

Impacts of Winter Grazing on Soil Health in Southeastern Cropping Systems

by

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ABSTRACT

Coastal Plain soils are often characterized by low soil organic carbon as a result of historically intense row cropping practices, highly weathered soils and the humid climate of the region which often breaks down organic matter faster than it can accumulate. A rotation of cotton (*Gossypium hirsutum* L.) and peanut (*Arachis hypogaea* L.) under conventional tillage is typical in this region, but an opportunity to encourage diversification of rotations that improve soil quality exists. If managed properly, combining annual winter grazing of cover crops in a cotton-peanut rotation under conservation tillage may provide additional soil organic matter to improve soil health and fertility. Studies are needed to establish guidelines for integrated crop-livestock (ICL) systems which maximize soil health benefits while maintaining yield and providing quality forage for livestock. A study was established at the Wiregrass Research and Extension Center in Headland, AL to determine the effects of an ICL system in which winter grazing livestock were incorporated into a cotton-peanut rotation with a winter cover crop mixture of ‘Cosaque’ oats, ‘FL401’ rye, ‘Sunrise’ crimson clover, and ‘T-raptor’ brassica. Three cattle removal dates (i.e., mid- February, mid-March, mid-April) and an ungrazed control were compared to assess the effect of grazing period length on soil organic carbon (SOC), permanganate oxidizable carbon (POXC), water stable aggregates (WSA), penetration resistance (PR), microbial biomass-carbon (MBC), and arbuscular mycorrhizal fungi (AMF) colonization rates. After the first two years of integrating winter grazing, selected soil health indicators did not change based upon length of grazing. The ungrazed control and treatments with shorter grazing periods resulted in increased biomass on the soil surface at the time of cover crop termination. Microbial biomass C was the only soil health indicator to exhibit a treatment effect having greater MBC in the ungrazed control treatments, likely due to greater cover crop biomass

present on the soil surface at termination. Water stable aggregates and PR were unaffected by the presence of livestock and length of grazing showing that negative physical impacts of winter grazing are not detectable in the early years of this study. Higher biomass may have increased cotton lint yield in 2019, possibly through conserving soil moisture during the cash crop growing season. However, peanut yield in 2020 was unaffected by presence of livestock or grazing time. The lack of consistent results may indicate that integrating winter grazing livestock does not negatively nor positively impact soil health in southeastern row crop production systems. However, more time under this management will be needed to thoroughly evaluate how winter grazing livestock impact soil health and yield.

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LIST OF ABBREVIATIONS

ICL	Integrated Crop-Livestock
SOC	Soil Organic Carbon
SOM	Soil Organic Matter
AMF	Arbuscular Mycorrhizal Fungi
TC	Total Carbon
CEC	Cation Exchange Capacity
POXC	Permanganate Oxidizable Carbon
MBC	Microbial Biomass Carbon
WSA	Water Stable Aggregates
PR	Penetration Resistance
OM	Organic Matter
WREC	Wiregrass Research and Extension Center
θM	Soil Moisture Content
WHC	Water Holding Capacity
AUC _{C.I}	Area Under the Curve for Cone Index

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LITERATURE REVIEW

INTRODUCTION

Technological advances in agriculture, such as chemical fertilizers and specialized machinery, have led to decreased food prices and increased availability of food, fuel, and fiber (Hilimire 2011). With these technological advances, millions have been saved from starvation and have greater access to protein sources, but these advances have led to the implementation of specialized agricultural systems. Specialized systems focus on the production of a single or very few commodities and have led to gains in productivity and labor-use efficiency (Peterson et al., 2019). However, there is a growing scrutiny of the long-term sustainability of specialized systems, since they may decrease biodiversity, lower soil quality, increase greenhouse gas emissions, and result in excessive nutrients and pesticides in water (Peterson et al., 2019; Franzluebbers et al., 2014). Specialized agricultural systems were considered a solution as urban development encroaches on available farmland and producers are pressured to find ways to feed a growing population (Balkcom et al., 2010). However, some producers are finding ways to limit negative impacts on their soil and the surrounding environment while remaining profitable through implementing conservation practices that sustainably intensify the land. Conservation practices that can improve agroecosystem health include conservation tillage, crop rotations, and cover cropping.

Sustainably intensifying land is an option for producers to meet demands of the growing population while lessening their environmental impact. Systems that sustainably intensify land may increase the number of crops in rotation or add cover crops between cash crops. Crop rotations are known to improve above and belowground biodiversity and break pest or weed cycles. With the introduction of certain crops to a rotation, the rotation can improve soil health by increasing residues and lessening the need for synthetic fertilizer. For example, introducing

corn into a rotation with cotton can increase the amount of organic matter inputs through increased residue on the surface. Adding a legume, such as peanut, into a rotation of non-legumes can reduce N requirements for the system. Producers can also enhance the sustainability of their cropping system by utilizing conservation tillage. Conservation tillage, which ranges from reduced tillage to no-tillage, retains 30% or greater cover from crop residue on the soil surface and has ecological benefits. Using conservation tillage can reduce wind and rain erosion, prevent runoff, and prevent organic matter decomposition, which can lead to improved soil health (Carter 2005; Rosa-Schleich et al., 2019). When used in combination with conservation tillage, cover crops can provide additional benefits like increased organic matter inputs to the soil, improved soil moisture, and reduced weed competition. Utilizing cover crops also contributes to increasing above and belowground biodiversity.

An integrated crop-livestock (ICL) system is a method that can be used to further diversify land sustainably. An ICL system is a type of management that incorporates livestock into a crop production system. There are many types of ICL systems. For example, grazing livestock can be incorporated on forages growing between rows of perennial crops or on cover crops growing between the cash crop growth period. While ICL systems are not new, as archeological evidence shows use since the Neolithic age, it has been replaced in favor of operations that focus on either specialized production of livestock or crops (Carvalho et al., 2010). There is a growing interest in diversifying land by implementing an ICL system, as monocultures and other specialized agricultural systems have garnered criticism for their impact on the environment (Riberio et al., 2019). Integrated crop-livestock systems provide opportunities for increased diversification, nutrient cycling, maximization of farmland use, and economic gains (Riberio et al., 2019). For example, grazing of winter cover crops can be

beneficial for producers interested in some soil health benefits, like erosion prevention or increased organic matter inputs, while simultaneously creating an economic return through animal gains. Row crop production systems which incorporate grazing of winter cover crops are feasible in the southern Coastal Plain region, where the hot, humid climate allows enough winter cover crop growth for grazing.

CONSERVATION SYSTEMS IN THE SOUTHEAST

Conservation Tillage

For many years, the use of conventional tillage has degraded soils all over the world and in the Southeastern US. Conventional tillage disturbs and turns the soil to incorporate crop residues and fertilizer, control weeds, and aerate the soil. It leaves little residue on soil surface exposing the soil to erosive forces like wind and rain. Mixing surface residues with soil leads to rapid decomposition of soil organic matter (SOM) through microbial activity which impacts soil physical and chemical properties such as aggregate stability and soil organic carbon (SOC) (Balkcom et al., 2013; Sarto et al., 2020). Cotton was historically produced in monocropping systems with conventional tillage which increased erosion and accelerated oxidation of the existing soil C (Motta et al., 2007). Unlike the Midwestern US where ancient glaciers protected the soils from natural forces, soils in the Southeast have been exposed to natural forces, creating highly weathered soils that are relatively acidic and C depleted (Shaw et al., 2009). The use of conventional tillage has further intensified the weathering of soils in the Southeast.

Replacing conventional tillage with conservation tillage is an option for producers to improve soil quality in the Southeast. Conservation tillage retains at least 30% of crop residue on the soil surface, protecting the soil from erosive forces and runoff. Mannering and Fenster (1977) found that conservation tillage decreases soil erosion in direct proportion to the amount of soil cover left after tillage. Two common types of conservation tillage include reduced tillage, which

allows for soil disturbances but keeps at least 30% of residues on the soil surface, or no-tillage, which does not disturb the soil and leaves all residues on the surface. Ecological benefits of reduced tillage include increased belowground biodiversity, increased nutrient availability, and higher carbon sequestration (Rosa-Schleich et al., 2019). One of the greatest benefits of conservation tillage is promoting SOM sequestration by increasing residue on the surface and decreasing soil disturbances. Reductions in soil disturbance prevent oxidation of organic C, allowing SOM to accumulate. Gamble et al. (2014) found that conservation tillage and residue inputs increased SOM in cotton-peanut-bahia grass rotation that included a winter cover crop of oat and rye. Economic advantages of conservation tillage include increased long-term yield, lower fuel cost, and reduced labor input (Rosa-Schleich et al., 2019). Some disadvantages associated with conservation tillage include the potential for higher herbicide costs and lower soil temperatures that delay germination (Belvins and Fyre 1993). Combining conservation tillage with other management systems, such as crop rotations and cover cropping, can further increase soil health benefits.

Crop Rotation

For centuries, producers have been aware that utilizing crop rotations can improve crop productivity. Depending on soil and crop type, organic matter inputs can increase when changing from a monocropping system to a crop rotation because the level of organic matter in the soil is considered a function of the net organic matter residue inputs from the cropping system (Chandler et al., 1997). Loss of SOC occurs when the rate of organic matter being mineralized is greater than the overall input from plants, so incorporating high residue crops with low residue crops can increase the net organic matter inputs to increase SOC and SOM (Mitchell and Entry 1998). For example, West and Post (2002) found that increasing rotation complexity, except when changing from continuous corn, increases SOC based on analysis of a global data set. Crop

rotations are also known to mitigate weed, insect, and pathogen pressure by breaking pest cycles that may occur in monoculture systems (McDaniel et al., 2014). For example, adding corn or sorghum in rotation with peanut can help mitigate nematode pressure and improve yields compared to a monoculture peanut system (Rodríguez-Kábana and Touchton 1984). The Federal Agricultural Improvement (FAIR) Act, which removed many program-based incentives for monoculture production of major field crops, led to an increase in crop rotations (Taylor et al. 1999). Producers are aware of the positive impacts crop rotations have on agroecosystem and soil health, so it is now a common practice throughout the US with an estimated 82% of the cropland in the US being in rotation (Taylor et al. 1999; Padgitt et al. 2000). There are further ways to diversify by incorporating cover crops outside of the cash crop growth season.

Cover Crops

In row crop production systems, cover crops are grown to cover and protect the soil between cash crop growing seasons. Utilizing cover crops in a row crop production system offers many benefits: prevention of erosion and compaction, accumulation of organic matter, suppression of weeds, and protection from plant nutrient loss (Schipanski et al., 2014). Cover crops can contribute to soil health before and after termination through increased aboveground and belowground organic matter inputs. Cover crop benefits vary in different regions with different soil types (Blanco-Canqui et al., 2015). Selection of cover crops should consider climate, soil type, and cropping system to maximize benefits of cover crops.

Small grains, such as rye and oat, are known to produce large amounts of above and belowground residues, making them suitable for use as cover crops in row crop production systems in the Southeast. The roots, which grow quickly and fibrously, promote good soil structure, improve drainage, protect the soil from erosion, and scavenge residual soil N (Schomberg and Endale, 2010). Small grains may also provide weed control benefits by

providing residue on the soil surface following termination or as a living cover crop (Poffenbarger et al., 2015). The most common cover crop utilized in the US in 2019 was cereal rye (CTIC, 2020). Balkcom et al. (2013) observed that rye supplied more biomass and more C to the soil compared to wheat over a six year corn-cotton rotation on a fine sandy loam in Alabama. Simoes et al. (2009) found that using rye as a winter cover crop in a cotton-peanut rotation provided an increase in net return for both cotton and peanut crops in sandy Coastal Plain soils. Oat, the third most popular cover crop used in 2019, also provides many benefits to agroecosystems, such as exceptional weed control due to the thick residue produced and the natural compounds released the roots which inhibit weed growth (CTIC, 2020). There is also evidence of increased cotton yield following oat grown on Goldsboro loamy soils according to Bauer and Reeves (1999) and on sandy soils of Georgia according to Schomberg et al., (2006). Small grains, like oat and rye, are excellent options for cover crops in row crop production systems and also make excellent forage for livestock. George et al. (2013) found that using ryegrass and oat as winter forage can offer benefits like reduced soil moisture loss and reduced nutrient leaching from the previous summer crop (George et al., 2013).

Legumes are another option for cover crops in row crop production systems. A major reason producers may plant legumes as a cover crop is for their ability to fix N. Winter legumes can provide a source of N for non-leguminous summer crops which reduces energy requirements and production cost in a cropping system (Touchton et al., 1984). The most commonly planted leguminous cover crop in the US in 2019 was crimson clover and is also a common winter cover crop in the Southeast (CTIC, 2020; Reeves, 1994). Crimson clover is slower to establish and produces less biomass than small grains, like rye, but offers the benefit of providing N (Schomberg et al., 2005). Crimson clover can provide significant amounts of N to the subsequent

non-leguminous crops, providing as much as 120 kg N ha⁻¹ (McVay et al., 1989). However, Sievers and Cook (2018) observed that hairy vetch, a type of leguminous cover crop, decomposed more rapidly than rye, a small grain, and released a majority of its N in the first two weeks after termination. The quick release of N can be lost to leaching or denitrification if the cash crop is not planted early (Sievers and Cook 2018). A study conducted in a sandy soil of the Coastal Plain, which has a low amount of N, indicated that legumes, including crimson clover, can be used as an N source for cotton (Touchton et al., 1984). McVay et al. (1989) found that adapting leguminous cover crops (crimson clover and hairy vetch) provided N fertilizer needs for the production of sorghum in the Coastal Plain. Leguminous cover crops are an excellent choice for producers to use when seeking to increase soil N, but there are further options when seeking other benefits.

Cover crops from the Brassicaceae family, such as radishes, are an option in row crop production systems for producers who are seeking a cover crop that produces biomass earlier than other common cover crops, such as rye, oat, winter wheat, crimson clover, and hairy vetch (Lawley et al., 2011). Other common cover crops produce most of their biomass in the spring while radishes emerge quickly in the fall if planted early (Lawley et al., 2011). The quick emergence of radishes also allows for early weed control. Several studies (Kruidhof et al., 2008; Swanton et al., 1996; Swanton et al., 1996; Wang et al., 2008) have shown different cultivars of radishes are able to suppress weed growth in the fall in various parts of the world in different cropping systems. In the Coastal Plain of Maryland, forage radish was able to provide near complete weed suppression in spring in no-till corn, but weed suppression did not continue through the summer (Lawley et al., 2011). Radishes are also known for their large taproot, which may penetrate through compacted soils in some instances. Soil compaction can negatively

impact plant root growth and water and nutrient uptake which hinders plant development (Chen and Weil, 2010). Utilizing a radish with a large taproot rather than fibrous roots, such as forage radish or rapeseed, can be used as a means of alleviating compaction (Chen and Weil, 2010). Williams and Weil (2004) saw an increase in soybean yields when using a combination of rye and forage radish as fall cover crops compared to using monoculture cover crops of radish or rye, regardless of deep tillage or no deep tillage. The increase was attributed to increased residue from rye which helps retain moisture and the larger root channels left by radish which may have allowed for low resistance pathways for soybean roots (Williams and Weil, 2004). Using a combination of cover crops is a great option for producers seeking multiple benefits.

A cover crop mixture can contribute diverse residues that vary in biochemical composition, which can impact soil health and, as a result, yield of the subsequent cash crop (Smith et al., 2014). As aforementioned, different cover crops offer various benefits to agroecosystems. Small grains can provide large amounts of residue, legumes can increase soil N and promote beneficial insect populations, and radishes can aid in alleviating compaction. On top of various benefits each cover crop contributes individually, finding the right combination of cover crops is important to maximize soil health. Combining a mixture of legume and non-legume cover crops can improve soil health by supplying C and natural N inputs, as well as lowering the potential of N leaching (Sainju et al., 2007). Poffenbarger et al. (2015) saw an increase in N content and a decrease in C:N ratio as hairy vetch biomass increased in a cover crop mixture. Proper C:N ratio is crucial to survival of microbes in soil. If the C:N ratio is too high (above 24:1), soil microorganisms will have to find additional N in the soil to break down cover crop residues which leads to slower decomposition of cover crops. If the C:N ratio is too low (below 24:1), cover crop residue will be decomposed more quickly, and microbes will leave

excess N in the soil. For example, rye reduced maximum soil temperature more effectively compared to hairy vetch according to Blanco-Canqui et al. (2015), which can be attributed to slower decomposition of rye due to its higher C:N ratio. A cover crop with a higher C:N ratio, such as rye, will decompose more slowly because microorganisms need to find additional N to consume rye. The N in the soil may then become unavailable until microorganisms die, releasing N. A benefit of a cover crop with a high C:N ratio is the slow decomposition keeps residue on the surface for a longer period of time which can limit soil moisture loss, reduce soil temperature, reduce erosion and leaching, and suppress weeds. A cover crop with a lower C:N ratio, such as hairy vetch, will be broken down more quickly and less N will be required for decomposition, leading to a temporary N surplus in the soil. An additional factor is the maturity of cover crops at the time of termination because more mature residues have greater C:N ratios (Schomberg et al., 2006). Based on the producer's goals, a cover crop or cover crop mixture can be selected and used to accomplish multiple goals. Cover crops can also be utilized for livestock consumption. In this type of system, selecting a cover crop monoculture or mixture to maximize forage availability and quality would be crucial.

Integrated Crop-Livestock Systems

Since the Neolithic age, ICL systems, a mixed farming system that combines row crop production with animal husbandry, have been utilized (Carvalho et al., 2010). Integrated crop-livestock systems provide opportunities for increased diversification, nutrient cycling, maximization of farmland use, and economic gains (Ribiero et al., 2019). Depending on climate and crop type, ICL systems can take many different forms. In the southeastern United States, mild winters allow for growers to incorporate grazing livestock on winter cover crops between the summer cash crop growth period. Nearly 80% of beef production operations in the Southeast are cow-calf operations in which a large amount of the operating cost comes from winter

feeding, so using an ICL system is a strategy that can reduce winter feed cost (Han et al., 2018). Additionally, many producers in this region already have livestock and row crops, so there exists an opportunity to merge row crop and livestock production systems.

There is a wealth of research on the benefits of cover crops, but research is lacking on the effect of ICL systems on soil properties and crop productivity (Franzluebbbers 2007). For producers, a major concern of adding grazing cattle is the potential negative effect on soil physical properties, like compaction, that could affect the subsequent cash crop's yield (Carvalho et al., 2010). Generalizing impacts of ICL systems on soil properties can be difficult due to differences in management styles; stocking rates, grazing intensity, soil type, and vegetation are all things to consider when evaluating an ICL system (Davinic et al., 2013). Cattle grazing can result in changes in air, water, and nutrient movement in the soil as a result of trampling, defoliation, defecation, and urination (Siri-Prieto et al., 2007). Negative impacts on soil properties have been reported in systems with high stocking rates and grazing intensities (Davinic et al., 2013). However, Franzluebbbers and Stuedemann (2014b) found that winter grazing on cover crops did not affect the subsequent cash crop's yield in a corn-soybean rotation on Ultisols of Georgia. In a four-year study evaluating grazing treatments on rye cover crops in a cotton production system in the Southeast, Schomberg et al. (2014) found that cotton yield was numerically lower for the grazed treatment, but the difference was not significant from the non-grazed system, from an economic standpoint. Even with the numeric decrease in cotton yield, Schomberg et al. (2014) saw greater returns in the grazed system compared to the non-grazed system by \$81 per hectare. Similarly, Franzluebbbers (2007) saw an increase in farm income when cattle were grazed on cover crops in a cotton-peanut rotation in the southeastern US. Integrated crop-livestock systems may have the potential to increase farm income in the

Southeast. However, more research is needed on ICL systems and how to properly manage them to obtain economic benefits while not negatively impacting crop production or the surrounding environment.

SOIL HEALTH INDICATORS

Soil health can be described as soil characteristics which determine its capacity to produce economic goods and services and regulate the environment (Lal 1993). Many soils around the globe are severely degraded as a result of natural and anthropogenic factors, such as erosion, insufficient added residues, and intensive tillage. Certain soil characteristics, such as porosity, C content, and microbial activity, are crucial to soil health and agricultural productivity. Many chemical, physical, and biological properties can serve as indicators of soil health, but SOM is viewed as the most significant single indicator of soil health because it is integrally tied to many other chemical, physical, and biological properties of soil (Weil et al., 2003; Reeves 1997). Soil organic matter is the carbon-containing fraction of soil made up of plant and animal residues at different stages of decomposition. Over time, plant and animal residues are broken down by microbial activity to detritus and then stabilized as humus. Soil organic matter is known for having the ability to provide ecosystem services that enhance nutrient cycling, water holding capacity, and soil structure. High soil organic matter concentrations can also reduce contaminant uptake in crops and prevent leaching of contaminants into groundwater (Diederich et al., 2019; Lehmann and Kleber 2015).

Approximately 58% of SOM is made up of SOC (Blanco-Canqui et al., 2013). Soil organic carbon serves as an energy source for microbial processes and is vital in respiration and nutrient storage (Reeves, 1997). Management practices can greatly affect SOC. For example, long-term conventional agricultural practices can decrease SOC stocks by 30-60% (Kopittke et al., 2019). Long term data from the Old Rotation at Auburn University revealed a substantial

decrease in SOC in continuous cotton with no winter cover crop, while continuous cotton with a winter legume cover crop substantially increased SOC (Reeves, 1997). Soil organic C cannot be increased without significant inputs of C from crop residues or manures; conservation tillage alone will only slow loss of SOC (Reeves, 1997). Adopting more complex crop management systems that increase residue or manure inputs may enhance soil C sequestration. However, soil C sequestration is also influenced by site history, crop selection, and climatic factors (Reeves, 1997; Sanderson et al., 2013).

In the Southeast, loss of SOM has been exacerbated by the chemical and physical nature of soils (Balkcom et al., 2013). Soils in the Southeast are highly weathered, easily erodible, carbon-depleted, and low in water holding capacity (Shaw et al., 2009; Simoes et al., 2009). Historic agricultural practices, such as intensive tillage, combined with the climate of the Southeast, has led to a further decrease in soil health. Mild winters, high temperatures, and high humidity in the Southeast accelerates breakdown of organic matter, reducing the ability to accumulate SOM (Balkcom et al., 2013). Hans and Pendleton (1945) compared soils along the Atlantic Coast and observed that for every 10° C increase in annual temperature, average SOM content decreases by two to three times, provided the precipitation-evapotranspiration ratio is kept the same. Crops produced in areas with low ability to build SOM may yield less than their economic potential (Siri-Prieto et al., 2007; Sainju et al., 2007). Although soils of the Southeast are not as naturally fertile as those of other regions, with conservation practices, like conservation tillage, crop rotations, and cover cropping, they can be highly productive (Balkcom et al., 2013). For example, long term data from The Old Rotation at Auburn University has shown increased crop yields of cotton and corn with increased SOC levels (Reeves, 1997). Building SOM is crucial to agricultural soils because SOM aids in retaining plant-available

nutrients, promoting the formation of soil structure, and improving water holding capacity (Lehmann and Kleber 2015).

Chemical

Soil chemical properties are extremely important to understanding and evaluating soil health. Various soil chemical indicators, such as SOC content, nutrient content, and soil pH, are related to basic soil functions like promoting microbial activity, controlling water and solute flow, cycling carbon and other nutrients, and providing physical stability. Soil chemical indicators that provide insight to how management practices affect SOM are often utilized as SOM is considered the single most important soil health indicator.

Soil Organic Carbon

Soil organic carbon is the total amount of organic C in the soil and it serves as an important soil health indicator because it is directly linked to SOM. The primary source of SOC is plant litter and microbial residues serve as a secondary source (Loren and Lal 2005). Soil organic C is dynamic. Its presence and retention are influenced by many factors such as climate, topography, and soil texture. Warm, humid climates, like those of the Southeast US, accelerate the rate of residue decomposition, thus decreasing the amount of SOC that can be retained. Sloping land generally has less SOC due to natural erosion that occurs on slopes. As a result, it is hard for land up-slope to retain SOM and build up soil structure to protect the soil from erosion. Soil texture, such as percentage of sand or clay, also impacts SOC retention. Clays and SOM bind tightly together, which slows the rate of SOM decomposition, thus increasing SOC sequestered in soil by encouraging the formation of stable aggregates.

Using SOC to quantify soil health is useful because it impacts many other soil health indicators. Water holding capacity, aggregate formation and stability, and cation exchange capacity (CEC) are just a few other properties that are intimately related to SOC content (Reeves,

1997). Increased organic matter inputs, decreased oxidation of SOC, decreased rate of SOM decomposition, or combinations of the three are required to increase SOC in soil (West and Post, 2002). Agricultural management practices can greatly influence SOC sequestration, but these changes may take longer periods of time to become evident (Plaza-Bonilla et al., 2014). Labile fractions of SOC may serve as more quickly responsive indicators of management changes. Long-term studies have shown how tillage impacts SOC. A global data analysis of 67 long-term agricultural experiments saw an average increase of 57 g C m^{-2} per year sequestered when management changed from conventional tillage to no-till (West and Post, 2002). No-till practices had significantly higher levels of SOC compared to both conventional tillage and reduced tillage in the study. In a long-term study in southeastern Coastal Plain soils, Gamble et al. (2014) observed 60% higher SOC in the top 5 cm of soil for conservation tillage treatments compared to conventional tillage treatments in cotton-peanut rotations containing a rye/oat winter cover crop. This information indicates that implementing multiple management practices simultaneously (e.g., cover crops and reduced tillage) has the potential to improve soil health at the soil surface.

Addition of cover crops to a crop rotation can also impact SOC. Sanju et al. (2006) saw an increase in SOC with the adoption of a cover crop mixture containing hairy vetch and rye compared to using a monoculture cover crop or no cover crop on a sandy loam in Georgia. Data from The Old Rotation at Auburn University showed an increase in SOC in a cotton-corn rotation with winter legume cover crops compared to a continuous cotton treatment containing no leguminous cover crops (Reeves 1997). Some research on the impacts ICL systems have on SOC in the Southeast has been conducted previously. Franzluebbbers and Studemann (2008) found that impacts on soil C were more influenced by tillage and cover crop presence than by grazing livestock. Grazing cattle in winter or summer in an ICL system did not negatively

influence soil C, especially when moderate grazing was used (Franzluebbers and Studemann 2008). Management of the ICL system may have a greater impact on SOC than the presence of grazing cattle alone. More long-term research could be used in this area to understand how ICL systems SOC.

Permanganate Oxidizable Carbon

Permanganate oxidizable carbon (POXC), also referred to as active C, is a chemical indicator that represents a portion of labile C. Using a simple method based on oxidation of organic matter by a weak potassium permanganate solution, POXC can be determined (Culman et al., 2013; Plaza-Bonilla et al., 2014; Weil et al., 2003). The POXC fraction can be more sensitive to changes in management and environmental variation than SOC (Wang et al., 2017). Permanganate oxidizable C makes up between 33% and 50% of SOM. This pool of SOC greatly influences key soil functions due to its short turnover time of days to years, which is much shorter than intermediately stable and stable SOC pools, which can take years to centuries to breakdown (Hurrismo et al., 2016). Due to its reactivity, the active soil C pool may provide greater insight to how management changes can impact nutrient cycling and soil health (Hurrismo et al., 2016). Permanganate oxidizable carbon is related to other C fractions such as total SOC and microbial biomass carbon (MBC), which are other indicators that are often used to quantify soil health (Culman et al., 2012).

After initiation of a new management technique, changes in POXC can be four times greater than changes in SOC (DuPont et al., 2010). Effects of tillage, cover crops, and land use are seen more rapidly in POXC than SOC, and this sensitivity has helped POXC become an indicator of ecosystem changes in soil (Culman et al., 2012). Conservation tillage has been documented to increase POXC levels. In a long-term trial comparing soil carbon sequestration in different tillage systems in a sandy clay loam Entisol and in a clay Vertisol, POXC was higher

for conservation tillage in both soil types (Melero et al., 2009). Franzluebbbers and Studemann (2015) found that a no-till system preserved the greatest amount of active carbon on sandy loam and sandy clay loam soils of Georgia. Crop rotations also impact active carbon. Cover crop presence and species can affect POXC. Ghimire et al. (2019) found that POXC was significantly greater when utilizing a six species cover crop mixture (of pea, oat, canola, hairy vetch, forage radish, and barley) compared to a monocrop treatment of canola and canola-pea mixture. The addition of oat, which procured greater biomass, as a cover crop or in a mixture resulted in greater POXC likely due to increased residue remaining on the soil surface (Ghimire et al., 2019).

Research on the effects an ICL system has on POXC is needed. Franzluebbbers and Studemann (2015) found that while grazing animals on cover crops in sandy loam soils did not negatively affect active carbon, there was minimal positive impact. Further research is needed to provide insight on impacts that grazing livestock on cover crops have on soil carbon pools like POXC. With minimal impact, negatively or positively, producers could still use ICL systems for the potential economic gain associated with utilizing their land year-round.

Physical

Soil physical properties are important indicators of soil health and are linked to soil structure. Soil structure is described as “the heterogeneous arrangement of solid and void space...and its ability to retain this arrangement when exposed to different stresses,” (Amézketa 2008). Physical indicators of soil health provide insight on air and water movement in the soil. Soil physical properties also influence rooting depth and volume, and, as a result, they impact nutrient availability and plant growth (USDA-NRCS). Maintaining good soil structure is key to agricultural productivity and depends on the presence of stable soil aggregates (Amézketa 2008; Tisdall and Oades 1982).

Water Stable Aggregates

Aggregate stability has been proposed as a soil physical property that can indicate soil quality (Arshad and Coen 1992). Aggregate stability is the ability of cohered soil particles to withstand disruption through erosive forces (Kemper and Rosenau, 1986). Water stable aggregates (WSA) is a measure of soil's ability to resist dispersive forces from rainfall. Aggregates that are water-stable are critical to growth of plant-roots and movement of water needed for crop production. Microbial communities play a key role in aggregate stability because microbes produce organic substrates that promote aggregation and can stabilize aggregates (Haynes and Swift 1990). For example, fungi are possibly stabilizers due to production of glomalin-related soil proteins that are known to bind mineral particles together. Glomalin-related soil proteins have been proven to increase WSA (Bedini et al., 2009). Water stable aggregation is crucial to soil health and successful crop production.

The degree to which an aggregate is stable against slaking by water is a sensitive indicator of soil health, and soil aggregate stability usually increases as SOM content increases (Weil and Magdoff, 2004). While aggregate stability is generally strongly correlated with SOM content, changes in management practices and differences in soil texture can affect the correlation. A review concluded the critical concentration of SOM needed for soil stabilization was dependent on the soil texture (Weil and Magdoff, 2004). For example, SOM content is critical in stabilizing soil structure for soils low in clay (Weil and Magdoff, 2004).

Conservation tillage can improve WSA compared to conventional tillage in the Southeast. Conventional tillage disrupts soil aggregates, increases aeration, and accelerates microbial breakdown of SOC (Causarano et al., 2008). Much of the data collected regarding tillage impact on WSA focuses on the positive impact conservation tillage has on SOM by limiting soil disturbance, thus slowing the SOC turnover rate. Because soil physical properties

are so closely related to SOM, it is reasonable to conclude that increasing SOM will improve soil physical health. Maintaining plant residues on the soil surface can retain soil humidity and moderate soil temperature which slows the breakdown of residues, leading to more stable aggregates (Weil and Magdoff 2004).

Cover crops grown in the winter in temperate regions can improve soil aggregation. (Weil and Magdoff 2004). In the Coastal Plain of Alabama, using crimson clover cover crop increased the percentage of WSA over winter fallow from 40% to 49% after five years when used between corn (Reeves, 1994). McVay et al. (1989) also observed greater aggregate stability following legume cover crops compared to fallow or wheat in the upper Coastal Plain.

More research is needed regarding ICL systems and aggregate stability. Franzluebbbers and Studemann (2008) completed a study on sandy loam and sandy clay loam soils in Georgia with two grazing management treatments: no grazing of cover crops and grazing cover crops with cattle consuming approximately 90% of available forage. The study concluded that grazing had little effect on soil bulk density and aggregate stability, so grazing could be recommended as a strategy to promote greater adoption of cover cropping throughout the southeastern US (Franzluebbbers and Studemann 2008). Carvalho et al. (2010) found that integrating livestock into a cash crop rotation improved soil aggregation and soil microbial activity when using moderate, controlled grazing intensities. One study on Red Latosols in Brazil found that ICL systems with moderate (maintaining 20 cm of forage) grazing that combined no-tillage had higher soil aggregation than the ungrazed treatment at 0 – 5 cm of depth (Souza et al., 2010). More research is needed on the impacts grazing livestock have on soil physical properties, like WSA, when used in an ICL system in the Southeast. The physical condition of the soil after grazing is a major concern for producers and can negatively impact the productivity of the cash crop.

Penetration Resistance

Soil penetration resistance (PR) is an index to quantify a soil's strength. Soil strength increases with compaction, and during the summer, compaction layers can become drier and harder making the soil less penetrable for roots in search of water and nutrients stored in the subsoil (Williams and Weil, 2004). Poor management of agricultural land can cause soil compaction, which leads to a reduction in yield for current and subsequent crop seasons (Carrara et al., 2007). Some soils are compacted from use of agricultural machinery or poorly managed animal production systems (Medina et al., 2012). Penetration resistance reflects changes in other soil physical properties, including bulk density, moisture content, porosity, and permeability (Medina et al., 2012). Compacted soil can negatively impact a crop because the decreased pore space limits nutrient and water availability, and hard pans from compacted soil can limit root development.

Conventional tillage disturbs the soil, and while it is known to have negative impacts on certain soil properties, it can alleviate compaction. Conservation tillage was once linked to compaction and poor cotton performance in the Southeast on silty clay soils, but research has shown that conservation tillage practices, like no-till, that also use cover crops produce cotton yields highly competitive to conventional tillage (Schwab et al., 2002). Conservation tillage practices have been shown to only have minimally higher soil PR and compaction compared to conventional tillage (Schwab et al., 2002). On sandy clay alluvial soils in Chile, a four-year study of two tillage treatments (no-till and conventional tillage) concluded that no-till significantly increased PR compared to the conventional tillage, but only in the top 2 cm of soil (Martínez et al., 2008). Cover crops can also influence compaction and PR. Forage radishes in Maryland's Coastal Plain alleviated compaction by creating root channels that the subsequent soybean crop could utilize as low resistance pathways to obtain water stored in the subsoil

(Williams and Weil 2004). The radish's large taproot may be how it was able to decrease soil compaction. More research on soil penetration and cover crops is needed in the Southeast.

Compaction is a major concern for producers who are interested in implementing an ICL system, but there is conflicting research on the impact grazing animals have on soil physical properties. Franzluebbers and Studemann (2015) found minimal evidence of negative effects due to grazing on soil compaction in sandy loam and sandy clay loam soils of Georgia, proposing that with proper management of cattle weight and forage mass, negative effects can be avoided. A study conducted on Ultisols in an ICL system with winter grazing observed that as stocking rates increased from 4 to 8 animals ha⁻¹, PR readings increased showing that proper management of grazing livestock is crucial for preventing soil compaction (Tollner et al., 1990).

Biological

Soil biological properties are useful for evaluating soil health because organisms are intimately linked to chemical and physical soil properties. Microbes play a critical role in key processes within the soil C and N cycle. Microbial communities are key regulators of SOM and nutrient availability, and different agricultural management practices may impact soil microbial communities (Sarto et al., 2020; Six et al., 2006). Because of their importance in soil processes, small reductions in soil microbial populations may have negative impacts on agroecosystems (DuPont et al., 2010).

Microbial Biomass Carbon

Microbial biomass carbon measures the amount of C within the living portion of SOM. Microbial biomass carbon, like POXC, can be used as an early indicator of changes in SOM dynamics and nutrient cycling (McDaniel et al., 2014). Soil MBC makes up less than 5 % of SOC in most soils, but it is the primary agent of organic C transformation (Dalal et al., 1998). The turnover rates of MBC can range from six months to five years, and changes in MBC could

be used to predict changes in SOM from management practices (Dalal et al., 1998). Studies have proven that MBC is sensitive to changes in tillage, cover cropping, and land use (Culman et al., 2012). For example, Motta et al. (2007) saw that changes in MBC were more evident between different management systems (tillage, cover cropping, crop rotation, and cropping intensity) below 3 cm depth on a silt loam soil in the Southeast compared to SOC, indicating that MBC may display greater responses to changes.

Soils with high microbial diversity are more likely to continue performing their ecosystem services after soil disturbance (Cardoso et al., 2013). Agroecosystems with higher microbial diversity can have higher nutrient efficiency, increased soil aggregate stability, improved OM concentration, and enhanced water regulation (Verbruggen et al., 2010). Producers are now looking to conservation practices that can enhance microbial activity, since ensuring that microbial communities are thriving is necessary to protect soil health (McDaniel et al., 2014).

Tillage can affect soil MBC. In fact, tillage, more so than other management practices, negatively impacts soil microbes and their functioning due to loss of biodiversity (Vukicevich et al., 2016). During a seven-year tillage study on a sandy loam soil of Western Australia, Roper et al. (2010) found that MBC was highest with a no-till treatment and decreased with increasing intensity of cultivation which included conservation tillage and rotary tillage. Melero et al. (2009) saw an increase in MBC with conservation tillage compared to conventional tillage (i.e., 766 mg kg⁻¹ and 378 mg kg⁻¹ respectively) at the 0-5 cm depth in a sandy clay loam Entisol and in a clay Vertisol of Spain. Similarly, Granstein et al. (1987) saw a 32% increase in MBC at the 0-5 cm surface layer in no-till plots compared to tilled plots in Mollisols of Idaho. These studies

add to the already popular notion that reducing soil disturbances can increase MBC, especially in the surface soil, which improves soil health.

There is evidence that crop rotations improve MBC. In a meta-analysis of over 122 experiments, McDaniel et al. (2014) found adding one or more crops into rotation increased MBC by 20.7%. Increases in MBC in systems that utilize crop rotations and cover crops is likely due to increased biodiversity as MBC may be sensitive to both the quantity and biochemistry of the crop inputs (McDaniel et al., 2014). Mendes et al. (1999) evaluated the effect of a winter cover crop in a vegetable crop rotation found an increase in MBC with both legume and cereal cover crops compared to winter fallow in the first year of the study. Motta et al. (2007) observed a higher intensity cropping system (i.e., cotton–double cropped soybean+wheat) increased MBC compared to cropping systems of lower intensity (i.e., continuous cotton, with winter fallow) at depths of 0-6 cm and 12-24 cm in silt loam soil, suggesting that cropping systems can improve MBC with depth as well as at the surface.

Integrated crop-livestock systems may provide increased MBC compared to other management systems. Franzluebbers and Studemann (2015) observed that grazing livestock resulted in a minimally positive impact on MBC in sandy loam soils in the Southeast and that using no-till was more important to preserving biologically active soil C following pasture termination. Salton et al. (2014) also observed MBC levels in native vegetative areas consistently had the highest MBC due to greater biodiversity, no tillage, accumulation of plant residues on the surface, and maintenance of fungal hyphae.

Arbuscular Mycorrhizal Fungi Colonization Rates

Arbuscular mycorrhizal fungi (AMF) are a type of microscopic fungi found in plants and soils all over the globe. They are obligate symbionts found in 80% of vascular plants and on every continent except Antarctica (Schubler et al., 2001). Once established in the host plant,

AMF form a network of hyphae that acts as an extension to the host plant's root system, providing access to nutrients the host plant would otherwise be unable to access. In return, the host plant provides energy in the form of photosynthetic products. The hyphae of AMF can reach beyond the nutrient depletion zone which aids the plant in nutrient uptake and some hyphae can be so small that they can grow into soil pores that even root hairs, would be unable to access on their own. One gram of soil can contain between one to twenty meters of AMF hyphae.

Sometimes referred as “living” fertilizers, AMF have the potential to improve yield and reduce the necessity for fertilizer (Zak et al., 2011). Arbuscular mycorrhizal fungi can increase available P uptake and other non-labile mineral nutrients that are needed for plant growth and development (Gianinazzi et al., 2010). While AMF are known to aid in P uptake, they may also play a key role in N and Zn uptake, pest resistance, drought tolerance, and aggregate stability (Schipanski et al., 2014). Because of their ability to extend the host plants rooting system, AMF are thought to have the ability to increase host-plant resistance to biotic and abiotic stresses (Njeru et al., 2014). Despite knowledge of the benefits AMF have to agricultural soils, they have yet to be fully utilized (Hart and Trevors 2005). In fact, over the last fifty years many of the agricultural practices that have increased yields all over the world have negatively impacted AMF and other soil microbes in agroecosystems (Hart and Trevors 2005).

There have been several studies designed to understand the potential of AMF to lessen the need for commercial fertilizer requirements (Zak et al., 1998). However, the variability in soil, crop planted, and management practices can affect crop response to AMF infection (Zak et al. 1998). Typically, the more intense the management practices, the less AMF functional diversity is found in agricultural soils (Verbruggen and Kiers 2010). Tillage has a strong impact on mycorrhizae. As soil is disturbed, mycelial networks formed by fungi are disrupted and

colonization of roots is reduced (Vukicevich et al., 2016). Tillage affects diversity of mycorrhizal fungi present in the soil, which can lead to one dominant AMF strain, and the resulting evolutionary pressures put on by tillage decreases mycorrhizae diversity of (Verbruggen and Kiers 2010). While some AMF, such as *Glomeraceae*, are able to reestablish themselves after hyphal disturbance, this process requires more energy, which can result in a larger carbon cost for the host plant (Verbruggen and Kiers 2010). In a two-year study of a wheat-oat rotation, Castillo et al. (2006) found that mycorrhizal colonization was higher under NT compared to conventional tillage.

Crop rotations may improve the relationship between AMF and host plants. Studies have shown that when wheat is cropped continuously, the relationship between AMF and wheat begins to resemble parasitism, which has a negative growth effect (Verbruggen et al., 2010). It is possible that the relationship changes from symbiotic to parasitic in a continuous cropping regimen due to the evolution of mycorrhizae accelerating, leading to a less mutualistic relationship (Verbruggen et al., 2010). Studies of cover crops incorporated into a corn rotation increased colonization in AMF (Schipanski et al., 2014). Species of winter cover crop can affect AMF populations in the soil. For example, members of the Brassicaceae family are non-hosts for mycorrhizae and may reduce colonization rates in the following cash crop; however, studies have varying results (Njeru et al., 2014).

More research is needed regarding impacts ICL systems have on AMF populations and diversity, but one study by Davinic et al. (2013) found that two types of ICL systems which integrated perennial and annual crops in rotation with cotton increased the abundance of AMF on a Texas clay loam soil compared to continuous monoculture cotton without grazing. Arbuscular mycorrhizal fungi play a key role in the formation of economically viable and environmentally

sustainable livestock agricultural systems due to their large impact on water and nutrient cycles (Sanderson et al., 2013).

OBJECTIVES

Developing systems that can improve soil health while simultaneously providing economic benefits is extremely important to the Southeast United States. There is an opportunity to incorporate winter grazing on cover crops between cash crops in the Coastal Plain region due to the climate, but there is a lack of research on ICL systems effects on soil health in this area. Research focusing on ICL systems, grazed cover crop systems in particular, is needed to establish recommended length of cattle grazing to optimize soil health, crop productivity, and animal productivity. Combining ICL systems with conservation tillage may mitigate potential negative impacts grazing animals can have on a cropping system, as well as provide economic benefits from the utilization of land year-round. Conservation practices are known to improve SOM, but additional research is needed on the chemical, physical, and biological effects of combining conservation systems and grazing livestock have on soil.

There are two objectives of this study: (1) determine the effect of cover crop grazing on soil health indicators in a conservation tillage cotton-peanut rotation and (2) determine dates at which cattle should be removed from grazed cover crops to optimize soil health benefits.

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IMPACTS OF WINTER GRAZING ON SOIL HEALTH IN SOUTHEASTERN CROPPING SYSTEMS

INTRODUCTION

Soils in the Southeast US are highly weathered, easily erodible, carbon-depleted, and low in water holding capacity (Shaw et al., 2009; Simoes et al., 2009). For many years, conventional agricultural practices, such as intensive tillage, lack of added residues, and monocropping, have degraded soils across the region. Further, the hot, humid climate in the Southeast causes accelerated breakdown of organic matter, which makes it challenging to improve soil health (Balkcom et al., 2013). The combination of intensive agricultural practices, soil type, and climate has resulted in lower soil organic matter (SOM) levels than in other regions of the US, and crops produced in the Southeast may yield less than their economic potential (Siri-Prieto et al., 2007; Sainju et al., 2007). Concerns regarding long-term sustainability of row crop production in the Southeast has led to a renewed interest in soil conservation practices. Implementing soil conservation practices can sustainably intensify land use and improve agroecosystem health. For example, conservation tillage and cover cropping are known to improve soil health by building SOM inputs into the soil, increasing above and belowground biodiversity and improving soil structure. There is a wealth of research regarding soil health benefits of conservation systems in the Southeast, but very limited research on the impacts conservation systems that further diversify land-use have on soil health.

Since the Neolithic age, integrated crop-livestock (ICL) systems, a mixed farming system that combines row crop production with animal husbandry, have been utilized (Carvalho et al., 2010). Integrated crop-livestock systems provide opportunities for increased diversification, nutrient cycling, maximization of farmland use, and economic gains (Ribiero et al., 2019). Depending on climate and crop type, ICL systems can take many different forms. In the

southeastern United States, mild winters allow growers to incorporate grazing livestock on winter cover crops between summer cash crop growing seasons. Nearly 80% of beef production operations in the Southeast are cow-calf operations, in which much of the operating cost comes from winter feeding. Using an ICL system to maximize forage availability is a strategy that can reduce winter feed cost (Han et al., 2018). Additionally, many producers in the Southeast already have livestock and row crops, so there exists an opportunity to merge row crop and livestock production systems. Unfortunately, there is limited research on ICL systems that are designed to promote soil health. Finding the best management practices in an ICL system to improve soil health and maximize economic gains, by using cropland year-round and potentially improving crop productivity through increased soil health, can improve livelihoods of producers in the Southeast.

Building SOM is crucial to agricultural soils because SOM is considered the single most important indicator of soil health. Soil organic matter is linked to many other chemical, physical, and biological properties of soil that quantify soil health. For example, SOM aids in retaining plant-available nutrients, promoting soil structure formation, and improving water holding capacity (Lehmann and Kleber, 2015). Conservation practices, such as conservation tillage, crop rotation, and cover cropping, are known to increase soil health by reducing erosion, increasing organic matter inputs, and improving biodiversity. Thanks to plentiful research, the use of conservation systems has increased dramatically across the country and in the Southeast. In the last five years, the average hectareage of cover crops planted in the US has increased by nearly 40% (CTIC, 2020).

Recent research has shown that adding grazing livestock to a production system can have benefits to agroecosystem health such as enhanced nutrient cycling, breaking weed or pest

cycles, and increased organic matter additions from manure (George et al., 2013; Salton et al., 2014). However, thorough research on how to manage ICL systems to improve soil health in the Southeast could lead to greater adoption by producers. Therefore, our objectives of this study were 1) to determine effects of cover crop grazing on soil health indicators in a cotton-peanut rotation under conservation tillage and 2) to determine dates at which cattle should be removed from grazed cover crops to optimize soil health benefits.

MATERIALS AND METHODS

Experimental Design

A research trial was established in the fall of 2018 at the Wiregrass Research and Extension Center (WREC) in Headland, AL (31°30'N, 85°17'W) on a soil classified as a Dothan fine sandy loam (fine-loamy, kaolinitic, thermic Plinthic Kandiudults). At WREC, the field was divided into twelve 0.61 ha paddocks. The site had been previously managed (> 8 years) in a peanut-rye/oat-pearl millet (*Pennisetum glaucum*) rotation under conventional tillage. Four grazing treatments were organized in a randomized complete block design and replicated three times. Grazing was initiated in early January. Treatments included: mid-February cattle removal, mid-March cattle removal, mid-April cattle removal, and an ungrazed control.

Prior to initiation of the experiment in 2018, the area was disked, subsoiled, and field cultivated prior to cover crop planting. A winter cover crop mixture of 'FL401' rye, 'Cosaque' oat, 'AU Sunrise' crimson clover, and 'T-raptor' brassica hybrid was planted. Seeding rates are provided in Table 1. Seeding rates varied between grazing and control plots based on guidelines for managing cover crops versus winter grazing according to Alabama Cooperative Extension System recommendations. Cover crops were planted using a Great Plains 1205 no-till drill (Great Plains Manufacturing Inc., Salina, KS) on 29 October 2018 and 4 November 2019 and terminated approximately two weeks prior to cotton planting and peanut planting in 2019 and

2020, respectively (Table 2). In all treatments, cover crops were chemically terminated with a tank mix of glyphosate at 1.25 kg ai ha⁻¹ and 1.6 kg ai ha⁻¹ of pendimethalin for burndown and rolled approximately one month after the last cattle removal treatment. Phosphorus, potassium and lime were maintained according to soil test recommendations from the Alabama Agricultural Experiment Station (Mitchell, 2012). Grazed plots received 26.9 kg ha⁻¹ N application and control plots received 67.2 kg ha⁻¹ N application. Nitrogen application varied between control plots and grazed plots because of different management recommendations for cover crops and for winter grazing according to Alabama Cooperative Extension System recommendations. In 2019, ‘Deltapine 1518’ cotton was planted with a 91.44-cm row spacing following cover crop termination. In 2020, ‘GA-06G’ peanut was planted with a 91.44-cm row spacing following cover crop termination. Cotton was planted 30 April 2019 and peanut was planted 4 May 2020 (Table 2). Conservation tillage, maintaining 30% or greater biomass on the soil surface, was used in combination with non-inversion subsoiling. Subsoiling is defined as tillage below a depth of 35 cm (ASAE Standards, 1999). Pesticides were applied according to Alabama Cooperative Extension System recommendations (Majumdar et al., 2020; Smith et al., 2020).

Stocker cattle of approximately seven to eight months in age in January 2019 were used to graze the experimental treatments under continuous grazing with a stocking rate of 1 kg body weight / 1 kg forage dry matter. Put-and-take cattle were used throughout the grazing season to maintain forage allowance (i.e., 1 kg dry matter per 1 kg body weight), and forage height was kept at approximately 15 cm. Grazing began in January and all cattle were removed from each designated treatment according to each cattle removal date assigned to that paddock. Cattle had *ad libitum* access to water and high-magnesium mineral during the grazing period. Cattle had no access to shade due to moderate seasonal temperatures.

Sampling

Soil and Biomass Sampling

Soil samples were collected using bucket augers following cover crop termination in spring of 2019 and 2020. Soil samples to be used for lab analyses of permanganate oxidizable carbon (POXC), water stable aggregates (WSA), and soil organic carbon (SOC), were collected from depth intervals of 0-5, 5-10, 10-15, and 15-30 cm two weeks after cover crop termination in 2019 and 2020. Due to variability in soil texture, two GPS marked locations were chosen for soil sampling within each paddock. These are referred to as sampling locations throughout the manuscript. The same GPS locations within each paddock were used for soil sampling in both 2019 and 2020. Ten sub-samples at each sampling location were combined to form a composite sample for laboratory analysis. A composite of ten samples from the 0-15 cm depth at each sampling location was collected and transported to the laboratory on ice for analysis of microbial biomass carbon (MBC).

Cover crop biomass sampling occurred within one week prior to cover crop termination. A composite of four random 0.25-m² subsamples were taken at each GPS-marked sampling location. Biomass samples were oven-dried for at least 48 hr at 60°C and weighed to obtain dry cover crop biomass.

Root Sampling

Root samples for arbuscular mycorrhizal fungi (AMF) colonization rates were collected during the cash crop growing period in 2019 and 2020. Cotton roots were sampled at the fourth true leaf stage and peanut roots were sampled approximately 60 days after planting. Sampling was conducted by uprooting five to ten plants from each GPS marked sampling location. Plants were placed in sealable plastic bags and transported to the laboratory on ice. Roots were then gently rinsed in water for two minutes. Using scissors and cutting as closely to the taproot as possible, lateral roots were cut and removed from the taproot and carefully placed in tightly

sealed glass vials with 0.5 M formalin–acetic acid–alcohol (FAA) that had been prepared prior to sampling. Vials were kept in a refrigerator at approximately 10°C until assessing AMF colonization rates.

Analysis

Soil Chemical Analyses

All soils were sieved to 2 mm for soil chemical analyses. A portion of the soils were ground using a coffee grinder and analyzed for total carbon via dry combustion with a CN LECO 2000 analyzer (LECO Corp., St. Joseph, MI) in 2019 and via combustion on a CHNS/SIR elemental analyzer (vario EL cube, Elementar, Lagensfeld, Germany) in 2020. No soil samples contained inorganic carbon; therefore, total carbon was used as a measurement of SOC.

Soil samples were analyzed for POXC as described in Weil et al. (2003). Air-dried soil (2.5 g) was placed in a 50 mL centrifuge tube with 18 mL of deionized (DI) water and 2 mL of 0.2 M potassium permanganate (KMnO₄) stock solution. Centrifuge tubes were placed in a shaker and shaken at 240 oscillations per minute for two minutes. Centrifuge tubes were then removed from the shaker and placed in a dark area for ten minutes to settle. The oxidation of active C results in the conversion of Mn (VII) to Mn (II). Lighter purple solutions correspond to higher POXC levels. Once settled, 0.5 mL of supernatant from each centrifuge tube was added to a new centrifuge tube with 49.5 mL of DI water for a 100-fold dilution and a 0.2 mL aliquot was transferred to a 96-well microplate with one replication. Four standards including 0.05, 0.1, 0.15, and 0.2 M KMnO₄ were prepared for the standard curve. The absorbance of the samples and standards was read at 550 nm on a spectrophotometric microplate reader (Biotek MQX200, Winooski, Vermont). POXC levels were determined using Equation 1, in which *abs* is absorbance, *a* is the intercept of the standard curve, and *b* is the slope of the standard curve (Weil et al., 2003; Culman et al., 2012a).

Equation 1:

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

Soil Physical Analysis

Water stable aggregates (WSA) were measured through methods described in Kemper and Rosenau (1986). A subset of each air-dried soil sample was sieved to only include 1- to 2-mm sized aggregates. The 1- to 2-mm aggregates were weighed (4 g) into cup-like sieves of 24 mesh cm^{-1} wire and rewetted to near field capacity using a household humidifier. The rewetted samples were then uniformly raised and lowered into pre-weighed individual metal containers of DI water at a rate of 35 times min^{-1} for three minutes. New pre-weighed metal containers were filled with diluted sodium hexametaphosphate $(\text{NaPO}_3)_6$ and NaOH dispersal solution and the aggregate samples were raised and lowered into the solution at 35 times min^{-1} until all remaining aggregates dispersed. All metal containers were then dried at 105°C overnight or until all water had evaporated. All metal containers were weighed again. The weight of soil remaining in the metal containers was compared to determine the percent of stable aggregates, with corrections for the dispersal solution.

Soil penetration resistance (PR) was measured *in situ* at each sampling location each year approximately one month after cash crop planting using a tractor-mounted hydraulic, five-probe penetrometer to obtain cone-index values described by Raper et al. (1999). The tractor was positioned so the center penetrometer rod was in the cash crop row. Two penetrometer rods on either side were located 22.5 and 45 cm away from the cash crop row to include both trafficked and non-trafficked rows. Cone-index values were recorded to 50 cm in the soil profile. Area under the curve for cone index ($\text{AUC}_{\text{C.I}}$) values were calculated by averaging cone index values

across all row positions and depths to quantify soil strength between different cattle removal treatments to simplify soil strength analysis. Soil moisture data was collected at the time of PR data collection at depths of 0-15 and 15-30 cm using soil probes. Area under the curve for cone index was calculated following Equation 3 in which i represents the row position, CI_i represents the average cone index value according to row position, d_i represents the distance between individual row position measurements, and k represents the total number of row positions (Balkcom et al., 2016)

Equation 3:

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

Soil Biological Analyses

Soil samples were analyzed for MBC through the chloroform fumigation-incubation method described in Jenkinson and Powlson (1976). Field-moist soil samples were sieved to 4 mm and weighed to 4 to 5 g. The samples were then placed in oven at 105°C for at least 48 hr and weighed again to calculate moisture content (Θ_M). A subsample was used to determine the water holding capacity (WHC). A funnel and filter paper (no. 42) were placed in pre-weighed 125 mL Erlenmeyer flasks. Field-moist (20 g) soil samples were weighed and placed into the funnel with filter paper. For quality control, two blanks which did not contain soil were included. Using a 100-mL beaker, 25 g of DI water was weighed and carefully added to soil samples in the funnels. Funnels were covered with aluminum foil and undisturbed for 24 hr. The remaining water collected in the 125 mL Erlenmeyer flask was then weighed and % WHC was determined. Once Θ_M and % WHC were calculated, the moist weight of 25 g of soil on a dry weight basis

was calculated. The moist soil equivalent to 25 g of soil on a dry weight basis was weighed and placed into a pre-weighed 150-mL beaker. Deionized water was added carefully to bring the sample to 50% of its WHC. Once the sample was brought to 50% WHC, the sample was placed in 1 L mason jar that contained 1.5 mL of water to maintain 100% humidity in the jar. Six blanks that contained only a pre-weighed beaker were prepared. All mason jars were closed and remained in the dark at room temperature (approximately 25°C) for five days for preincubation. After preincubation, two replicates of the samples were fumigated with ethanol-free chloroform at room temperature for 24 hr in a desiccator while the third sample served as the unfumigated control. Fumigated and unfumigated samples and the six blanks were transferred to mason jars containing a scintillation vial with 5 mL of sodium hydroxide (0.5 M NaOH). The jars were incubated in the dark at room temperature (25°C) for 10 days. At the end of the incubation period, hydrochloric acid (0.25 M HCl) was used to titrate the unreacted NaOH to determine the amount of CO₂ released from the soil samples during the ten-day incubation period. Soil MBC was calculated using Equation 2 using a conversion factor of 0.41 (Voroney and Paul, 1984).

Equation 2:

$$\text{Biomass C} \left(\frac{\mu\text{g}}{\text{g soil}} \right) = \frac{[[\text{HCl blank} (\mu\text{L}) - \text{HCl soil} (\mu\text{L})] * (0.25 \text{ M HCl} * 6 \div \text{soil Dry Wt. (g)})]}{0.41}$$

Root Analysis

Root samples were analyzed for AMF colonization rates using acid fuchsin staining as described in Berch and Kendrick (1982). Roots were removed from glass vials with forceps and placed onto a clean petri dish. Roots were rinsed gently three times with tap water, and placed in

new, clean test tubes containing 10% KOH solution. Test tubes were then placed in a hot water bath at 90°C for approximately 90 minutes for dissociation. Once dissociated, roots were removed from test tubes, placed into a clean petri dish, and rinsed gently with water three times. Roots were then placed in a petri dish and immersed in lactic acid for three minutes to neutralize the 10% KOH. Roots were then transferred to a clean 55 mm by 75 mm microscope slide (Fisher Scientific, Pittsburgh, PA) and stained with 0.5% Acid Fuchsin. Each slide was then heated by using a clothespin to hold the slide above a flame until lightly smoking three times. Once cooled, roots were transferred to a clean microscope slide where lactic acid glycerol was added to remove the stain from roots. Once acid fuchsin staining appeared to be removed, two to three drops of lactic acid glycerol were applied to the center of a clean microscope slide. Two to three root segments approximately 5 cm in length were arranged on the center of the slide parallel to one another. Two to three additional drops of lactic acid glycerol were applied to the root segments and a cover slip was carefully applied. The cover slip was gently pressed upon with forceps to remove any bubbles. Filter paper was used to remove excess lactic acid glycerol. Roots were then checked under an optical microscope at 160X magnification for percent colonization according to methods in McGonigle et al. (1990) (Fig. 1). Moving in one direction (left to right or right to left), fifty eyesight views were obtained. A constant distance between the eyesight views was maintained. If AMF crossed the vertical eyepiece crosshair, the eyesight was marked as “AMF present”. The eyepiece crosshair was rotated to ensure it was perpendicular to the root at all times. Each eyesight view was marked as either “AMF present” or “no AMF” and percent colonization was calculated by dividing the amount of the eyesight views that were marked as “AMF present” by the fifty eyesight views that were taken.

Data Analysis

Data were analyzed using mixed models in SAS® PROC GLIMMIX with Kenward-Roger degrees of freedom. Year, treatment, depth, and their interactions were considered fixed effects. Random effects were rep within year and treatment within rep within year. Repeated measures for depth were accounted for using the autoregressive ((AR)1) covariance structure. Mean separations were performed using Tukey's honestly significant difference (HSD) test ($\alpha = 0.1$). Relationships among soil health indicators were determined using Pearson's correlation. Correlations were described as weak ($R < 0.30$), moderate ($R = 0.31$ to 0.70), and strong ($R > 0.70$).

RESULTS AND DISCUSSION

Cover Crop Biomass

Cover crop biomass did not exhibit a treatment-year interaction but was affected by treatment ($P < 0.0001$) and year ($P = 0.0303$) independently (Table 3). The ungrazed control treatment produced greater biomass compared to all other cattle removal treatments (Fig. 2). This was expected as cover crops in the ungrazed control were not consumed by livestock. The mid-February cattle removal treatment had higher cover crop biomass than both the mid-March and mid-April treatments, which were not significantly different from each other (Fig. 2). Grazing livestock were removed from the paddock earlier in the mid-February treatments than in the mid-March and mid-April treatments which allowed for more biomass to develop. While there is a lack of statistically significant difference in biomass from the mid-March and mid-April cattle removal treatments, the mid-April treatment had numerically less biomass by 809 kg ha^{-1} , which is expected as livestock were able to consume the cover crop mixture for a longer period. In 2019, cover crop biomass was greater than 2020 producing 4384 kg ha^{-1} and 3378 kg ha^{-1} , respectively. Differences in biomass between years may be related to variation in weather, soil moisture, seed quality, and insect or weed pressure.

Soil Organic Carbon

Soil organic carbon was not affected by treatment or year and did not exhibit any interactions between treatment, year, or depth (Table 3; Table 4). Lack of differences in SOC between treatments may be attributed to soil type, climate, and duration of the experiment. In Coastal Plain soils, the surface horizon texture varies from loamy sand to sandy loam (Causarano et al., 2008). Coarser soils, like those in the southeastern Coastal Plain, tend to accumulate SOM very slowly (Hassink and Whitmore, 1997). Conversely, soils with a higher clay content are better able to preserve SOM, since metal oxides and aluminosilicate clays form stable complexes that protect SOM (Torn et al., 1997). The decomposition of SOM is further accelerated for coarse textured soils examined in this study by the warm, humid climate of the Southeast. Therefore, SOM increases will likely take additional time under current conservation management practices.

Depth was the only significant factor affecting SOC ($P < 0.0001$; Table 3), and SOC was stratified by depth class. Soil organic carbon decreased as depth increased (Fig. 3). In the top 5 cm, SOC averaged 8.35 g kg^{-1} while the 5-10 cm, 10-15 cm, and 15-30 cm depths averaged 7.67 g kg^{-1} , 6.93 g kg^{-1} , and 6.26 g kg^{-1} of SOC, respectively. Using the assumption that approximately 58% of SOM mass is made up of SOC, the measured SOC values equate to 1.44%, 1.32%, 1.20%, and 1.08% SOM at 0-5 cm, 5-10 cm, 10-15 cm, and 15-30 cm depths, respectively. The greater SOC at shallower depths is likely linked to biomass present on the soil surface, as above and below ground plant residues are the primary source of SOC (Loren and Lal, 2005). Increased SOC at the soil surface has been observed in other studies conducted on Coastal Plain soils in the Southeast (Balkcom et al., 2013). Causarano et al. (2008) observed that management changes (pasture, conservation tillage, and conventional tillage) had the greatest impact on SOC in the top 5 cm of Coastal Plain soils. In a three-year study, Siri-Prieto et al. (2007) saw a 38% increase of

SOC in the top 5 cm of soil when using non-inversion paratilling compared to chisel and disk tillage in an ICL system on Coastal Plain soils. Non-inversion tillage minimizes soil surface disturbances that lead to mineralization of carbon in the soil. In the current study, conservation tillage practices likely allowed SOC from crop residue to accumulate at the soil surface.

Greater amounts of SOC near the soil surface is considered a positive quality, as SOM present at the soil surface is important for soil aggregation, which prevents erosion and promotes water infiltration into the soil (Franzluebbers 2002). In no-till systems, SOC is stratified with depth while in conventional tillage systems, SOC is more evenly distributed (Motta et al., 2002). Franzluebbers (2002) suggests that the stratification ratio (SR), a ratio of a given soil property at the surface layer to that at a deeper layer, may be useful to indicating soil health. A high SOC SR ($SR > 2$) can indicate improvements in soil health because the increased level of SOC at the surface can improve soil chemical, physical, and biological properties (Franzluebbers 2002). Causarano et al. (2018) saw higher SOC SR in pastures and fields under conservation tillage than in fields using conventional tillage in the Coastal Plain and Southern Piedmont. In the current study, the SR of SOC from 2019 and 2020 was 1.3, indicating that there is an opportunity to further improve soil health with additional time under conservation management.

Numerically, SOC decreased within each treatment from 2019 to 2020, but the decrease was not significant. The numerical difference in SOC content by year may be associated with the different methods used to measure SOC between 2019 and 2020.

Permanganate Oxidizable Carbon

Permanganate oxidizable carbon was not affected by treatment and did not exhibit any interactions with year or depth (Table 3). Year and depth each affected POXC independently (Table 3). While there was no difference in POXC between cattle removal treatments, the lack of difference indicates there was little negative or positive response to length of cattle grazing.

While this may change over time, the lack of response may indicate that utilizing an ICL system does not negatively impact soil health for producers in the Southeast who are looking to utilize land year-round for economic benefits.

Soil organic carbon and POXC had a weak ($R=0.26717$) but significant ($P=0.0002$) correlation (Table 5; Fig. 4). While POXC is a portion of the active SOM pool that makes up between 33% and 50% of SOM, the weak correlation may be due to soil texture and other environmental factors. The active C fraction may be more quickly degraded by microorganisms in coarse-textured soils, since metal oxides and aluminosilicate clays that form stable complexes are not present in high concentrations to protect SOM. Lucas and Weil (2012) conducted research in croplands of the Mid-Atlantic region of the US and found that in coarse-textured soil (i.e. sand and loamy sand soils) POXC was not predictive of crop responses while SOC was predictive of crop responses. However, POXC was predictive of crop responses in finer textured soils (i.e. silt loam and channery loam/silt loam soils of the Piedmont region) (Lucas and Weil 2012). The greater predictive responses of SOC than POXC in sandy soils than may be linked to improved soil physical properties, such as greater water holding capacity, that are known to improve as SOC increases.

Permanganate oxidizable C was affected by depth ($P=0.0204$; Table 3). As depth increased, POXC decreased numerically (Fig. 3). The shallowest depth, 0-5 cm, had 20.6% greater POXC than the 15-30 cm depth (Table 3). Greater POXC in the top 5 cm of soil is likely due to accumulation of crop residues and manure which contribute readily decomposable C sources at the soil surface. With depth, POXC was less stratified than SOC over both years of this research. Correlations between SOC and POXC closer to the surface were observed in this study. The 0-5 cm, 5-10 cm, and 10-15 cm depths all had significant correlations with SOC, and

the 5-10 cm depth had the strongest correlation ($R=0.38274$; Fig. 5). The 15-30 cm depth showed no correlation ($R=0.00126$; $P=0.9932$; Fig. 5). These observations support a suggestion that the relationship between SOC and POXC varies with depth (Wang et al., 2017). As aforementioned, active forms of soil C make up a portion of the SOC, so a correlation between POXC and SOC would be expected. However, because active C fractions are more readily available for use as food and energy for microbial organisms, these fractions would likely be depleted and unable to extend to deeper depths in the soil profile.

A significant decrease (29%) in POXC occurred from 2019 to 2020. Timing of sampling could be a factor that affected the difference in POXC between 2019 and 2020. In 2019, cover crops were terminated on April 18th and soil samples were collected on May 14th; in 2020, cover crops were terminated on April 14th and soil samples were collected on May 1st (Fig. 6). The two-week difference between the elapsed time from cover crop termination to soil sampling between 2019 and 2020 may have resulted in less decomposition of POXC by microorganisms at the time of soil sampling in 2020. Similarly, cover crop biomass, which saw a 22% decrease from 2019 to 2020, may have affected the decrease in POXC. Permanganate oxidizable C and other labile C fractions act as fuel for soil microbes, influencing nutrient cycling and other biologically related soil properties (Weil et al., 2003). Active forms of C are also known to be easily affected by environmental conditions, so differences in soil temperature and moisture between years may have also contributed to temporal differences in POXC.

Water Stable Aggregates

Grazing time and its interactions with year and depth did not affect WSA (Table 3). Both year and depth independently affected WSA ($P < 0.0001$). The lack of influence cattle removal treatments had on WSA may indicate this type of ICL system has minimal impacts on aggregate stability for Coastal Plain soils in early years of adoption based on the conditions observed for

these two growing seasons. While research is limited on the impact grazing animals have on soil physical properties when used in an ICL system, the research available indicates minimal influence on soil physical properties like WSA. For example, Franzluebbbers and Stuedemann (2008) found that grazing had little effect on aggregate stability in a study on sandy loam and sandy clay loam soils in Georgia between an ungrazed and grazed cover crops with cattle consuming approximately 90% of available forage. Their study demonstrated that grazing had little effect on aggregate stability even when 90% of available forage was removed by grazing (Franzluebbbers and Stuedemann 2008). Carvalho et al. (2010) found that integrating livestock into a cropping system with moderate grazing intensities (maintaining 20 and 40 cm forage height) resulted in increased soil aggregation compared to intensively grazed treatments (maintaining 10 cm forage height). Carvalho et al. (2010) maintained moderate grazing intensity treatments to forage heights greater than were maintained in the current study which may have resulted in greater aggregate stability. In the current study, greater residues in control and mid-February treatments did not contribute to increased aggregate stability. With greater biomass present, which results in greater OM, may increase WSA. More time under current management may be needed to see changes in WSA or a relationship between OM inputs and WSA.

Depth affected WSA in the current study, and the 0-5 cm depth had lower WSA than all other depths (Fig. 3). All other depths were not different from each other. As depth increased, WSA increased numerically (Fig. 3). Typically, the soil surface contains less clay and SOM than the subsurface soils. Since clay acts as a binding agent of soil aggregates (Causarano et al., 2008) and sandy soils are more easily aerated leading to rapid decomposition of SOM, soil aggregation may have less potential to form stable aggregates at the soil surface in the current study.

Similarly, McVay et al. (1989) found Coastal Plain soils had lower WSA compared to finer-textured soils in the Limestone Valley.

Estimated WSA values in 2019 and 2020 were 95% and 91%, respectively. As with POXC, the decrease in biomass and sample timing in 2019 and 2020 may have affected the WSA values between years. In 2019, soil samples were collected at a later date than in 2020 (Table 2). The two-week difference between the elapsed time from cover crop termination to soil sampling may have allowed biomass on the soil surface to undergo further decomposition. Therefore, microbial activity which promotes soil aggregation through release of organic substrates may have resulted in lower WSA in 2020 compared to 2019. A study conducted by Cosentino et al. (2006) indicated microbial activity stabilized aggregates by increasing cohesion and hydrophobicity of soil particles.

Water stable aggregates were not correlated to SOC content (Table 7). However, it is well understood that SOM affects aggregate stability. It is possible that other soil properties, such as clay content, had a greater influence on WSA than SOM. Kamprath and Welch (1962) saw a significant correlation ($R=0.77$) between OM and clay content in eighteen Coastal Plain soils.

Penetration Resistance

Penetration resistance, which was evaluated using an area under the curve approach to represent soil strength, was not influenced by treatment or year and did not exhibit a year-treatment interaction. The lack of treatment effect on PR indicates that non-inversion subsoiling following grazing may have reversed the potential negative impacts cattle grazing may have had on soil strength. Utilizing non-inversion subsoiling could impact other soil physical properties, like WSA by breaking up aggregates, but is useful in this type of system to potentially limit soil compaction from the grazing livestock. Additionally, intrinsic soil properties may have influenced soil strength more than cattle removal treatments (Williams and Weil, 2004; Balkcom

et al., 2016). Numerically, the control and mid-March cattle removal treatments produced the lowest PR over both years, but the control treatment was the only treatment to have the consistently lowest PR in 2019 and in 2020. A lack of consistency in PR over time under an ICL system was also observed by Tracy and Zhang (2008). Tracy and Zhang (2008) found PR measurements showed no consistent trend from 2002 to 2006 on silty, clay loams, and suggested that winter grazing can reduce feeding costs without negatively impacting soil compaction and crop productivity. Further, Franzluebbbers and Stuedemann (2015) found little evidence of compaction due to winter grazing on sandy loam and sandy clay loam soils, proposing that ICL systems can be utilized on Ultisols in the Southeast without negatively impacting soil compaction. More data over time may provide greater insight as to how grazing livestock and different grazing lengths impact soil strength.

Soil PR had a moderate, negative correlation with SOC ($R=-0.36737$; $P=0.0102$; Table 6). As SOC increased, the $AUC_{C.I}$ decreased. Because SOC makes up approximately 58% of SOM and SOM is known to influence soil structure, this relationship between PR and SOC was expected. Lower SOM levels make a soil more susceptible to compaction (Hoorman et al., 2011). Maintaining or increasing SOC may help prevent compaction when combined with practices like non-inversion subsoiling, which was used in this study. Soil strength is known to decrease with increased soil moisture. However, there was no difference between treatments for soil moisture readings collected at the time of sampling, indicating that $AUC_{C.I}$ values lack of treatment differences were not influenced by soil moisture at sampling (Wang et al., 2017).

Contour graphs provide a visual representation of soil strength profiles collected with the multiprobe penetrometer according to soil depth and distance from row middle (Fig. 7), but they do not allow for statistical analysis (Balkcom et al., 2016). While there was no difference in PR

measurements between treatments or years, some visual observations can be made based off contour graphs. Increased force was required to push the multiprobe penetrometer through the soil below approximately 40 cm depth at all distances from the row middle in mid-March and mid-April treatments in 2019 (Fig. 7). Penetration resistance in the mid-February cattle removal treatment was numerically higher in 2020 compared to 2019 at the 30 to 50 cm depth. For the center penetrometer position, the effect of subsoiling is clearly demonstrated within each treatment to a depth of 30 cm. The mid-March and mid-April saw a decrease in soil strength decreased from 2019 to 2020 at the 35 to 50 cm, possibly linked to the non-inversion subsoiling practice used which may have alleviated compaction at that depth in those treatments.

Microbial Biomass Carbon

Microbial biomass C was not affected by year and did not exhibit a year-treatment interaction; however, MBC was influenced by treatment ($P=0.017$; Table 3). The ungrazed control treatment had greater MBC than the mid-February and mid-April cattle removal treatments (Fig. 8). The MBC in the mid-March cattle removal treatment was not different compared to all other treatments (Fig. 8). Numerically, the ungrazed control contained approximately 30% greater MBC than mid-February and Mid-April cattle removal treatments. Greater MBC measured in the control can be attributed to greater crop residues remaining on the soil surface compared to all other treatments. The control treatment produced the greatest amount of biomass on the surface, which provided a food source for microbial organisms. Additionally, biomass may have provided protection from soil moisture loss which could have contributed to greater biological activity. Increasing soil moisture mobilizes nutrients, stimulating microbial activity (Van Horn et al., 2014). Franzluebbers and Studemann (2015) observed that grazing livestock resulted in a minimally positive impact on MBC in sandy loam soils in the Southeast and that using no-till was more important to preserving biologically active soil C following

pasture termination. The lack of negative impacts on biological activity over the seven-year study supported the recommendation to utilize winter grazing livestock in the Southeast US (Franzluebbers and Studemann 2015). George et al. (2013) saw greater MBC in grazed, non-irrigated plots compared to grazed, irrigated plots and non-grazed plots with and without irrigation, speculating that greater MBC in grazed, non-irrigated plots could be due to increased organic matter inputs from manure or increased soil moisture. George et al. (2013) reported greater soil moisture within grazed plots, both irrigated and non-irrigated, which may be related to increased diversity of OM inputs. Organic matter inputs, such as manure from grazing livestock, may increase aggregate stability which increases water holding capacity. Carvalho et al. (2010) saw an increase in MBC as grazing intensity increased on winter-grazed oat and ryegrass which was partially attributed to increased OM additions from the grazing livestock. Contradictory results of how MBC is impacted by grazing livestock in an ICL system may be due to differences in methods used to quantify MBC (i.e., chloroform fumigation-incubation method, chloroform fumigation-extraction method), soil texture, climate, and stocking rate. More time under this management system may provide greater insights to how MBC is influenced by grazing time.

Microbial biomass C had a significant ($P=0.0029$), moderate ($R=0.42127$) correlation with SOC for the 0-15 cm depth (Table 6). Soil MBC is a portion of the SOC pool that is composed of living organisms, like bacteria and fungi, so a positive correlation between MBC and SOC is expected. A similar correlation was seen in a long-term tillage study on sandy clay loams and clay soils in Spain where MBC had a moderate correlation ($R=0.352$) with SOC (Melero et al., 2009). The positive correlation between SOC and MBC and the effect grazing

time had on MBC in the first two years of this study adds to the evidence that MBC serves as a soil health indicator that is more responsive to changes in management.

Arbuscular Mycorrhizal Fungi

Arbuscular mycorrhizal fungi (AMF) colonization rates were not influenced by grazing treatments in 2019 ($P=0.8082$) or 2020 ($P=0.3739$) (Table 8). Years were analyzed separately because different cash crops were planted each year (cotton in 2019 and peanut in 2020).

Research on AMF colonization rates and ICL systems is extremely limited, especially in the Southeast. Davinic et al. (2013) found that two types of ICL systems which integrated perennial and annual crops in rotation with cotton increased the abundance of AMF on a Texas clay loam soil compared to continuous monoculture cotton without grazing. Arbuscular mycorrhizal fungi play an important role in water and nutrient availability for plants, as well as soil aggregation due to their release of glomalin-related proteins that are known to improve soil aggregates, so their presence is beneficial to agroecosystem health.

AMF are known to improve soil aggregates through production of glomalin-related soil proteins that bind mineral particles together. Glomalin-related soil proteins can increase WSA (Bedini et al., 2009). Cosentino et al. (2006) found that fungal biomass was correlated to WSA, but no correlation between AMF colonization rates and WSA were seen in this study. Because of different crops in rotation, more time under this ICL system may be needed to compare colonization rates and distinguish differences. Arbuscular mycorrhizal fungi colonization rates were not correlated to any other soil health indicator in this study (Table 7).

Yield

Grazing time affected cotton lint yield in 2019 (Fig. 9). Cotton lint yield of the ungrazed control and mid-February cattle removal treatments were greater than the mid-April and mid-March treatments (Fig. 9). Greater cotton lint yield may be related to greater cover crop residue

remaining on the soil surface that minimized the soil moisture loss and added OM to the soil, which enhances nutrient cycling. Cotton lint yield's coefficient of variation for mid-March and mid-April cattle removal treatments (40.4 and 42.1, respectively) were also greater than the control and mid-February cattle removal treatment (34.8 and 32.8, respectively). The lower yields and greater variability in yield in the mid-March and mid-April treatments could also be linked to compaction observed at the 35 to 50 cm depths (Fig. 7.). However, the contour graphs which represent soil strength do not allow for statistical analysis. Grazing time did not affect peanut yield in 2020, but peanut yield decreased numerically with increasing grazing time (Table 9).

There is conflicting research on the effects grazing livestock have on cash crop yield. For example, in a four-year study evaluating winter grazing treatments in a cotton production system, Schomberg et al. (2014) observed that cotton yield was numerically lower in an ungrazed compared to grazed treatment, but the difference was not significant. Franzluebbers and Stuedemann (2014) found that winter grazing did not impact yield of corn or soybean on an ICL system on Ultisols in Georgia. Negative impacts on yield have been reported in several studies of ICL systems that used high grazing intensities and stocking rates, but due to differences in climate, soil type, vegetation, and many other factors, it can be challenging to generalize impacts an ICL system has on yield. More research is needed to properly evaluate how the type of ICL system used in this study affects cotton and peanut yield. Future data from the upcoming years may provide this information.

CONCLUSION

After the first two years of integrating winter grazing livestock into a cotton-peanut rotation, selected soil health indicators did not consistently indicate how grazing livestock and length of grazing impact soil health. Of the selected soil health indicators analyzed in this study,

MBC was the only indicator to exhibit a treatment effect. Ungrazed cover crops had greater MBC than the mid-February and mid-April cattle removal treatments, likely due to greater biomass present on the soil surface. The control and treatments with short grazing periods resulted in increased biomass on the soil surface at the time of cover crop termination. Higher biomass levels may have increased cotton lint yield in 2019, possibly by limiting soil moisture loss during the cash crop growing season. Physical soil health indicators (WSA and PR) were unaffected by the presence of livestock and length of grazing, but PR may have been influenced by tillage practices used, like subsoiling. Negative physical impacts of winter grazing are not detectable in the early years of this study. There was no effect of grazing time on SOC storage, which was expected as SOC is known to be a less responsive indicator of soil health. The lack of detectable difference in SOC between grazing times may be explained by soil type, climate, and duration of the experiment. Coarse-textured soils in the southeastern Coastal Plain tend to accumulate SOM very slowly and more time under this management may be needed to see differences. Although POXC has been reported to be more responsive to changes in management than SOC, POXC did not show a response to cattle removal treatments in the current study. The lack of consistent results may indicate that integrating winter grazing livestock does not negatively impact soil health in southeastern row crop production systems. However, more time under this system is required to thoroughly evaluate how winter grazing livestock impact soil health and yield.

TABLES AND FIGURES

Table 1. Seeding rates of cover crop mixture by species for ungrazed control paddocks and grazed paddocks.

Cover Crop Species	Ungrazed Control Paddocks	Grazed Paddocks
	kg ha ⁻¹	
Cereal Rye	33.6	50.4
Oat	33.6	50.4
Crimson Clover	16.8	18.8
Brassica hybrid	3.36	3.36

Table 2. Dates of field operations at the Wiregrass Research and Extension Center from 2018 to 2020.

Operation	2018	2019	2020
Cover crop planting	29 October	4 November	-
Grazing begins	-	11 January	13 January
Mid-February cattle removal treatment	-	15 February	12 February
Mid-March cattle removal treatment	-	15 March	9 March
Mid-April cattle removal treatment	-	5 April	9 April
Cover crop termination	-	18 April	14 April
Cash crop planting*	-	30 April	4 May
Soil Sampling	-	14 May	1 May
Cash crop harvest	-	12 October	1 October

*Cash crops in 2019 and 2020 differed: cotton was planted in 2019 and peanut was planted in 2020.

Table 3. Summary of analysis of variance (ANOVA) for biomass, soil moisture, soil organic carbon (SOC), permanganate oxidizable carbon (POXC), water stable aggregates (WSA), area under the curve for cone index ($AUC_{C.I.}$), and microbial biomass carbon (MBC) in response to grazing treatment, depth, year and their interactions.

Source of Variance	ANOVA, Pr > F							
	df	Biomass	Moisture	SOC	POXC	WSA	$AUC_{C.I.}$	MBC
Treatment (T)	3	<0.0001	0.0481	0.1600	0.1438	0.1042	0.1654	0.0170
Depth (D)	3	-	0.0366	<0.0001	0.0204	<0.0001	-	-
Year (Y)	1	0.0303	0.0071	0.6904	0.0084	<0.0001	0.1654	0.8034
T x D	9	-	0.2227	0.6261	0.9030	0.3897	-	-
T x Y	3	0.8554	0.9349	0.8985	0.6753	0.7775	0.8439	0.3543
D x Y	3	-	0.0251	0.9532	0.1040	0.4351	-	-
T x D x Y	9	-	0.6553	0.4771	0.4718	0.9399	-	-

Table 4. Means of soil organic carbon (SOC), permanganate oxidizable carbon (POXC), and water stable aggregates (WSA) across depths under the curve for cone index ($AUC_{C.I.}$), and microbial biomass carbon (MBC) by treatment across 2019 and 2020 at Wiregrass Research and Extension Center (WREC).

Soil Health Indicator	Treatment			
	Control	Mid-Feb	Mid-March	Mid-April
SOC (g kg ⁻¹)	7.71	7.16	7.36	6.96
POXC (mg kg ⁻¹)	337	289	295	310
WSA (%)	93.24	92.42	90.93	91.73
$AUC_{C.I.}$	149	181	169	189
MBC ($\mu\text{g g}^{-1}$)	249.3 a ¹	190.9 b	194.3 ab	193.8 b

¹ Values followed by the same letter in a row are not significantly different between treatments according to Tukey's HSD at $\alpha= 0.1$.

Table 5. Means of soil organic carbon (SOC), permanganate oxidizable carbon (POXC), and water stable aggregates by depth.

Soil Health Indicator	Depth			
	0-5 cm	5-10 cm	10-15 cm	15-30 cm
SOC (g kg ⁻¹)	8.35 a ¹	7.66 b	6.93 c	6.26 d
POXC (mg kg ⁻¹)	318 a	296 ab	268 b	264 b
WSA (%)	90.72 a	93.12 b	94.07 b	94.07 b

¹ Values followed by the same letter are not significantly different within a given row according to Tukey's HSD at $\alpha=0.1$.

Table 6. Summary of analysis of Pearson Correlation Coefficients of soil organic carbon (SOC), permanganate oxidizable carbon (POXC), and water stable aggregates (WSA) across all depths using 192 observations (N). ns indicates Pearson Correlation Coefficients are not significant at $\alpha=0.1$.

Pearson Correlation Coefficients, N = 192 Prob > r under H0: Rho=0			
Variables	SOC	POXC	WSA
SOC	1.0000	0.26717 0.0002*	-0.15636 0.0303*
POXC		1.0000	-0.13342 0.0651*
WSA			1.0000

*Values are significant at $\alpha=0.1$.

Table 7. Summary of analysis of Pearson Correlation Coefficients for biomass, soil organic carbon (SOC) at 0-15 cm, permanganate oxidizable carbon (POXC) at 0-15 cm, water stable aggregates (WSA) at 0-15cm, area under the curve for cone index (AUC_{C.I.}), microbial biomass carbon (MBC), and arbuscular mycorrhizal fungi (AMF) colonization rates at Wiregrass Research and Extension Center (WREC). ns indicates Pearson Correlation Coefficients are not significant at $\alpha= 0.1$.

Pearson Correlation Coefficients, N = 48 Prob > r under H0: Rho=0							
Variables	Biomass	SOC	POXC	WSA	AUC _{C.I.}	MBC	AMF Colonization Rates
Biomass	1.0000	ns	ns	ns	ns	ns	ns
SOC		1.0000	ns	ns	-0.36737 0.0102**	0.42127 0.0029*	ns
POXC			1.0000	-0.56076 <0.0001*	ns	ns	ns
WSA				1.0000	ns	ns	ns
AUC _{C.I.}					1.0000	-0.28327 0.0511*	ns
MBC						1.0000	ns
AMF Colonization Rates							1.0000

*Values are significant at $\alpha= 0.1$.

Table 8: Arbuscular mycorrhizal fungi (AMF) colonization rates between treatments within year. Years were analyzed separately due to different cash crops planted (cotton in 2019 and peanut in 2020) at the Wiregrass Research and Extension Center (WREC).

Year	Treatment	AMF Colonization Rate (%)
2019	Control	72
	Mid-February	74
	Mid-March	74
	Mid-April	72
2020	Control	69
	Mid-February	70
	Mid-March	67
	Mid-April	73

Table 9: Peanut yield (kg ha^{-1}) between treatments in 2020 at the Wiregrass Research and Extension Center (WREC).

Treatment	Peanut Yield kg ha^{-1}
Control	6130
Mid-February	5911
Mid-March	5723
Mid-April	5706

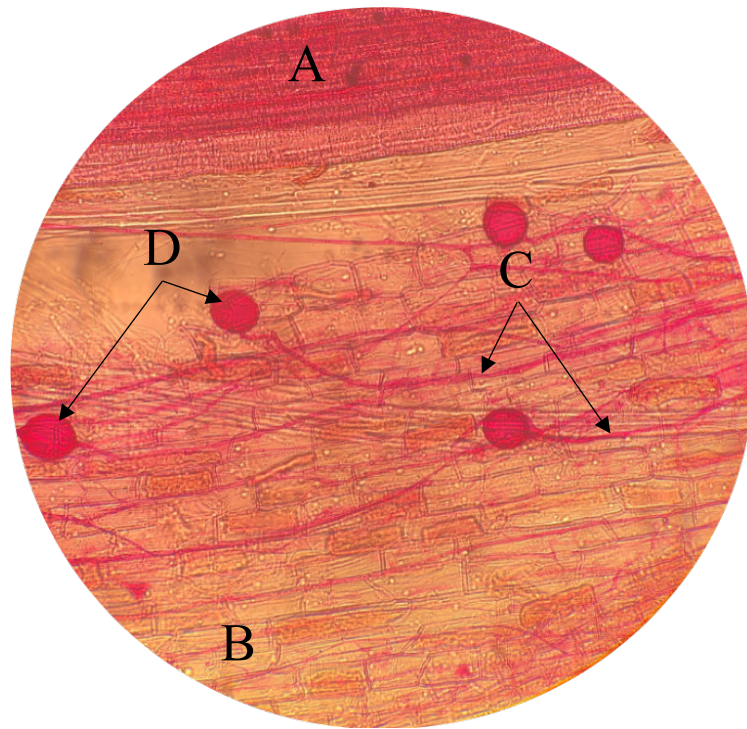


Figure 1. Image of cotton root with arbuscular mycorrhizal fungi (AMF) infection under optical microscope (160X magnification) showing a) cotton root xylem, b) cotton root cortical cell, c) AMF hyphae, and d) AMF vesicles.

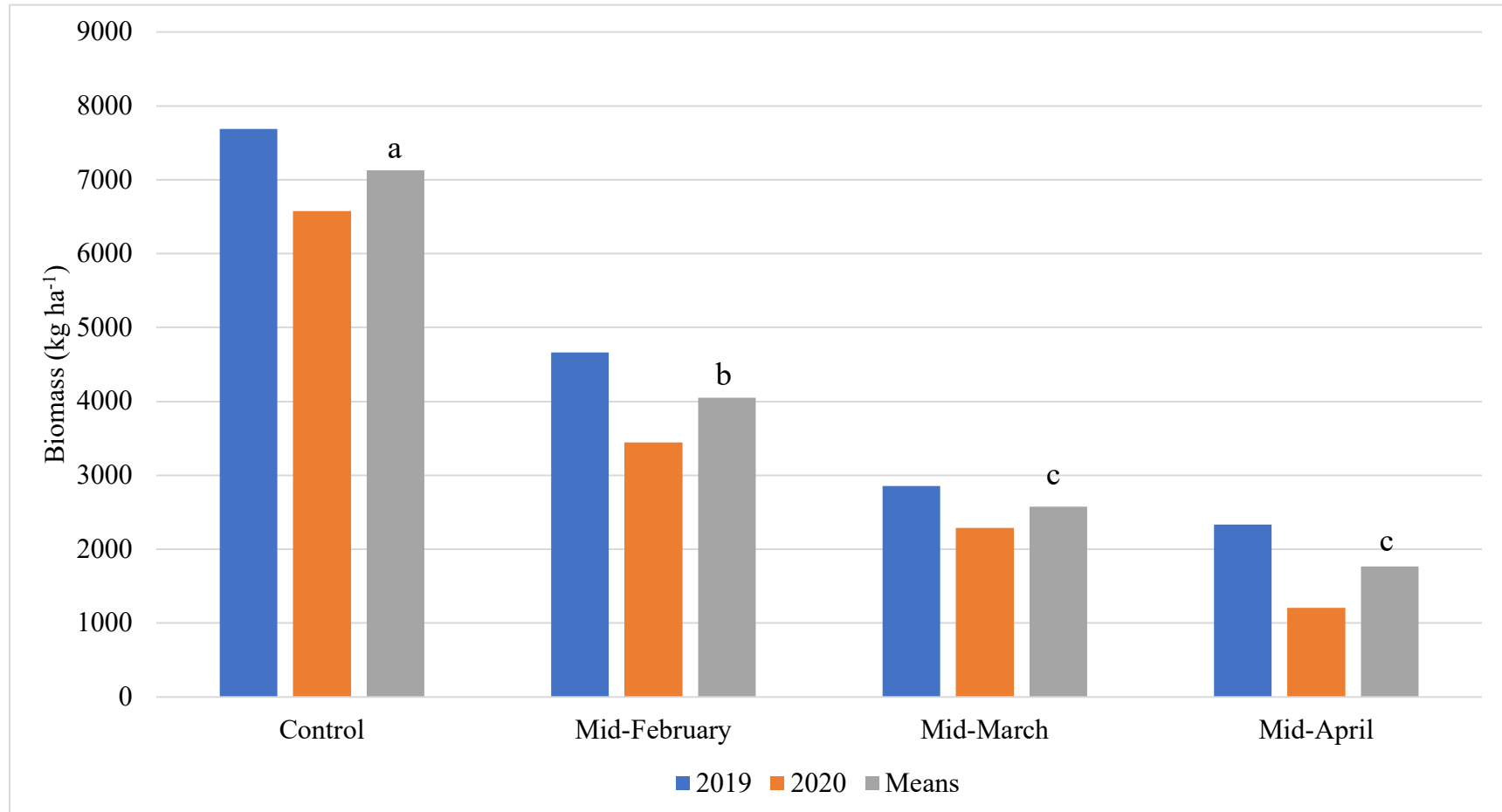


Figure 2. Comparison of cover crop biomass between years by treatment. Columns with the same letter do not differ between cattle removal treatments ($\alpha = 0.1$).

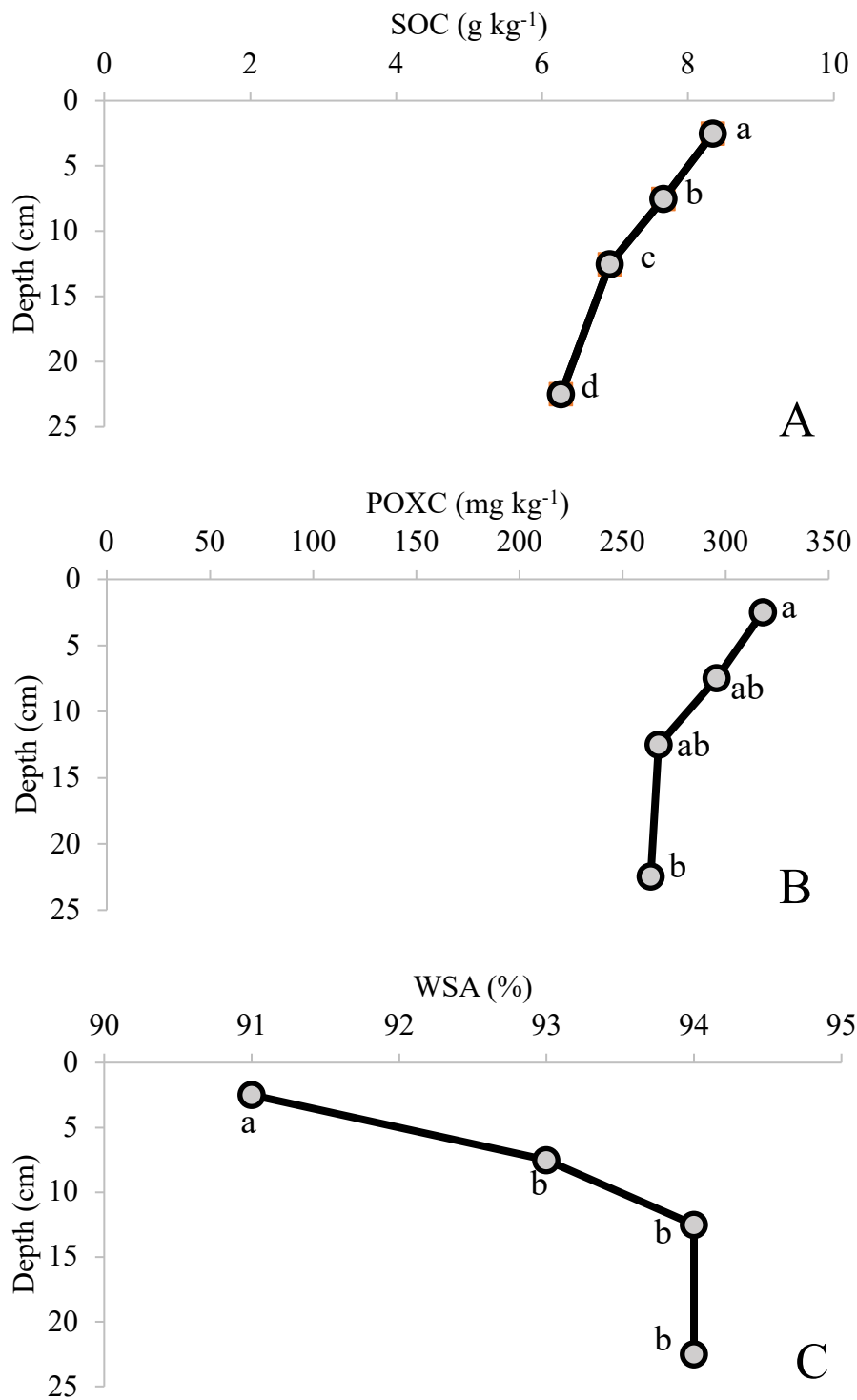


Figure 3. a) Soil organic carbon (SOC), b) permanganate oxidizable carbon (POXC), and c) water stable aggregates (WSA) by depth at Wiregrass Research and Extension Center (WREC). Depths with the same letter do not differ within the soil health indicator ($\alpha = 0.1$).

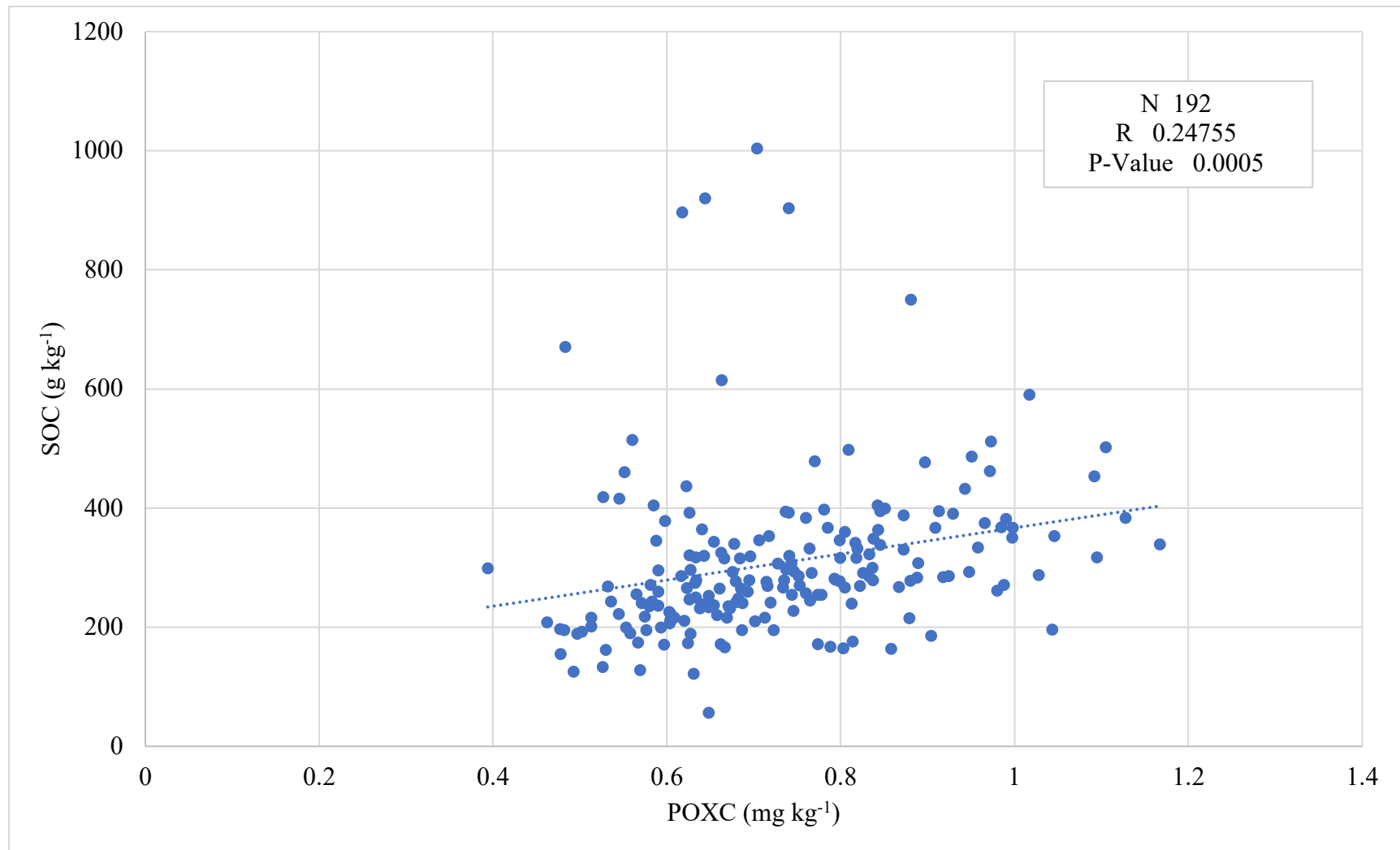


Figure 4. Correlation between soil organic carbon (SOC) and permanganate oxidizable carbon (POXC) of all depths based on Pearson's correlation coefficient (R) using 192 observations (N).

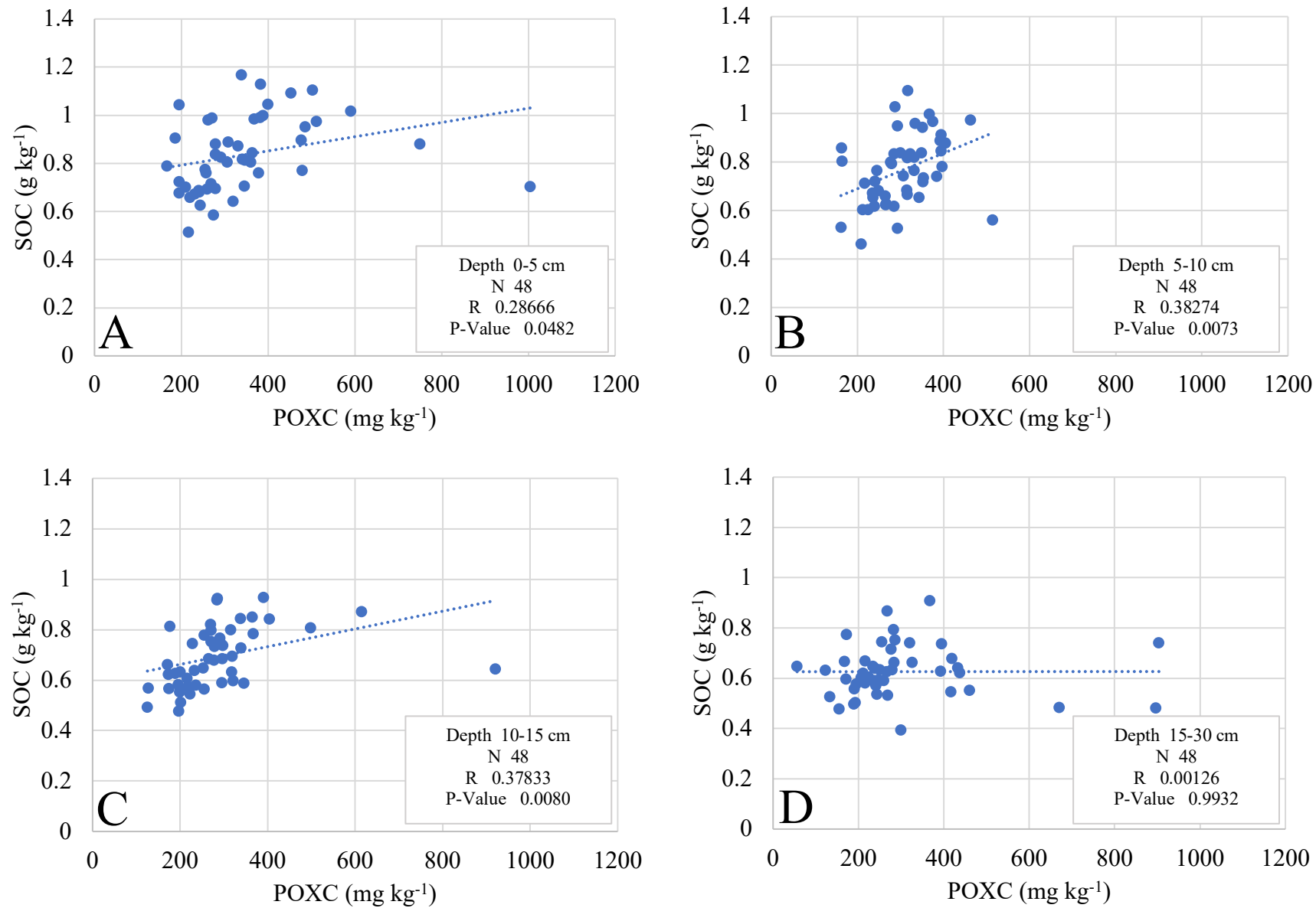


Figure 5. Correlation between soil organic carbon (SOC) and permanganate oxidizable carbon (POXC) at a) 0-5 cm depth, b) 5-10 cm depth, c) 10-15 cm depth, and d) 15-30 cm depths based on Pearson's correlation coefficient (R) with 48 observations (N).



Figure 6. Images comparing biomass on the soil surface at the time of soil sampling in 2019 (in a mid-March cattle removal treatment plot) and 2020 (a control cattle removal treatment plot).

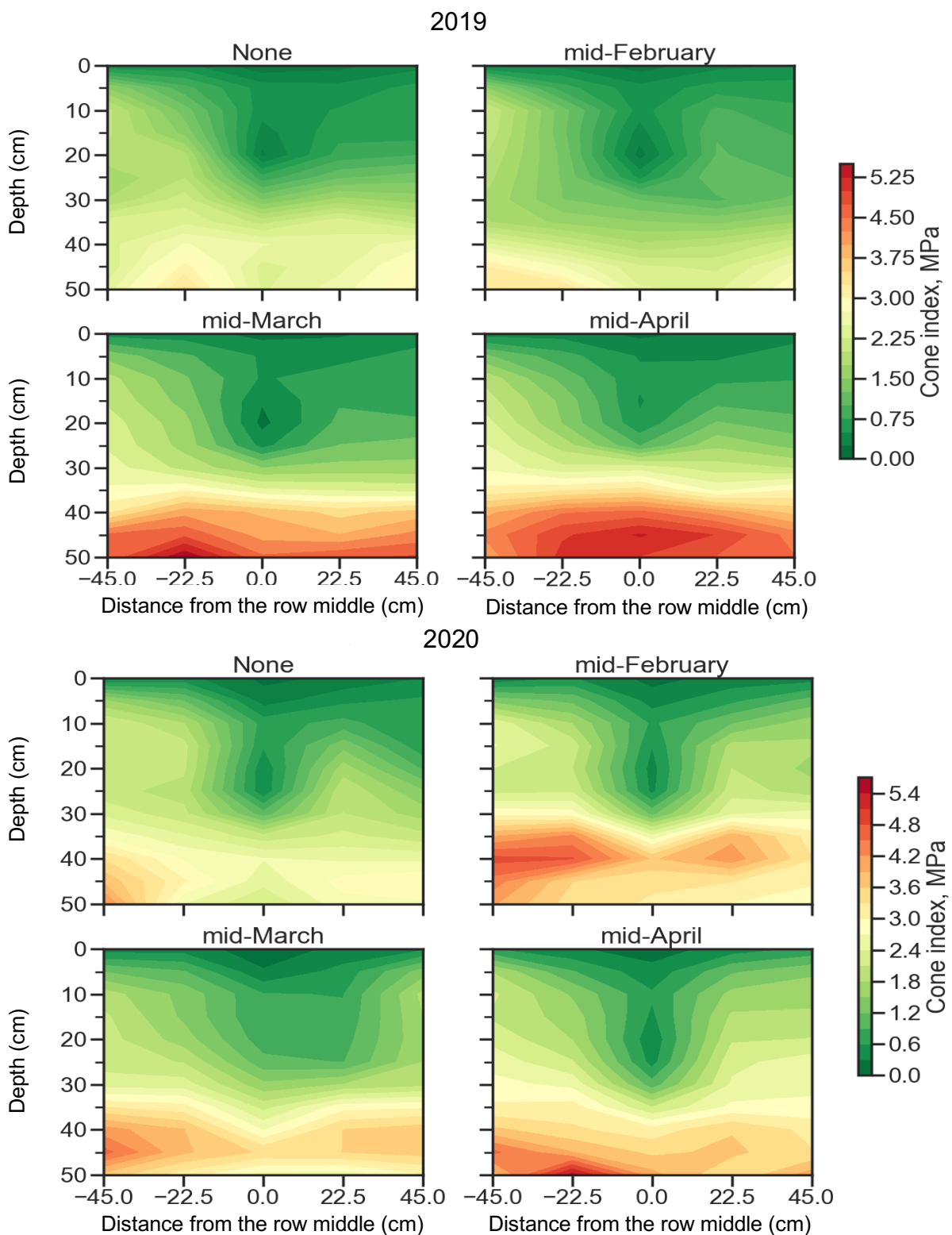


Figure 7. Area under the curve for cone index ($AUC_{C.I.}$) to represent penetration resistance (PR) between treatments by depth and distance from row middle at Wiregrass Research and Extension Center (WREC) in 2019 and 2020.

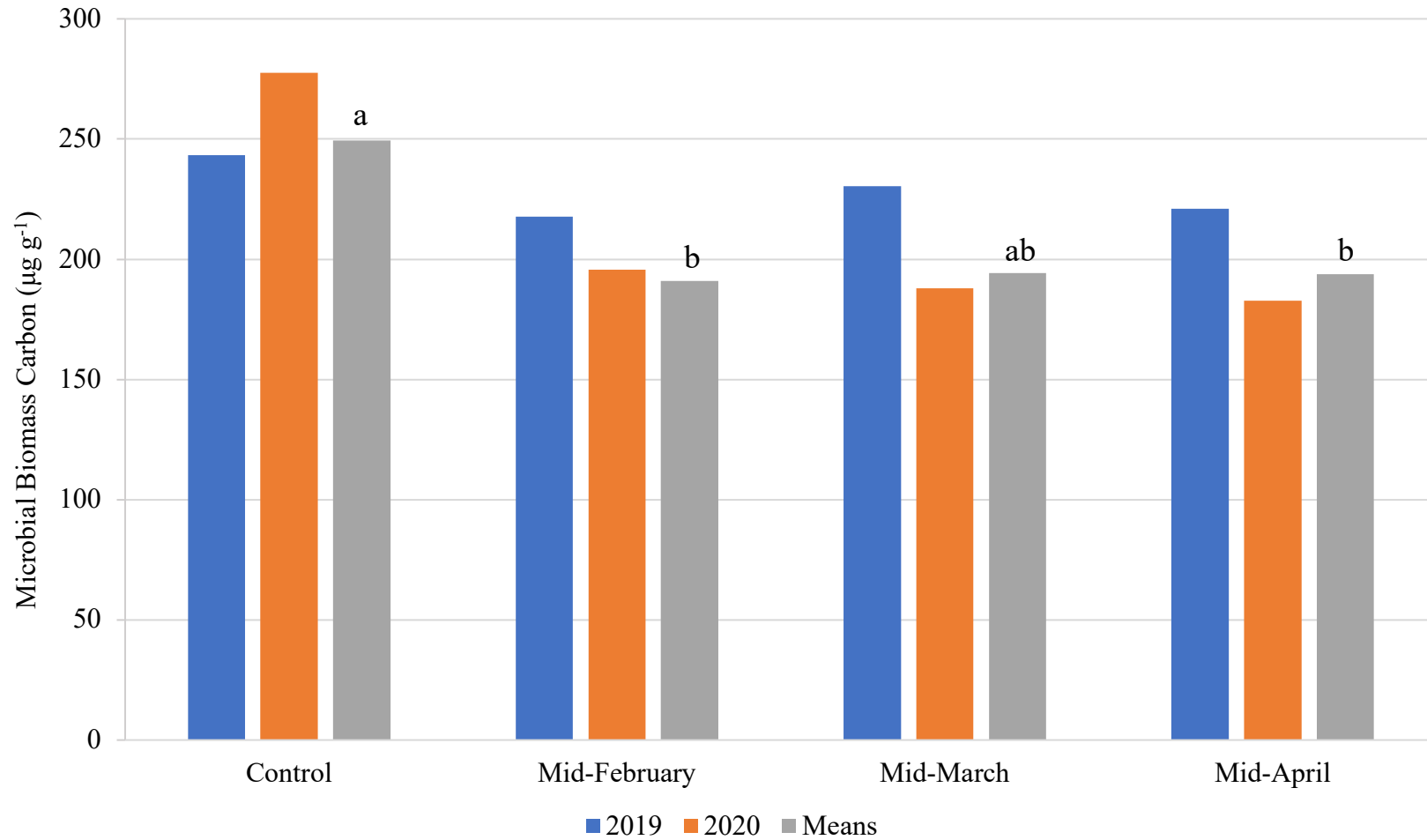


Figure 8. Microbial biomass carbon (MBC) between treatments from 2019 and 2020. Columns with the same letter do not differ between cattle removal treatments ($\alpha = 0.1$).

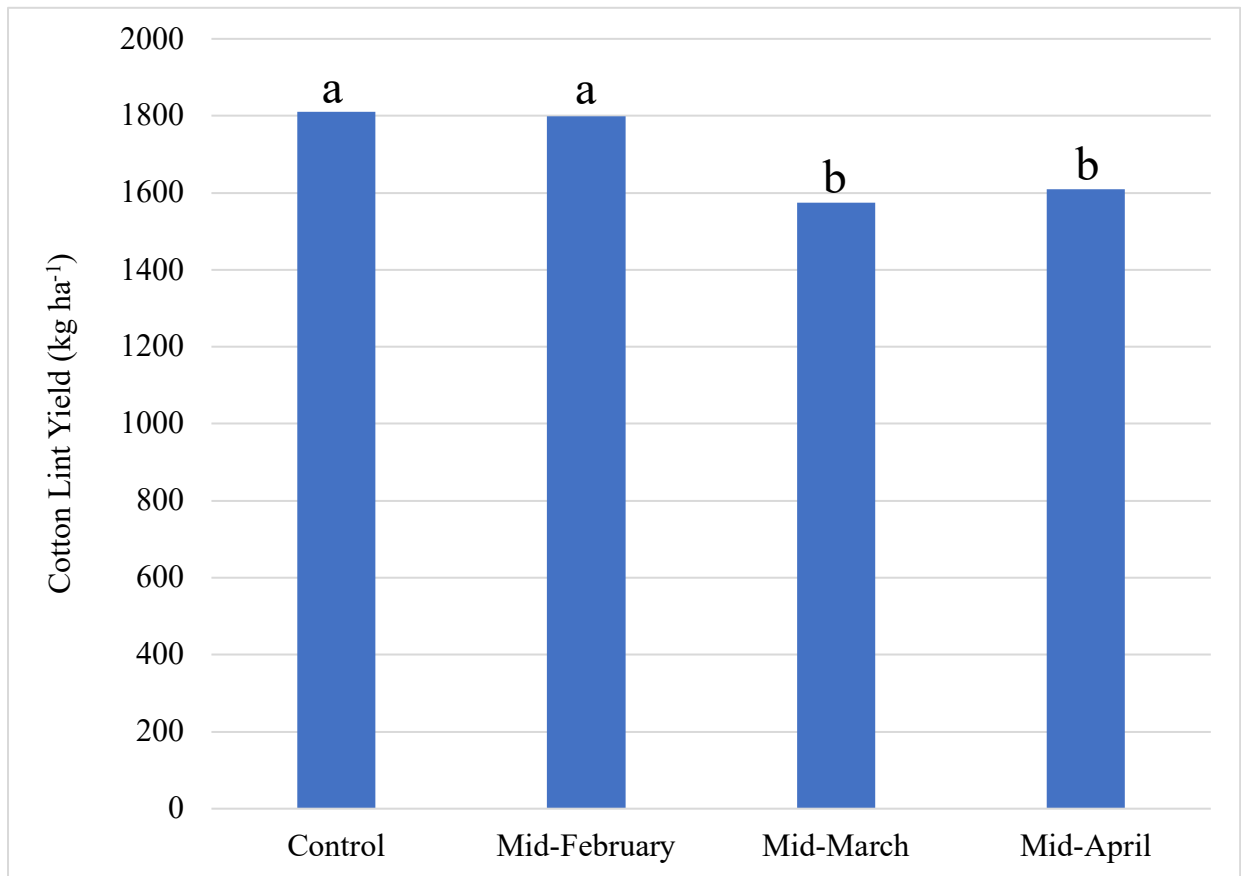


Figure 9. Cotton lint yield (kg ha⁻¹) between treatments in 2019. Columns with the same letter do not differ between cover crop treatments ($\alpha = 0.1$).

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