curve, and *b* is the slope of the standard curve (Weil et al., 2003).

EQUATION 3.3:

$$mg\ POXC\ kg^{-1} = \left[0.02\ M - \left(a + (b * abs)\right)\right] * 9000\ \frac{mg\ C}{mol} * 0.02\ \frac{L\ solution}{kg\ soil}$$

Soil organic C and total nitrogen were determined with combustion analysis. Soils were air-dried and ground to a fine powder with a coffee grinder. For the 2018 and 2019 seasons, a CN LECO 2000 analyzer (Leco Corp., St. Joseph, MI) was used, and for 2020 and 2021 a $CHNS/S_{IR}$ elemental analyzer was used (Elementar, Lagensebold, Germany).

Data Analysis

Soil variables measured across depths (SOC, POXC, and WSA) were subjected to repeated measures analysis of variance using PROC GLIMMIX in SAS Version 9.4 (SAS Institute Inc., Cary, NC). The analysis was conducted separately by location and soil depth. Treatment, year, and their interaction were used as fixed effects and replication was used as random effect. The first order autoregressive covariance structure AR(1) was used to account for repeated measures among years. For all analysis, degrees of freedom were calculated using the Kenward-Rodger method and the Tukey adjustment was used to adjust mean differences for multiplicity (Littell et al., 2006). Mean separations were determined at $\alpha = 0.1$ for these variables.

All other data ($AUC_{C.I.}$, soil moisture, cover crop biomass, and cash crop yield) were analyzed individually by year and location and were log transformed prior to analysis. Mean separations were determined using Tukey's significant difference (HSD) test ($\alpha = 0.1$). Treatment, year, and depth, and the interactions between variables were analyzed as fixed effects, and block replications were treated as a random factor.

RESULTS

Cover Crop Biomass

Across all site-years at both WREC and TVREC cover crop treatment affected cover crop biomass according to year at both WREC and TVREC (Table 3.3). At TVREC, cover crop biomass varied according to treatment in 2018, 2019, and 2021 (Table 3.3). In 2018, the radish cover crop was winter killed and no aboveground biomass was collected. The rye-clover and 3-way mixture (which was primarily comprised of rye and clover aboveground biomass) were greater in biomass than the clover monoculture and clover-radish mix. In 2019, the radish cover crops survived colder temperatures, but biomass production was 716 kg ha^{-1}, which is equivalent to 12-19% of the biomass produced from every other cover crop treatment. In 2021, the radish treatment was lower than every other treatment at 1,528 kg ha^{-1}. The rye-clover mixture was higher in biomass than the clover, radish, and clover-radish mixture in 2021. This observation was made in both 2018 and 2021, which indicates that the incorporation of rye into a clover cover crop may increase biomass. The rye-clover mixture preformed consistently better than most other treatments and may be a potential way to build soil health in the Tennessee Valley region of Alabama based on cover crops tested. At TVREC, 2019 and 2021 had 64% greater biomass than in 2018 and 2020 (Table 3.3).

Cover crop biomass was affected by cover crop treatment at WREC in 2019 and 2021 but all cover crop treatments produced equivalent biomass in 2018 and 2020 (Table 3.3). In 2019, all mixes containing clover had greater biomass than any treatment not containing clover. All three clover mixes had an average biomass of 7,404 kg ha^{-1}, which is much higher than biomass production observed in 2020 and 2021 at WREC. Additionally, the rye-clover mix prodcued

more than double the biomass of the clover monoculture treatment. In 2021, a similar trend was seen. Clover monoculture and all mixtures containing clover performed better than the radish monoculture. Treatments that contained clover averaged 3,156 kg ha^{-1} of biomass and produced approximately 2.75 times more biomass than the radish treatment. At this location, clover and clover mixes performed consistently well across all four years. As reported by Cates et al. (2018), cover crop biomass is directly related to soil health benefits such as nutrient retention and soil carbon which may indicate that the utilization of clover cover crops in the Coastal Plain region could lead to increased soil health. At WREC, 2018 and 2019 produced an average of 119% greater biomass production than in 2020 and 2021 (Table 3.3). Seasonal difference in biomass production may be due to planting date which is dictated by weather and harvest of the cash crop, and only a difference of one week in planting date can affect cover crop biomass especially in a warm climate (Saini et al., 2008; Ruis et al., 2020). Cover crop planting dates show that an earlier planting date can correspond to higher biomass production (Table 3.2). Additionally, weather can influence plant growth, pest pressure, soil moisture, and frost damage. The duration of cover crop growth is also affected by the following cash crop because this will determine the cover crop termination date. As shown in Table 3.2, planting dates are slightly different for cotton and peanut/soybean years, and a later cover crop termination date led to higher biomass production (Ruis et al., 2020).

A meta-analysis by Ruis et al. (2020) studied average biomass production values for monoculture cover crops across a variety of climates. In humid and warm regions most similar to the climate in this study, average crimson clover production was 4,000 kg ha^{-1}, radish was 3,000 kg ha^{-1}, and rye was 8,000 kg ha^{-1}. During the highest production years at WREC, there were similar values for crimson clover and radish monocultures. However, rye biomass values never

exceeded 6000 kg ha^{-1} in the current study (Table 3.3). A study by Saini et al. (2008) found that, under ideal planting date conditions at WREC and TVREC, the greatest measured rye biomass was 8,522 kg ha^{-1} at WREC and 10,953 kg ha^{-1} at TVREC. Saini et al. (2008) applied much more nitrogen (N) fertilizer than this study, which likely led to higher biomass production of rye. Saini et al. (2008) observed more similar crimson clover biomass production to this study. At WREC they reported a maximum of 5,447 kg ha^{-1} and at TVREC they reported 4,002 kg ha^{-1} under ideal planting date conditions. A third study by Balkcom et al. (2013) found in a similar area of Alabama that rye produced an average 8,857 kg ha^{-1} over a 6-year period. This study used equivalent fertilization and management but resulted in much higher rye biomass than this one, which could be due to differences in planting date or climate conditions.

In the current study, many monocultures and mixes were examined to determine whether a diverse mixture of cover crops could be beneficial for increasing biomass production and soil health benefits. Previous studies have shown that cover crop mixtures do not always perform better than monocultures in terms of biomass production. In a meta-analysis by Florence and McGuire (2020) which evaluated differences between cover crop monocultures and mixes, only 2% of comparisons produced significantly higher biomass with mixtures than the monocultures. In 72% of the comparisons, there was no difference between mixtures and monocultures for biomass production. In the current study, a mixture of two species with a higher biomass than both of the constituents' monocultures occured in only one out of eight site-years (Table 3.3). However, there was also no year in which a monoculture had greater biomass than a mixture containing the respective species. Late planting dates led to less-than-ideal environments for maximum biomass production. Additional studies to evaluate these cover crop species under a

range of environmental conditions will be useful for understanding cover crop biomass production.

Soil Organic Carbon

At TVREC, SOC was affected by treatment at the 0-5 cm and 5-10 cm depths (Table 3.4). At the 0-5 cm depth, cover crop treatments containing rye and clover averaged 15% greater SOC than the fallow and radish monoculture treatments (Table 3.5). At the 5-10 cm depth, the rye-clover mix and rye-radish mix both had higher SOC than the fallow. Also, the rye-radish mix SOC concentrations at 5-10 cm were greater than the radish monoculture and fallow treatments. At the lowest depth measured (10-15 cm) at TVREC, SOC was not affected by treatment (Table 3.4). These results are consistent with a study by Balkcom et al. (2013) that found differences in SOC with cover crops in the top 10 cm of soil but not at the 10-15 cm depth. The radish monoculture treatment was not different from the fallow control at any depth (Table 3.5). The low radish biomass production may have contributed to this observation. Radish utilization as a cover crop is also limited by a low tolerance for cold weather which can be problematic in cotton-soybean systems since harvest is later than the optimum planting date for radish particularly at TVREC. Carbon contents of small grain and legume biomass have more carbon than brassicas, which may have contributed to this difference along with decreased biomass (Ghimire et al., 2017). There were no years where cover crop mixes had higher SOC than its monoculture constituents. However, at the 5-10 cm depth, the rye-clover and rye-radish mixes were the only treatments different from fallow. In this case, mixtures containing rye increased SOC at this depth, whereas none of the monoculture treatments showed increases compared

tofallow. In addition to treatment, SOC was affected by year at every depth at TVREC. For the 0-5 cm depth, an increase in SOC occured over time across all treatments, as SOC was higher in 2021 (23.9 g kg^{-1}) than 2018 (21.0 g kg^{-1}), 2019 (20.7 g kg^{-1}), and 2020 (20.6 g kg^{-1}; Figure 3.2). This indicates that SOC was building over time in the top layer of soil and could continue to increase soil C if the cover crops were implemented over a longer period of time.

At WREC, SOC was not affected by cover crop treatment at any depth, but all depths were affected by year (Table 3.4). At the 0-5 cm depth, SOC was greater in 2020 and 2021 than 2018 and 2019, indicating that carbon might be building over time at this depth, regardless of cover crop treatment (Figure 3.2). At the deepest depth measured (15-30 cm) the opposite effect was seen. These differences seen at the lower depths may be due to seasonal variability or it takes time for carbon to move down through the soil profile. Coastal Plain soils increase in clay with depth, and therefore the illuvial movement of organic carbon lower in the profile will take longer as depth increases. Additionally, these plots are managed with conservation tillage and the above ground cover crop residue is not incorporated into the soil except during peanut digging, which only impacts the top 15 cm of soil. Therefore, organic matter additions begin at the 0 cm depth and have to move down the profile without mechanical intervention below 15 cm. As Liebmann et al. (2020) found, only small amounts of recent litter additions to the soil surface move down to the subsoil, and subsoil SOC is instead tied to soil organic matter (SOM) processes of sorption and mobilization that take longer than 3.5 years to migrate SOM downward through the profile. Steele et al. (2012) also found SOC varied by year in a Coastal Plain soil and some years produced a significant cover crop effect while others did not. Soil organic carbon decomposition rates are affected by clay content, which may attribute to the lack of differences between treatments seen on the coarser-textured Coastal Plain soil (Balkcom et al., 2013).

Compared to a no cover crop control, many studies have found that cover crops can increase SOC (Sainju et al., 2002; Higashi et al., 2014; Olson et al., 2014; Nascente et al., 2015; Wulanningtyas et al., 2021). A review by Causarano et al. (2006) found that across 41 sites in the southeast United States, cropping systems utilizing cover crops had higher SOC than cropping systems without cover crops and this difference was greater in conservation tillage systems. However, there have been studies on similar soil types that did not show an increase in SOC with cover crop use and conservation tillage (Motta et al., 2007; Balkcom et al., 2013). Despite the short-term nature of this study, cover crop treatments containing rye and clover were able to increase the SOC concentration at TVREC compared to fallow. The low biomass production of the radish monoculture treatment at TVREC contributed to its inability to build SOC compared to winter fallow. At WREC, no cover crops resulted in increased SOC. This can be partially explained by the coarser-textured Coastal Plain soil, which makes it difficult for SOC to be retained by the soil. Additionally, peanut digging operations can disrupt soil aggregates and make it more difficult to build SOC. In soils with higher clay content, like those in the Tennessee Valley, SOC is retained at higher rates.

Permanganate Oxidizable Carbon

Permanganate oxidizable C was affected by treatment at the 0-5 cm and 10-15 cm depths at TVREC (Table 3.4). In the 0-5 cm depth, all cover crop treatments that did not contain radish (i.e., clover, rye, and rye-clover mix) were 14% higher in POXC than the radish monoculture and fallow treatments (Table 3.6). Additionally, all cover crop treatments except the clover-radish mix and the radish monoculture were higher in POXC than winter fallow. Treatment did not

have a significant effect on POXC at the 5-10 cm depth, but at the lowest depth measured (10-15 cm), there was an interesting difference (Table 3.4). The clover monoculture treatment was 41% higher in POXC than the clover-rye mix (Table 3.6). One potential explanation for this is rye biomass decomposes slower due to a greater carbon to nitrogen (C:N) ratio. Permanganate oxidizable C was also influenced by year at all depths at TVREC. Similar to SOC, POXC is dependent on the decomposition rate of organic matter, which is dependent on yearly climatic factors.

At WREC across all years, POXC was not affected by treatment at any depth (Table 3.4). The climate at this location is warmer, which increases organic matter decomposition (Kirschbaum 1995; Franzluebbers et al., 2001). This effect may make it difficult to detect differences in POXC for cover crop treatments. However, this is contrary to findings by Steele et al. (2012) that found a larger POXC effect in response to cover crops on Coastal Plain soils than on Piedmont soils. These conflicting results may be partially explained by the duration of cover crop implementation, as cover crops were established for 13 years in the Steele et al. (2012) study. In the current study, POXC was affected by year in addition to cover crop treatment at the 5-10 cm, 10-15 cm, and 15-30 cm depths. Again, climate's influence on soil organic matter can vary with year to year temperature differences.

Wang et al. (2017) found that on a Coastal Plain soil, radish cover crops were able to increase POXC compared to winter fallow in the top 15 cm of soil, contrary to findings in this study. On the WREC Coastal Plain soil in 2021, the opposite effect was seen across all depths. The fallow treatment had higher POXC than the radish monoculture. In addition, at TVREC there was no difference in POXC between radish monoculture and fallow across all years and depths. However, the cover crop planting date for this study was much later than Wang et al.

(2017), which may have led to higher radish biomass production and therefore increased differences in POXC.

Many studies have observed that POXC responds to management practices quicker than SOC, but this four-year study showed that certain cover crop treatments improved POXC and SOC at the TVREC location (Weil et al., 2003; Culman et al., 2012; Steele et al., 2012). Permanganate oxidizable carbon and SOC were strongly and positively correlated ($R=0.88216$; $P<0.0001$) across all soil depths, but POXC did not appear to respond quicker than SOC. A similar cover crop experiment on a South Carolina Ultisol by Parajuli et al. (2021) also found strong correlations between POXC and SOC at the 0-15 cm depth.

Aggregate Stability

On the finer-textured soil at TVREC, no differences in WSA were observed across treatments (Table 3.4). This soil has a higher carbon and clay content, which may make it difficult to detect differences in WSA. The WSA values from this location were in the range of 95-98% which is similar to other research findings conducted on similar soil types (Table 3.7; Franzluebbers et al., 2000). Since this soil's aggregate stability is already high, the amount of organic carbon added by a cover crop might not be enough to impact WSA. This is similar to results reported by Steele et al. (2012). At TVREC, WSA was affected by year at every depth.

At the 5-10 cm depth at WREC, there was a trend toward higher WSA in the rye monoculture than the clover-radish mix (Table 3.7). The greater rye C:N ratio and therefore lower decomposition rate compared to the mixture may explain this result. Climate factors like precipitation and temperature, fungal activity, and soil moisture content can have impacts on

WSA and can create seasonal variability (Perfect et al., 1990; Bossuyt et al., 2001; Legout et al., 2005; Wang et al., 2016).

In the coarser-textured Coastal Plain soil at the WREC location, treatment influenced aggregate stability in certain site-years, but cover cropping was not able to improve WSA compared to the winter fallow (Table 3.7). A study by Steele et al. (2012) found that the Coastal Plain soil showed a larger WSA response to cover crop management than the Piedmont soil after 12 years. It may take more years to observe difference in WSA due to cover crop utilization.

Penetration Resistance

Penetration resistance was representedby cone index values and summarized with an area under the curve approach.. Across eight site-years, four years had differences in cone index values affected by cover crop treatment (Table 3.8). At both WREC and TVREC, 2019 and 2021 exhibited treatment effects. These results corresponded to differences in cover crop biomass at WREC during the same years (Table 3.3). In 2019 at WREC, the rye-clover mix had a higher $AUC_{C.I.}$ value than winter fallow, rye, and the rye-radish mix (Table 3.8). Additionally, the fallow and rye monoculture treatments were lower than every treatment but rye-radish. All three of these treatments had soil moisture levels similar to every other treatment except radish. At the same location in 2021, the rye-radish mix $AUC_{C.I.}$ value was higher than rye, radish, rye-clover, and the 3-way mixture. At the time of sampling, there were no differences in soil moisture, indicating that treatment differences were not confounded with soil moisture. Results indicate that $AUC_{C.I.}$ values can be variable within the same location and treatments and may also depend

on the growing season. Rye also had a lower $AUC_{C.I.}$ value than radish and the rye-radish mix, indicating that radish may not alleviate penetration resistance.

At TVREC in 2019, the rye, rye-radish mix, and the 3-way mix treatments all had lower $AUC_{C.I.}$ values than the fallow (Table 3.8). There were no soil moisture differences observedat the time of penetration resistance sampling indicating that these differences were not confounded with treatments. Additionally, the radish treatment had a higher $AUC_{C.I.}$ value than rye and the rye-radish mix. During this site-year, radish biomass was lower than every other cover crop treatment, and therefore the increased penetration resistance may be attributed to low biomass. Winter cover crops are planted and terminated in the off season between cotton and soybean phases, and it is possible that radish biomass production could increase during a different time frame. However, for this system, radish is less likely to reduce soil compaction. In 2021, there was a similar treatment effect, but soil moisture in the top 0-15 cm was different. Higher soil moisture values corresponded to lower $AUC_{C.I.}$ values. Radish and fallow had higher $AUC_{C.I.}$ values than every other treatment but also had two of the lowest soil moisture contents. The rye-clover mix had lower $AUC_{C.I.}$ values than radish, fallow, and the clover-radish mix. Many have cited a study by Williams and Weil (2004) as evidence that brassica cover crops are able to alleviate soil compaction. However, this conclusion is drawn from minirhizotron root images, and few studies report direct effects of brassica cover crops on soil penetration resistance.

Despite differences found in these four site-years, there were four other site-years where cover crop treatments did not affect penetration resistance compared to the winter fallow and to each other. This indicates that penetration resistance can be variable within a short-term experiment on Southeastern soil types. Differences between treatments at TVREC and WREC can be partially attributed to soil type as well as cropping system. At WREC, peanuts are planted

every other year in the rotation, and in order to harvest, the soil undergoes a disturbance in the top layer of soil. This may lead to decreased compaction in the top of the soil profile at WREC (Figure 3.3). Additionally, the site at TVREC was managed with no tillage for many years prior to this experiment and the site at WREC was strip tilled prior to planting, which could decrease soil compaction in the top layer of soil.

Cash Crop Yield

Although this study ran for four years, cotton yield data was only collected from WREC for one year due to crop failure in 2018. In the second year of cotton production at WREC, there were no differences according to treatment (Table 3.9). At TVREC, cotton yield was affected by cover crop in 2018 and 2020. In 2018, the rye-radish mix had higher cotton yield than the clover monoculture and clover-radish mixture, but no cover crop treatments were different than the fallow control. Similarly, the rye-radish mix was higher than the clover monoculture, but many treatments yielded better than the control in 2020. The rye monoculture, rye-clover mix, rye-radish mix, and clover-radish mix yielded approximately 25% higher than the no cover crop control (1670 kg ha^{-1}). A study by Raper et al. (2000) in the same region of Alabama found an increase in cotton yield with rye cover cropping and conservation tillage. Schomberg et al. (2006) conducted a study on a Georgia Coastal Plain soil that found cover crops increased cotton yield, but crimson clover hindered cotton growth compared to other cover crops tested. The authors attributed this difference to limited N leading to poor nodulation (Schomberg et al., 2006). In both years of the current study, the clover monoculture treatments produced cotton yields similar to the fallow control.

Peanut production at WREC was affected by cover crop treatments in 2019 (Table 3.9). No cover crop treatments improved peanut yield compared to the control, but the rye monoculture, radish monoculture, and rye-radish mix were equivalent to the control. All treatments that contained clover yielded lower than the fallow control. Typically, it is not recommended to plant a legume cover crop prior to a legume cash crop, because it is likely the cover crop will have similar diseases and pests that can carry over to the cash crop. This may have contributed to the decreased peanut yield following clover. Soybean yield at TVREC was affected by cover crop treatment in 2019, but no cover crops increased yield compared to the fallow control (Table 3.9). The only treatment difference observed in 2019 was the clover-radish mix yielded higher than the rye-radish mix.

Cash crop yield was not affected by cover crop treatments at either location in 2021.

CONCLUSION

Many cover crop monocultures and mixtures were evaluated in this study, but few differences existed between the mixtures and their constituents in the parameters measured. Rye and clover were consistently high in biomass production whether planted as a monoculture or in a mixture, but radish crops were not able to reach ideal biomass levels within the confines of the cotton-legume crop rotation used in the current study. Cover crop influence on soil health indicators and cash crop production appears to depend more on total biomass production and not on the number of species used. The effect of cover crops on selected soil health indicators varied according to location. Soil organic C and POXC tended to increase in the top soil depth with rye cover crops in a silt loam soil. In a loamy sand soil, there were no differences in POXC or SOC

according to cover crop treatment. Additional years of cover crop utilization might lead to detectable differences in SOC and POXC, but in this case, four years was not enough time for cover crops to have an effect. Soil organic C and POXC were strongly and positively correlated and both soil health indicators can be responsive to short-term conservation management.

Soil physical properties were not always affected by cover crop treatment. Aggregate stability did not show many meaningful differences in either soil type evaluated in the current study. Treatments containing rye tended to decrease soil strength and the radish monoculture treatment tended to increase soil strength in a silt loam soil. These results are consistent with biomass production. In a loamy sand soil, there were inconsistent differences in soil strength, which may be due to the peanut cropping system which requires a disturbance to the soil to harvest the crop. In the third year of the study, several cover crop treatments increased cash crop yield compared to the fallow control in the silt loam soil. No cover crop treatments increased cash crop yield compared to the fallow control in the loamy sand soil.

The effects that cover crops had on selected soil health indicators varied between the two locations evaluated in this study. Although both experiments were conducted in the Southeast, differences in soil types and climate affected how cover crops influenced soil health and crop yield. More time may be needed to see more dramatic cover crop effects at both locations.

TABLE 3.1. Cover crop seeding rates at Wiregrass Research and Extension Center (WREC) and Tennessee Valley Research and Extension Center (TVREC).

	Seeding Rate (kg ha^{-1})		
Treatment	**Rye**	**Radish**	**Clover**
Rye	100	-	-
Radish	-	9	-
Clover	-	-	22
Rye-Clover	50	-	22
Rye-Radish	50	9	-
Radish-Clover	-	9	22
Rye-Radish-Clover	34	4	11

TABLE 3.2. Dates of field operations at Wiregrass Research and Extension Center (WREC) and Tennessee Valley Research and Extension Center (TVREC).

Year	Operation	WREC	TVREC
2017	Cover crop planted	7 Nov	5 Oct
2018	Cover crop terminated	12 April	18 April
	Soil samples collected	27 April	4 May
	Cotton planted	3 May	3 May
	Cover crop planted	19 Nov	19 Oct
2019	Cover crop terminated	22 April	23 April
	Soil samples collected	4 May	7 May
	Peanut/Soybean planted	22 May	20 May
	Cover crop planted	18 Nov	5 Nov
2020	Cover crop terminated	27 April	16 April
	Soil samples collected	14 May	6 May
	Cotton planted	28 May	2 May
	Cover crop planted	24 Nov	22 Oct
2021	Cover crop terminated	6 April	13 April
	Soil samples collected	7 May	28 April
	Peanut/Soybean planted	20 May	18 May

TABLE 3.3. Cover crop biomass at Wiregrass Research and Extension Center (WREC) and Tennessee Valley Research and Extension Center (TVREC), 2018-2021.

		Cover Crop Biomass			
		Year			
Location	**Treatment**	**2018**	**2019**	**2020**	**2021**
		kg ha^{-1}			
WREC	Rye	5330	3822 bcd*	2144	1524 ab
	Clover	3828	4247 bc	1676	2731 a
	Radish	5919	2878 cd	1881	841 b
	Rye-Clover	3415	9334 a	2271	3045 a
	Rye-Radish	3695	2012 d	2136	1102 ab
	Clover-Radish	5698	6599 ab	1808	3323 a
	Rye-Clover-Radish	3915	6280 ab	2574	3526 a
	Average	4440 A**	4482 A	2051 B	2021 B
TVREC	Rye	2574 abc	4078 a	2438	4973 ab
	Clover	1518 c	5560 a	1605	3658 b
	Radish	- †	716 b	1986	1528 c
	Rye-Clover	3208 a	6047 a	2594	7313 a
	Rye-Radish	2788 ab	4218 a	2207	4152 ab
	Clover-Radish	1657 bc	3745 a	1946	3774 b
	Rye-Clover-Radish	3689 a	5216 a	2911	5403 ab
	Average	2445 B	3617 A	2203 B	4028 A

*Values followed by the same lowercase letter are not significantly different with a year and location class according to Tukey's HSD at $\alpha = 0.1$.

**Values followed by the same uppercase letter are not significantly different within a location class according to Tukey's HSD at $\alpha = 0.1$.

†Cover crop was winter killed and no biomass data was collected.

TABLE 3.4. Summary of analysis of variance (ANOVA) for soil organic carbon (SOC), permanganate oxidizable carbon (POXC), and water stable aggregates (WSA) in response to year, treatment, and their interaction according to location and depth at the Wiregrass Research and Extension Center (WREC) and Tennessee Valley Research and Extension Center (TVREC).

Location	Depth (cm)	Source of Variance	df	ANOVA (Pr > F)		
				SOC	POXC	WSA
WREC	0-5	Year (Y)	3	<0.0001	0.0666	0.0478
		Treatment (T)	7	0.5300	0.0565	0.0661
		Y x T	21	0.0740	0.2418	0.3707
	5-10	Y	3	0.0027	0.0006	0.0034
		T	7	0.5833	0.7423	0.0397
		Y x T	21	0.3121	0.9201	0.4797
	10-15	Y	3	0.0053	<0.0001	<0.0001
		T	7	0.8229	0.3147	0.1208
		Y x T	21	0.6490	0.1166	0.3021
	15-30	Y	3	0.0567	<0.0001	0.0001
		T	7	0.6455	0.0652	0.8618
		Y x T	21	0.4077	0.4190	0.1664
TVREC	0-5	Y	3	<0.0001	0.0003	<0.0001
		T	7	<0.0001	0.0003	0.1925
		Y x T	21	0.2931	0.9325	0.8863
	5-10	Y	3	0.0500	0.0016	<0.0001
		T	7	0.0025	0.8212	0.6612
		Y x T	21	0.8582	0.2575	0.5040
	10-15	Y	3	<0.0001	<0.0001	<0.0001
		T	7	0.2458	0.0220	0.3639
		Y x T	21	0.8495	0.2144	0.7518

Significant effects are determined at Tukey's HSD at α=0.01.

TABLE 3.5. Effect of cover crop treatment on soil organic carbon (SOC) according to location and depth at Wiregrass Research and Extension Center (WREC) and Tennessee Valley Research and Extension Center (TVREC).

		SOC			
		Depth			
Location	**Treatment**	**0-5 cm**	**5-10 cm**	**10-15 cm**	**15-30 cm**
		g kg^{-1} soil			
WREC	Fallow	6.38	5.52	5.15	5.00
	Rye	6.92	5.89	5.49	5.58
	Clover	6.80	6.08	5.33	5.58
	Radish	6.57	5.63	5.71	5.74
	Rye-Clover	7.09	6.06	5.22	5.14
	Rye-Radish	7.12	6.12	5.65	5.42
	Clover-Radish	6.82	5.82	5.46	5.44
	Rye-Clover-Radish	7.44	6.35	5.56	5.33
TVREC	Fallow	18.3 b*	9.66 c	7.50	-
	Rye	21.8 a	10.8 abc	7.73	-
	Clover	22.0 a	10.6 abc	7.70	-
	Radish	19.5 b	10.0 bc	7.40	-
	Rye-Clover	23.8 a	11.1 ab	7.52	-
	Rye-Radish	22.5 a	11.6 a	8.09	-
	Clover-Radish	22.1 a	10.9 abc	7.76	-
	Rye-Clover-Radish	22.6 a	10.7 abc	7.79	-

*Values followed by the same letter are not significantly different with a year and location class according to Tukey's HSD at $\alpha = 0.05$.

TABLE 3.6. Effect of cover crop treatment on permanganate oxidizable carbon (POXC) according to location and depth at Wiregrass Research and Extension Center (WREC) and Tennessee Valley Research and Extension Center (TVREC).

		POXC			
		Depth			
Location	**Treatment**	**0-5 cm**	**5-10 cm**	**10-15 cm**	**15-30 cm**
		mg g^{-1} soil			
WREC	Fallow	235	239	240	175
	Rye	242	224	202	197
	Clover	277	234	215	192
	Radish	213	202	199	170
	Rye-Clover	301	241	231	216
	Rye-Radish	248	218	198	166
	Clover-Radish	286	227	206	163
	Rye-Clover-Radish	279	225	194	218
TVREC	Fallow	681 c*	391	252	-
	Rye	800 a	409	273	-
	Clover	808 a	402	336	-
	Radish	700 bc	414	259	-
	Rye-Clover	803 a	424	238	-
	Rye-Radish	784 ab	438	290	-
	Clover-Radish	762 abc	414	291	-
	Rye-Clover-Radish	778 ab	416	260	-

*Values followed by the same letter are not significantly different with a year and location class according to Tukey's HSD at $\alpha = 0.05$.

TABLE 3.7. Effect of cover crop treatment on water stable aggregates (WSA) according to location and depth at Wiregrass Research and Extension Center (WREC) and Tennessee Valley Research and Extension Center (TVREC).

		WSA			
		Depth			
Location	**Treatment**	**0-5 cm**	**5-10 cm**	**10-15 cm**	**15-30 cm**
		%			
WREC	Fallow	82.9	83.0 ab*	80.1	83.2
	Rye	86.4	86.7 a	78.8	85.3
	Clover	84.4	82.9 ab	77.7	84.8
	Radish	83.8	83.9 ab	76.4	84.7
	Rye-Clover	84.3	82.2 ab	74.0	84.4
	Rye-Radish	87.1	85.2 ab	79.4	85.5
	Clover-Radish	84.6	81.7 b	80.5	84.8
	Rye-Clover-Radish	87.2	86.2 ab	79.7	86.4
TVREC	Fallow	96.7	96.5	96.9	-
	Rye	97.4	96.9	96.2	-
	Clover	96.6	96.9	96.6	-
	Radish	95.9	97.3	97.3	-
	Rye-Clover	96.7	97.5	97.2	-
	Rye-Radish	96.6	97.5	97.2	-
	Clover-Radish	96.6	97.0	96.8	-
	Rye-Clover-Radish	97.3	97.1	96.9	-

*Values followed by the same letter are not significantly different with a year and location class according to Tukey's HSD at $\alpha = 0.05$.

TABLE 3.8. Influence of cover crop treatment and significant years on area under the curve for cone index ($AUC_{C.I.}$) and volumetric soil moisture by depth at the time of $AUC_{C.I.}$ measurement at Tennessee Valley Research and Extension Center (TVREC) and Wiregrass Research and Extension Center (WREC).

		Year					
		2019			2021		
			Soil Moisture			Soil Moisture	
Location	Treatment	AUC C.I.	0-15 cm	15-30 cm	AUC C.I.	0-15 cm	15-30 cm
		—MPa cm^{-1}—	——————%——————		—MPa cm^{-1}—	——————%——————	
WREC	Fallow	210 c*	6.50 ab	8.84	227 abc	8.67	9.62
	Rye	205 c	6.84 ab	9.00	207 c	9.12	9.40
	Clover	270 ab	6.64 ab	8.33	235 abc	8.94	9.53
	Radish	227 abc	6.35 b	8.89	253 ab	8.25	9.35
	Rye-Clover	263 ab	6.97 ab	8.53	219 bc	9.15	9.55
	Rye-Radish	225 bc	7.29 a	9.11	260 a	8.82	9.41
	Clover-Radish	275 a	6.79 ab	8.32	241 abc	8.78	9.33
	Rye-Clover-Radish	256 ab	6.98 ab	8.23	220 bc	9.29	9.51
TVREC	Fallow	175 a	17.31	17.09	235 a	14.78 b	17.49
	Rye	152 c	18.23	17.79	186 bc	18.22 a	18.62
	Clover	167 abc	18.09	17.31	199 bc	16.27 ab	17.95
	Radish	173 ab	17.43	17.10	238 a	15.05 b	17.54
	Rye-Clover	158 abc	18.65	17.79	183 c	17.20 a	18.19
	Rye-Radish	152 c	18.83	17.26	188 bc	17.60 a	18.27
	Clover-Radish	162 abc	18.32	17.41	203 b	16.37 ab	18.12
	Rye-Clover-Radish	155 bc	18.04	18.19	187 bc	17.67 a	18.95

*Values followed by the same letter are not significantly different with a year and location class according to Tukey's HSD at $\alpha = 0.1$.

TABLE 3.9. Effect of cover crop treatment on cash crop yields at the Wiregrass Research and Extension Center (WREC) and the Tennessee Valley Research and Extension Center (TVREC), 2018-2021.

		Year			
		2018	**2019**	**2020**	**2021**
Location	**Treatment**	**Yield**			
		Cotton†	Peanut	Cotton	Peanut
		kg ha^{-1}			
WREC	Fallow	-††	5500 ab*	1474	3827
	Rye	-	4652 abc	1657	4809
	Clover	-	4204 bc	1699	4449
	Radish	-	5730 a	1479	3896
	Rye-Clover	-	4210 bc	1512	4380
	Rye-Radish	-	5129 abc	1540	4194
	Clover-Radish	-	3884 c	1483	3783
	Rye-Clover-Radish	-	4233 bc	1643	4772
		Cotton	Soybean	Cotton	Soybean
		kg ha-1			
TVREC	Fallow	1850 ab	3725 ab	1670 b	3763
	Rye	1897 ab	3826 ab	2051 a	3777
	Clover	1623 b	4092 ab	1835 ab	3774
	Radish	1860 ab	3937 ab	1951 ab	3949
	Rye-Clover	1738 ab	3744 ab	2031 a	3855
	Rye-Radish	1995 a	3675 b	2160 a	3823
	Clover-Radish	1658 b	4171 a	2054 a	3900
	Rye-Clover-Radish	1780 ab	3900 ab	1963 ab	3849

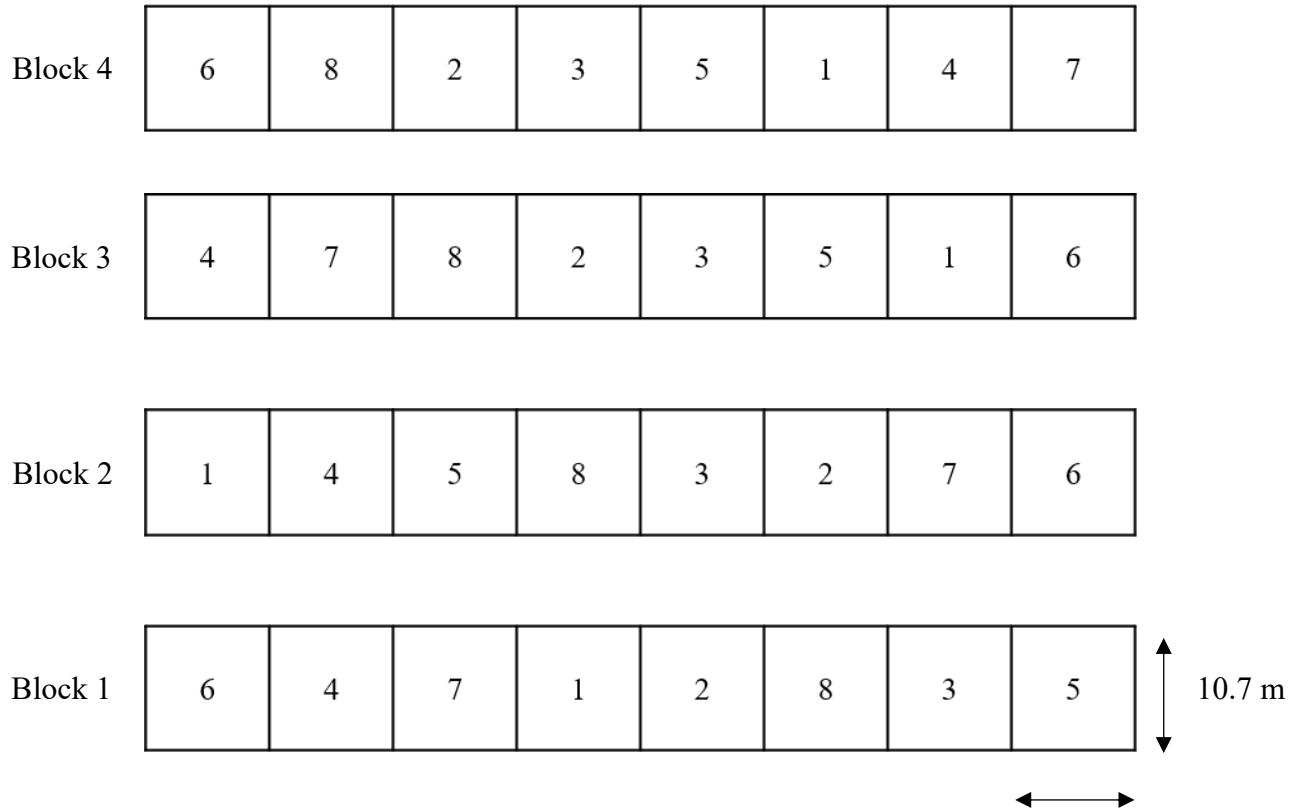
*Values followed by the same letter are not significantly different with a year and location class according to Tukey's HSD at α=0.1.

†Cotton yields are reported as lint yield.

††Yield data was not collected due to crop destruction by Hurricane Michael.

†††Yield TBD.

FIGURE 3.1. Field layout of cover crop mixture trials.



Treatment Key	
1	Fallow
2	Rye
3	Clover
4	Radish
5	Rye-Clover
6	Rye-Radish
7	Clover-Radish
8	Rye-Clover-Radish

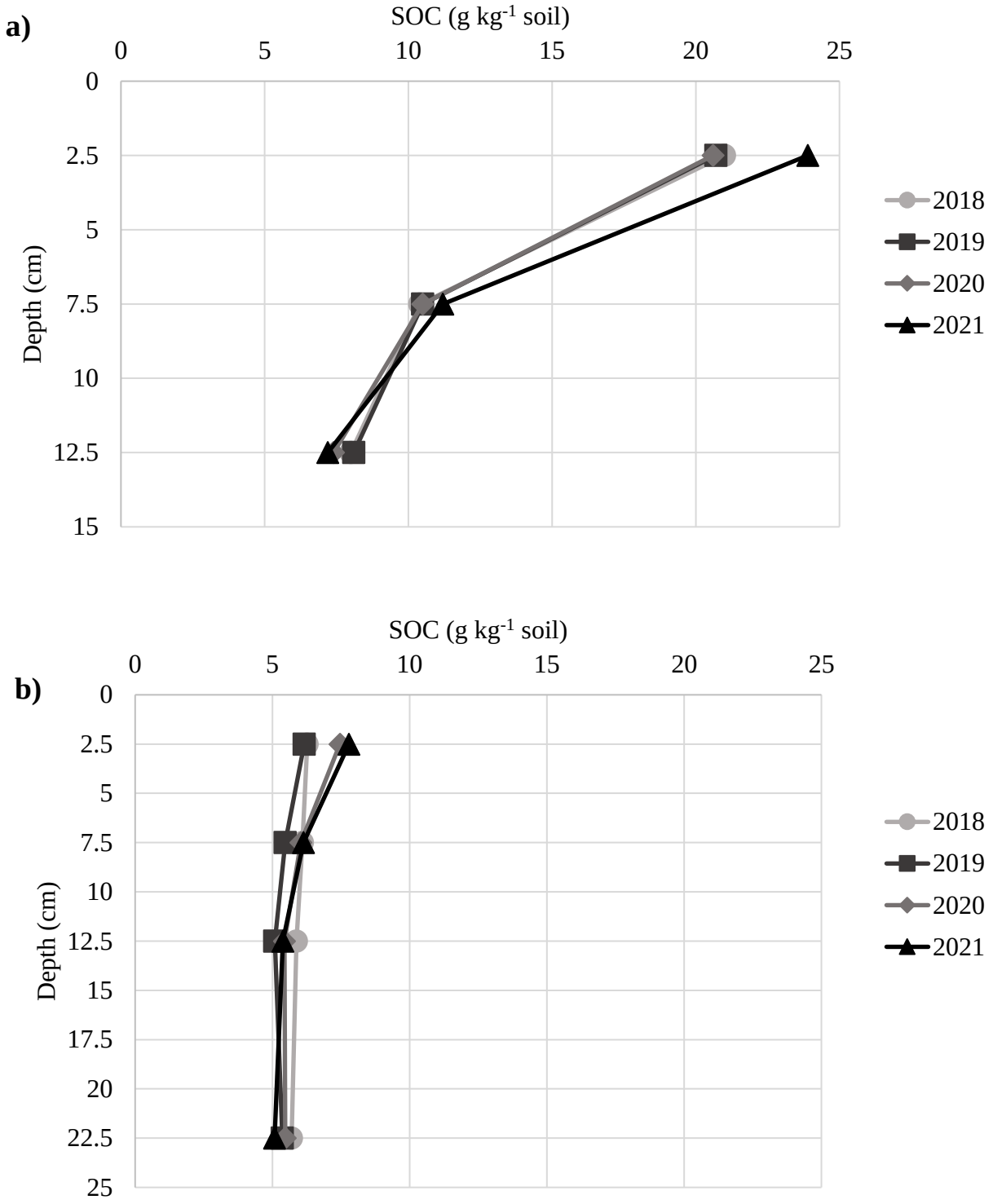


FIGURE 3.2. Effect of year and depth on soil organic carbon (SOC) at the a) Tennessee Valley Research and Extension Center (TVREC) and b) the Wiregrass Research and Extension Center (WREC).

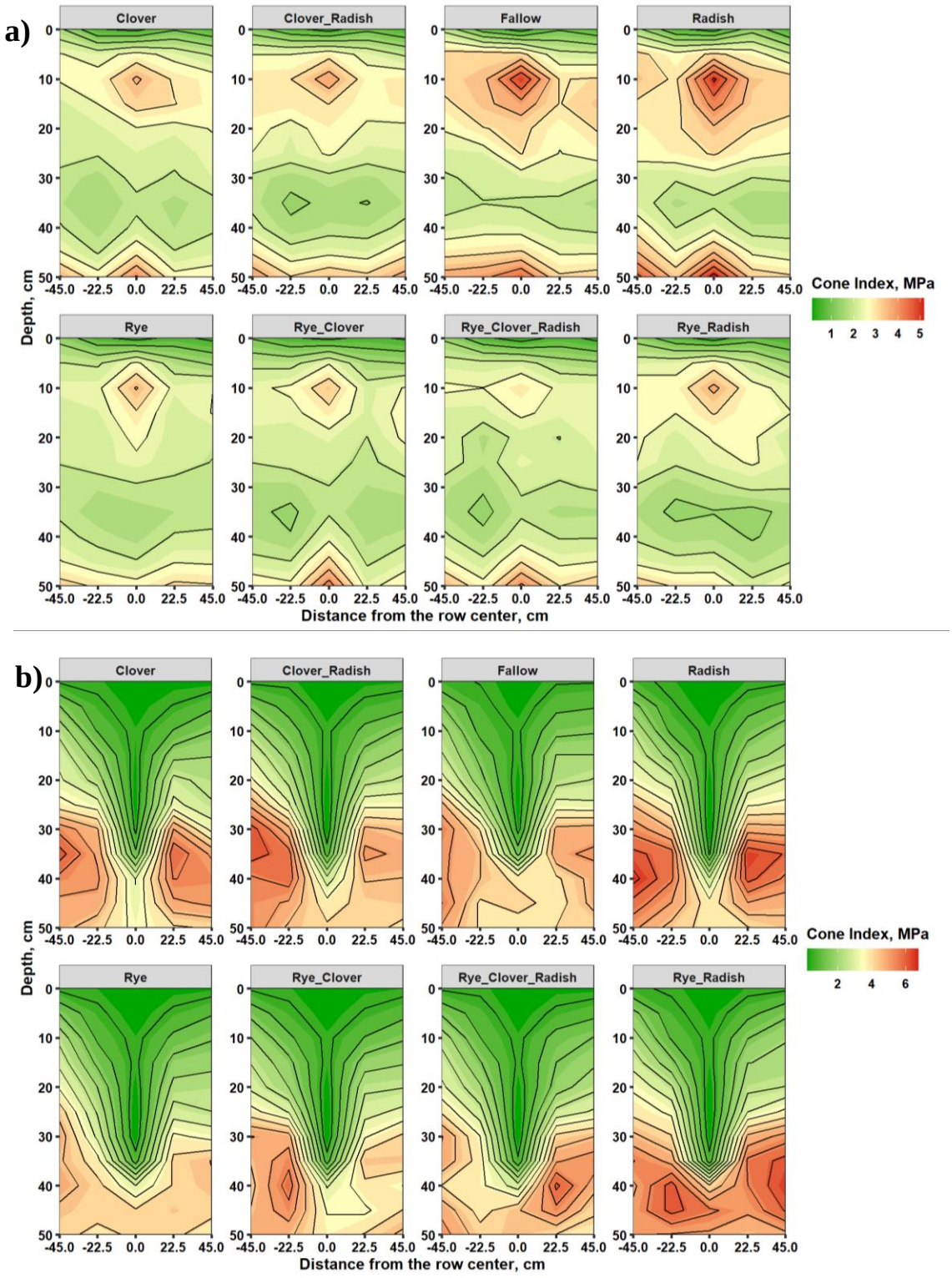


FIGURE 3.3. Contour graphs of cone index values according to cover crop treatment in 2021 at a) Tennessee Valley Research and Extension center (TVREC) and b) Wiregrass Research and Extension Center (WREC).

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APPENDIX

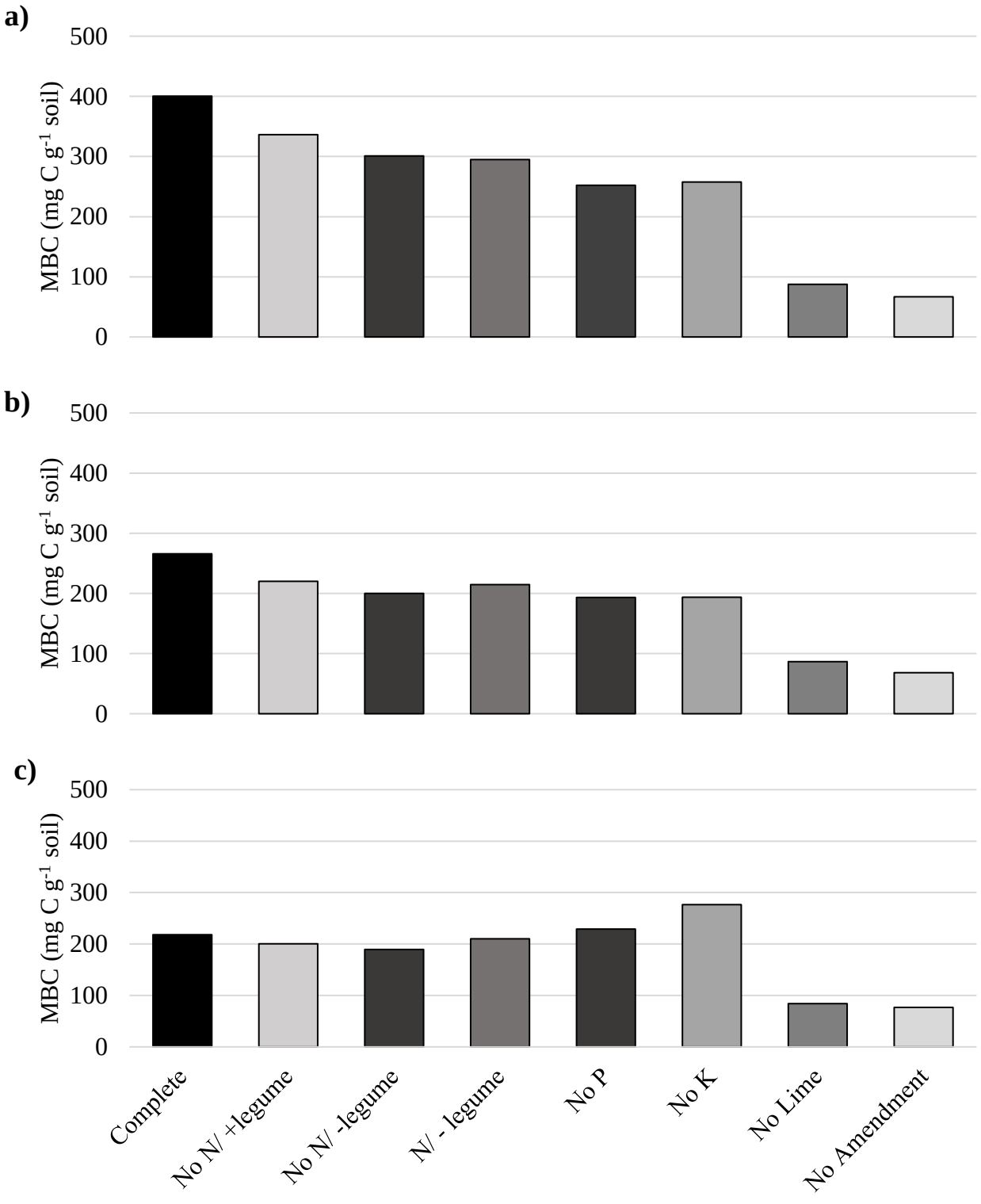

FIGURE A.1. Effect of treatment by sampling date on microbial biomass carbon (MBC) at the Cullars Rotation from 0-10 cm on a) April 27, 2020, b) July 7, 2020, and c) October 6, 2020.

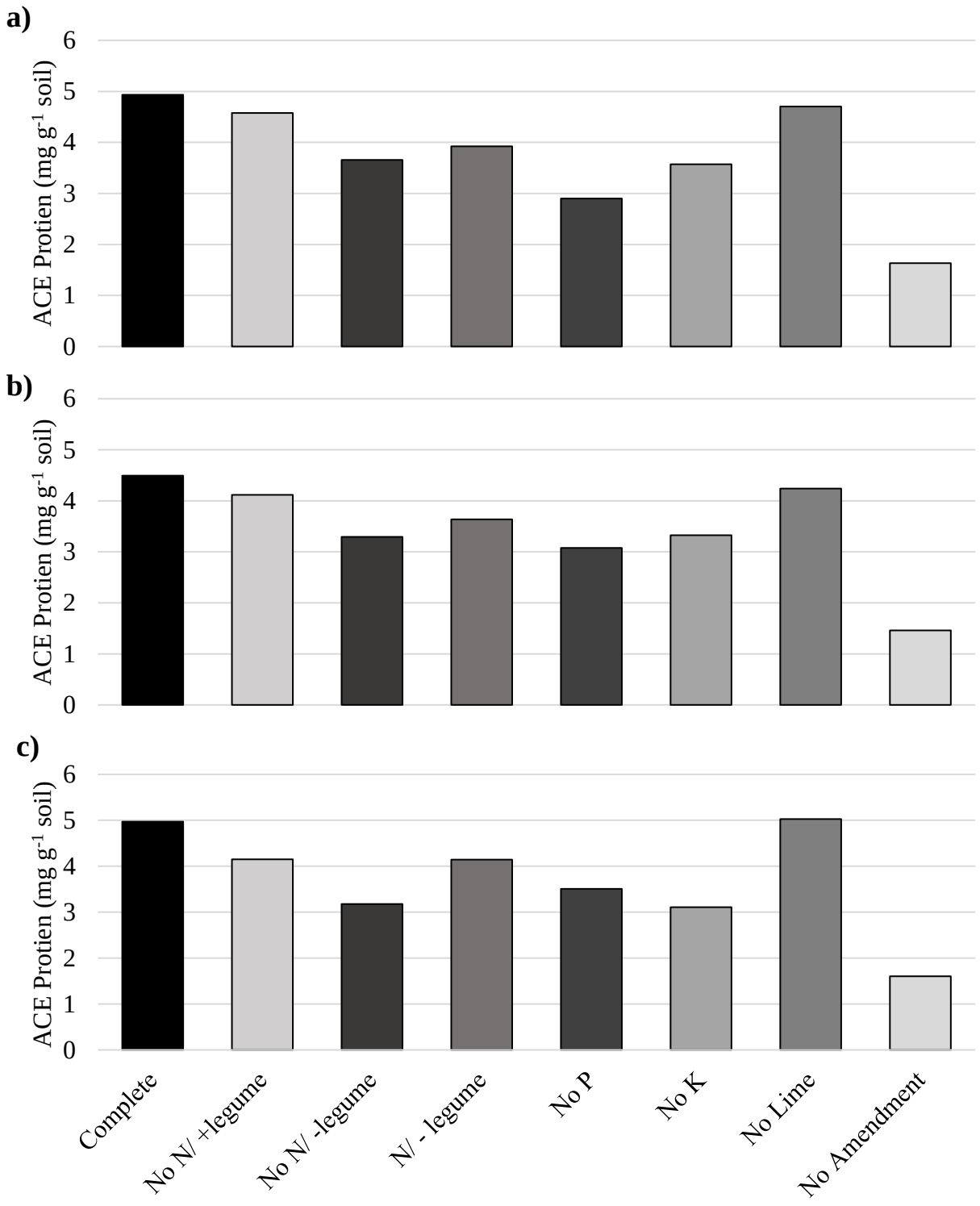

FIGURE A.2. Effect of treatment by sampling date on autoclaved citrate extractable (ACE) protein at the Cullars Rotation from 0-10 cm on a) April 27, 2020, b) July 7, 2020, and c) October 6, 2020.

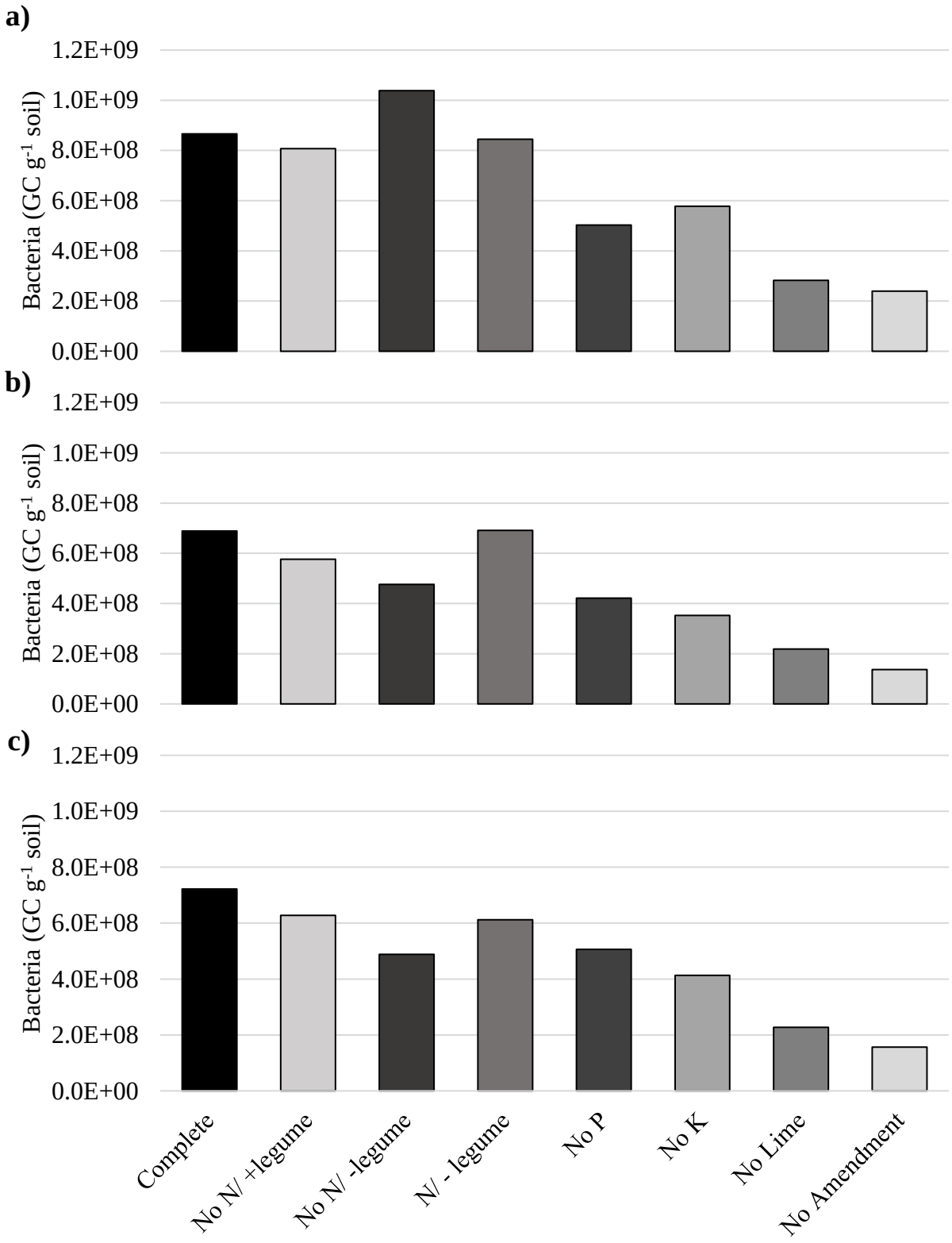

FIGURE A.3. Effect of treatment by sampling date on bacteria gene copies (GC) protein at the Cullars Rotation from 0-10 cm on a) April 27, 2020, b) July 7, 2020, and c) October 6, 2020.

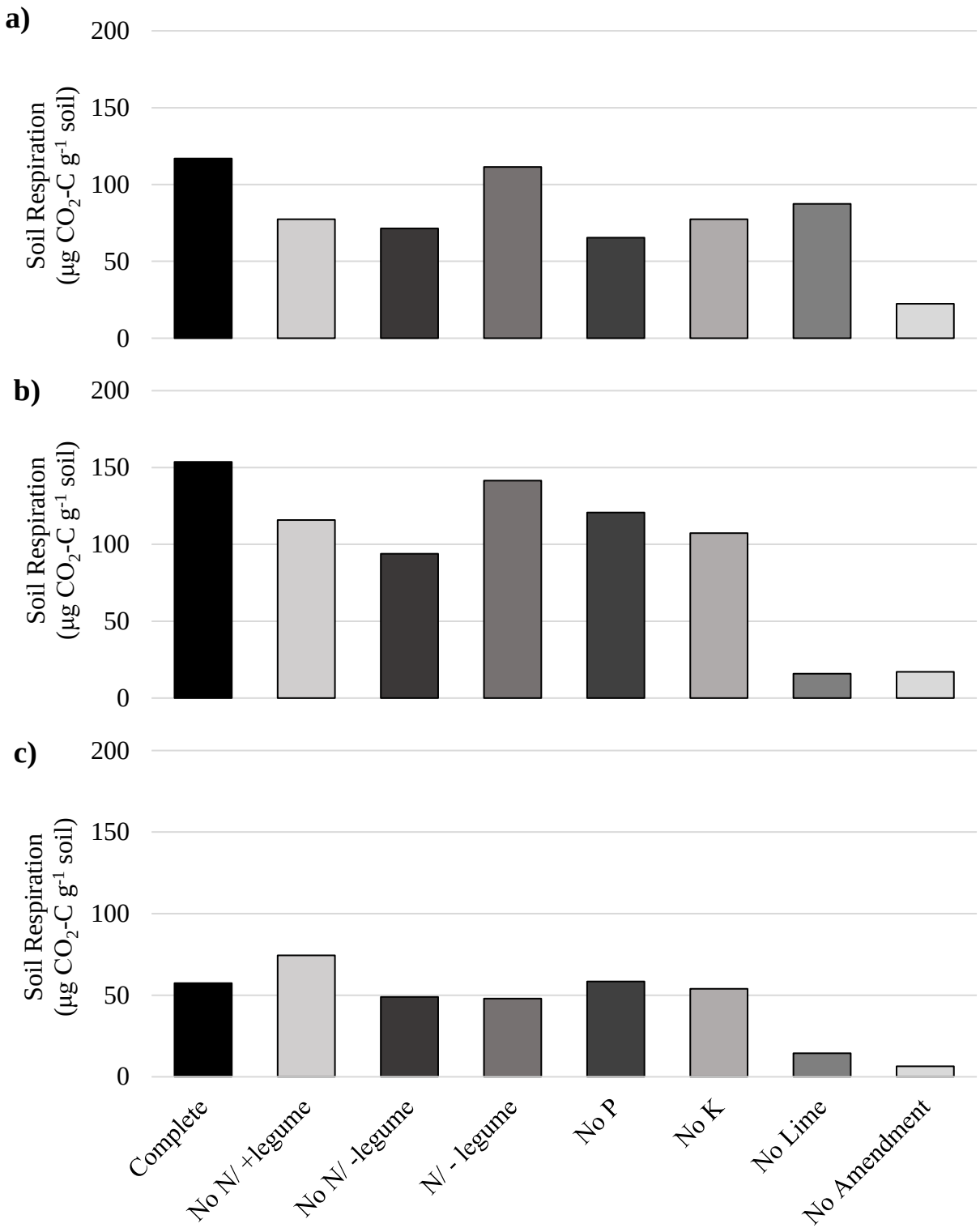

FIGURE A.4. Effect of treatment by sampling date on soil respiration at the Cullars Rotation from 0-10 cm on a) April 27, 2020, b) July 7, 2020, and c) October 6, 2020.