Influence of Cover Crop Mixtures on Soil Health and Weed Control in Cotton Production Systems

by

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ABSTRACT

The restoration of soil health is a crucial step in maximizing productivity in the historically-eroded Ultisols of the Southeast. The utilization of winter cover crops can potentially improve soil health by increasing soil organic matter, improving soil structure, and enhancing nutrient-use efficiency. Studies were established at the Tennessee Valley Research and Extension Center (TVREC) and Wiregrass Research and Extension Center (WREC) to examine the impact of cover crop monocultures and mixtures on dynamic soil health indicators in cotton (*Gossypium hirsutum*) production systems. Eight treatments including monocultures and combinations of cereal rye (*Secale cereale*), crimson clover (*Trifolium incarnatum*), and Daikon radish (*Raphanus sativus*) were arranged in a randomized complete block design with winter fallow controls. Cover crop biomass was collected at termination, and soil samples were collected two weeks following termination. Measured soil health indicators included permanganate oxidizable carbon (POXC), total carbon (TC), water stable aggregates (WSA), and soil strength ($AUC_{C.I.}$). Stratification of TC with depth occurred at TVREC, and TC under crimson clover, rye-clover, and rye-radish was higher than TC under the winter fallow control. In both 2018 and 2019, POXC at TVREC was not different between treatments at the 0-5 cm and 5-10 cm depths, while POXC was higher under crimson clover compared to the rye-crimson clover mixture at 10-15 cm. There were no differences in TC and POXC between treatments at WREC, but POXC was higher in 2019 than in 2018. WSA values from both locations were not different between treatments within the same depth class in both 2018 and 2019. No differences in $AUC_{C.I.}$ occurred between treatments at TVREC, while the rye monoculture was less compacted than the crimson clover monoculture and

crimson clover/radish mixture at WREC. Additional years under these cover crop treatments may be required to detect changes in soil health.

Winter cover crops are also a common tool for integrated weed management in both conventional and conservation agricultural systems. Two trials were established at E.V. Smith Research Center in Shorter, AL in November 2016 to evaluate the efficacy of several cover crop systems as a supplemental form of weed control in cotton production systems. The first trial consisted of twelve treatments. Cover crops included a rye monoculture, a mixture of rye, oats (*Avena sativa*), wheat (*Triticum aestivum*), crimson clover, and Daikon radish, and winter fallow. Each cover crop system was evaluated under four herbicide regimes including PRE only (pendimethalin and fomesafen), POST only (dicamba fb glyphosate), PRE+POST, and herbicide-free. *Amaranthus* control was lower in all herbicide-free treatments compared to all PRE+POST, and cotton lint yield was lower in the herbicide-free treatments compared to treatments with herbicide applications. Lint yield was higher in PRE+POST treatments compared to herbicide-free treatments, regardless of cover crop. Lint yield under PRE only treatments were not different from PRE+POST while POST only treatments had lower lint yield than PRE+POST treatments in 2018. The second trial includes rye monocultures as whole-plot and row-middle only treatments, a clover-radish mixture in the whole plot and within the cotton row only, and three-species mixtures as whole-plot treatments and with precision placements; all treatments managed with a PRE+POST herbicide regime. In the cover crop placement trial, weed control was often similar between treatments in the same year, and cotton yield was only influenced by year. Results indicate that cover crops alone will not eliminate the need for chemical weed control.

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List of Abbreviations

POXC	Permanganate Oxidizable Carbon
TC	Total Carbon
SOM	Soil Organic Matter
SOC	Soil Organic Carbon
WSA	Water Stable Aggregates
CEC	Cation Exchange Capacity
POC	Particulate Organic Matter
MBC	Microbial Biomass Carbon
K_{sat}	Saturated Hydraulic Conductivity
Θg	Gravimetric Water Content
$AUC_{C.I.}$	Area Under the Curve for Cone Index
WREC	Wiregrass Research and Extension Center
TVREC	Tennessee Valley Research and Extension Center
PRE	Preemergent herbicides
POST	Postemergent herbicides
WF	Winter Fallow
R-W	Rye as a whole plot treatment
CR-W	Clover-Radish as a whole plot treatment
RCR-W	Rye-Clover-Radish as a whole plot treatment
R-BR	Rye between rows only
CR-IR	Clover-Radish inside rows only
R-BR/CR-IR	Rye between rows with Clover-Radish inside rows

I. LITERATURE REVIEW

INTRODUCTION

Agricultural land management consisting of conventional tillage has historically led to the depletion of productive topsoil and soil organic matter across an estimated 38% of global agricultural land (Reeves, 1997; Simoes et al., 2009). Southeastern Ultisols are particularly prone to degradation due to the warm, humid climate of the region (Schomberg et al., 2006; Causarano et al., 2008; Franzluebbers and Stuedemann, 2010). In addition to being highly degraded, sandier Southeastern soils often have poor water holding-capacity and are highly compacted below the plow layer (Schomberg et al., 2006; Balkcom et al., 2013). In recent decades, conservation agriculture has been promoted as a key to improving agricultural productivity and remediating soil degradation. Management under conservation systems consists of regular crop rotation, reduced tillage, and the implementation of cover crops.

Despite the highly erodible nature of Southeastern Ultisols, the region has high productivity potential due to mild winters and above-average annual rainfall (Franzluebbers, 2010). With the need to produce more food on less arable land in conjunction with a rising global population, improved productivity is of great importance. Conservation agricultural systems can promote increased productivity through reduction of soil erosion and runoff, enhanced water-holding capacity, soil organic matter accumulation, improved nutrient availability, potential supplemental cultural pest control, and overall improvement of soil health through enhancement of various ecosystem services (Weil et al., 2003; Franzluebbers and Stuedemann, 2010; Murrell et al., 2017).

Soil health refers to the continued ability of soil to function as part of a living ecosystem and coincides with the remediation of degraded soils (Fine et al., 2017; Stott, 2019).

CONSERVATION AGRICULTURE

Conservation Tillage

Conservation tillage is a management system in which 30% or more of the soil surface remains covered by residue from the previous crop. This may include no-till, strip-till, ridge till, and mulch till systems (Bosch et al., 2005; Mulvaney et al., 2011). Conservation tillage is often used as a method of reducing the risk of erosion, encouraging the accumulation of soil organic matter, and improving water infiltration and retention of soil moisture. Areas that are at high risk for losses from soil erosion or drought are highly suited for conservation tillage because accumulation of organic matter builds soil fertility and improves overall soil aggregation (Blevins et al., 1971; Ismail et al., 1994; Bauer and Reeves, 1999; Mulvaney et al., 2011; Aulakh et al., 2015; Clark et al., 2017).

Conservation tillage is a common practice in cotton (*Gossypium hirsutum*) production with between 35 and 44% of acres in the Southeast utilizing strip-till or no-till systems in 2010-2011 (Wade et al., 2015). Due to variable precipitation rates and the erosive nature of Southeastern Ultisols, soil water retention and soil stability are gradually improved by conservation tillage (Mahboubi et al., 1993; Bosch et al., 2005; Simoes et al., 2009; Campbell et al., 2014). No-till and strip-till are the most common conservation tillage methods used in cotton. In no-till systems, the seed is planted directly into the cover crop residue, while in strip-tilled systems the seed is planted in a narrowly tilled strip. Throughout the season, the remaining crop residue provides a vapor barrier preventing water loss, reduces rainfall impact, and increases infiltration, which can potentially increase plant available water and reduce the need for irrigation (Bosch et al., 2005; Price et al., 2011). This increase in plant available water can in some cases improve crop productivity and economic gains (Tolk and Evett, 2012).

Challenges associated with conservation tillage systems include potential reduced weed control and lower soil temperatures, which may delay cover crop planting (Mulvaney et al., 2011). Conservation tillage on its own can also exacerbate weed pressure. Inversion tillage can serve as a form of cultural weed control through burial of the weed seedbank. Reduced or no-till systems keep weed seed close to the surface, allowing more weeds to germinate (Aulakh et al., 2015). The adoption of conservation tillage became more common with the introduction of broad-spectrum herbicides and again with the introduction of herbicide-resistant crop varieties. Because of this, the productivity of conservation tillage systems is often dependent on herbicides. Recent developments of herbicide-resistant weeds is considered a major obstacle in conservation systems (Price et al., 2011).

Cover Crop Utilization

As a component of conservation systems, cover crops are established when fields would otherwise remain fallow for the primary purpose of developing a layer of plant residue to prevent erosion and build soil organic matter over time (Reeves, 1997; Dabney et al., 2001; Bosch et al., 2005). A layer of plant residue reduces the impact of rainfall on the soil surface to reduce erosion and promote infiltration. Cover crops also shade the soil surface, which promotes soil water retention by reducing evaporation from the topsoil (Dabney et al., 2001). Additional benefits of cover crops include nutrient scavenging, improved soil structure, carbon sequestration, and contributions to integrated pest management programs (Bosch et al., 2005; Blanco-Canqui et al., 2015). Winter cover crops are particularly impactful in Southeastern cotton production systems under conservation agriculture due to limited residue left by cotton following harvest (Bosch et al., 2005; Vann et al., 2018).

Specific cover crop species provide different benefits to the crop production system. Soil shading and weed suppression are proportional to cover crop biomass, so cereal rye (*Secale cereale*) and other small grains are particularly beneficial due to high biomass accumulation (Murrell et al., 2017). Crimson clover (*Trifolium incarnatum*) and other legume species are less frequently planted than small grain cover crops but can add plant available nitrogen to the soil (McVay et al., 1989; Schomberg et al., 2006). Deep-rooted brassicas also have potential to alleviate compaction and scavenge plant nutrients by penetrating deep into the soil profile (Schomberg et al., 2006; Chen and Weil, 2010; Blanco-Canqui et al., 2015). Chen and Weil (2010) found that Daikon radish (*Raphanus sativus*), a commonly planted brassica cover crop, had roots that extended deeper into a highly compacted soil than a rye monoculture, thus demonstrating potential for brassica cover crops to alleviate compaction and scavenge nutrients deep in the soil profile.

Cover crop mixtures have been widely promoted in recent years for the combined benefits of different types of cover crops and the diversification of ecosystem services by cover crops (Clark, 2007; Reberg-Horton et al., 2011; Finney et al., 2016; MacLaren et al., 2019). However, the performance of individual cover crop species in mixtures can be variable. Murrell et al. (2017) and Finney et al. (2016) found that small grains tended to dominate late-planted cover crop mixtures while brassicas were less successful in mixtures compared to monoculture and legume biomass varied depending on planting date. However, the addition of a small grain cover crop, has been shown to increase biomass compared to many legume monocultures (Reeves et al., 2005; Reberg-Horton et al., 2011; Webster et al., 2013).

INFLUENCE OF COVER CROPS ON WEED CONTROL

Weed pressure is often among the most limiting factors in crop production systems due to competition for water and nutrients. The generally intensive management of monoculture agronomic systems results in the increased prevalence of highly competitive weed species (Price et al., 2011; Webster and Nichols, 2012). Herbicide resistant crop varieties have contributed to overuse of some herbicide chemistries thus contributing to development of herbicide resistance in some weed species. According to Webster and Nichols (2012), only eight of the top fifteen most problematic weeds in cotton and soybean (*Glycine max*) systems were similar in both 1995 and 2009, which indicates a shift towards more competitive weeds. This contributes to a reduction in plant biodiversity in agronomic systems, which can amplify weed management challenges.

The development of broad-spectrum herbicides and herbicide resistant crops has reduced the need for inversion tillage, which provides weed control via burial of weed seed. This has contributed to higher rates of conservation tillage. Because of this, the productivity of conservation tillage systems is dependent on reliability of herbicides, making herbicide resistance of weeds a major issue facing conservation tillage. A noteworthy example is glyphosate resistant palmer amaranth (*Amaranthus palmeri*) (Price et al., 2011, 2016; Webster et al., 2013). Such challenges have subdued the efficacy of standard weed management under conservation systems (Price et al., 2011; Mirsky et al., 2013). To address herbicide resistance of weeds, resistance management methods are frequently encouraged. These methods include utilization of multiple herbicide modes of action and integrated weed management programs (Price et al., 2011, 2012; Duzy et al., 2016).

Integrated approaches to weed control include intensified crop rotations and inversion tillage between conservation systems in conjunction with high-residue cover crops (Price et al., 2011; Price et al, 2016). In addition to improved soil quality, cover crops can provide some level of early season cultural weed control compared to winter fallow systems (Brainard et al., 2011; Price et al., 2011, 2016; Duzy et al., 2016). Cereal rye (*Secale cereale*), one of the most widely utilized winter cover crops in the Southeast, and other high biomass small grains are particularly notable as weed-suppressing cover crops (Reeves et al., 2005; Vann et al., 2018).

Mechanisms of weed suppression from cover crops can include light quality interference for weed germination, smothering of pre-existing weeds, and allelopathic compounds (Teasdale and Mohler, 2000; Price et al., 2012; Aulakh et al., 2015). A high biomass mulch layer is critical for optimum weed suppression. This is because actively growing and terminated cover crops shade the soil surface to block germination-inducing red light from reaching the seed (Teasdale and Mohler, 1993; Mulvaney et al., 2011; Mirsky et al., 2013). Teasdale and Mohler (2000) found that weed emergence decreased exponentially with greater mulch area and that proportion of weed emergence was directly related to proportion bare ground. However, the threshold for high cover crop biomass varies. Mirsky et al. (2013) found that consistent weed suppression was achieved when cereal rye biomass was at least 8000 kg ha^{-1} at termination while biomass of at least 4500 kg ha^{-1} has also been recommended as a biomass threshold for weed suppression (Reiter et al., 2008). Such results indicate that a successful, high-biomass cover crop can be a key component of weed control in conservation systems.

Substantial biomass accumulation can vary with climatic conditions and management. Small grain cover crops, including cereal rye, will generally produce high biomass across a variety of environments. Many legume and brassica cover crops may develop less biomass than small grains because they often require earlier planting for high biomass development, which can be restricted by late cash crop harvest (Clark, 2007; Lawley et al., 2012). Regardless of winter cover crop species, biomass maximization typically requires timely planting, late termination, and nitrogen additions through fertilization or the inclusion of legumes (Clark, 2007). Management requirements, including planting and harvest dates, for the primary crop often limit biomass production (Reberg-Horton et al., 2011; Mirsky et al., 2013).

Conservation agriculture is not without its own weed management challenges. Conventional inversion tillage provides some level of weed control by uprooting winter weeds and burying the weed seedbank, which can prevent germination. Conservation systems can in turn reduce weed control by keeping the weed seed closer to the surface, allowing more weeds to germinate. Under extensive weed pressure, this may contribute to additional herbicide requirements (Blanco-Canqui et al., 2015). A study by Aulakh et al. (2015) demonstrated that weed control and overall peanut (*Arachis hypogaea*) yield varied by weed species present rather than by tillage method and cover crop presence. An additional study conducted by Reeves et al. (2005) found that small grain winter cover crops plus a PRE herbicide system provided similar weed control to a PRE+POST herbicide system, leading to similar cotton yields. Results from such studies demonstrate that high biomass cover crops may not provide enough weed control to eliminate the need for POST emergent herbicides and that weed pressure is largely influenced by weed seedbank dynamics.

SOIL ORGANIC MATTER DYNAMICS

Soil organic matter (SOM) is a major component of soil health and productivity. It is greatly influenced by land management practices, with less disturbed soil often having higher organic matter contents. The influence of organic matter ranges from improved structure and aggregation to slow release of plant nutrients, improved availability of plant nutrients, increased cation exchange capacity (CEC), and improved conditions for microbial activity. Because of this, building SOM is a cornerstone for improving soil health (Franzluebbers, 2010; Stott, 2019; Weil et al., 2003). However, significant changes in SOM often take at least 3 years to appear in the humid climate of the Southeastern United States (Causarano et al., 2008; Franzluebbers and Stuedemann, 2010).

Soil organic matter comprises the largest terrestrial carbon pool, consisting of approximately 58% carbon (Stott, 2019). Organic matter is largely influenced by land management along with inherent soil and environmental properties, such as soil texture and climate (Weil and Magdoff, 2004; Causarano et al., 2008; Reicosky et al., 2011).

SOM is highly reactive and complex material, made up of various fractions with different turnover rates (Burke et al.; Lal, 2016). However, the role of SOM in agricultural production is often overlooked when compared to the availability of macronutrients (Reicosky et al., 2011; Brevik, 2012; Lal, 2016).

Similar to clay, SOM has a large surface area with high charge density, which allows it to form protective organo-mineral complexes with clay minerals that can encapsulate SOC for millennia (Lal, 2016). SOM is sensitive to management practices, such as residue management and tillage, along with inherent soil properties and climate. Although the inherent texture of a given soil can influence SOM sequestration, increases in organic matter can alleviate some soil quality issues that may arise from soils too high in sand or clay (Burke et al., 1989; Hassink and Whitmore, 1997; Weil and Magdoff, 2004; Lal, 2016). SOM has low particle density, so increased SOM can reduce soil bulk density, thus improving soil structure. Because SOM contributes to CEC, it is also known to influence pH buffering (Weil and Magdoff, 2004; Franzluebbers, 2010).

Soil microorganisms also heavily influence the development and transformations of SOM and the cycling of nutrients from plant residue and organic amendments (Tabatabai et al., 2005). Soil organisms utilize the photosynthetic energy in organic residues to mediate the mineralization of various soil nutrients, including nitrogen and phosphorous to promote crop growth and development (Reeves, 1997; Weil and Magdoff, 2004).

Influence of Conservation Tillage on SOM

The minimization of soil disturbance through conservation tillage preserves crop residue and SOM at the soil surface. Keeping plant residue at the soil surface slows decomposition and promotes the accumulation of SOM (Causarano et al., 2008; Reicosky et al., 2011). As a result, SOM is stratified with depth in reduced-tillage or no-tillage soils, with SOC concentration higher in the top 3 to 5 cm of soil under conservation tillage when compared to conventionally tilled soil (Franzluebbers et al., 2009; Gamble et al., 2014). For example, Gamble et al. (2014) found that in the top 5 cm of soil, SOC content was 58% greater under strip-tillage compared to conventional tillage in a cotton-peanut-bahiagrass rotation in the Coastal Plain, while the opposite was true at the 10 to 30 cm depths, where SOC was higher under conventional tillage. In addition to management, SOM stratification is influenced by climate. Soils with inherently low SOM in warm, humid environments tend to have higher levels of stratification than soils in cool, dry environments and higher SOM (Causarano et al., 2008; Franzluebbers, 2010). Hubbard et al. (2013) found that in highly erodible Coastal Plain soil, total C and N increased under cover crops, which contributed to improved soil structure including reduced bulk density and an increase in volumetric soil water content. Results from such studies indicate that soils with inherently lower SOM will likely receive more benefit from greater higher concentration of SOM near the soil surface (Causarano et al., 2008; Franzluebbers et al., 2009).

Despite the far-reaching influence of SOM on soil health, SOM alone may be too complex to be a stand-alone soil health indicator. Instead, various soil carbon fractions are often analyzed as part of soil health evaluations (Wander, 2004; Franzluebbers and Stuedemann, 2010). Soil carbon can be quantified on its own or in different, more specific fractions. Soil carbon fractions can generally be divided into passive and active pools. The passive pools have turnover rates as long as hundreds of years while active, labile pools can decompose within days (Wander, 2004; Weil and Magdoff, 2004). Because increases in total SOM can take several years, it is useful to identify indicators that predict SOM increase in a shorter time period. Several labile soil carbon fractions are recommended to detect changes in soil health relatively early following management changes. These soil carbon fractions include POC, microbial biomass carbon (MBC), and biologically active permanganate oxidizable carbon (POXC) (Weil et al., 2003; Causarano et al., 2008; Stott, 2019).

Particulate Organic Carbon

Particulate organic carbon (POC) consists of partially decomposed, sand-sized (>53 μm) organic residues that are not yet mineralized and can act as a substrate for microbial activity (Cambardella and Elliot, 1992; Causarano et al., 2008; Franzluebbers et al., 2009). It is a relatively large SOC pool compared to POXC and MBC. Culman et al. (2012) found that soil POC concentrations were about four times the concentration of POXC and MBC. Although it is sensitive to management changes and is considered a labile form of carbon, its decomposition rate places it between the active and passive fractions of soil carbon (Weil et al., 2003; Wander, 2004; Culman et al., 2012b).

POC is measured by first isolating particulate soil organic matter as a whole by dispersal and passage of soil through a 53 μm sieve. Soil particles remaining on the sieve are then dried and analyzed for total organic carbon (Cambardella and Elliot, 1992; Franzluebbers et al., 2000a). The presence of surface crop residue and the level of soil disturbance is a dominant factor in the development of POC, which leads to particulate carbon levels being higher closer to the soil surface (Franzluebbers et al., 2009; Culman et al., 2012b).

The role of surface residue in POC accumulation also indicates that conservation tillage systems can lead to higher POC levels over time when compared to conventional systems (Franzluebbers et al., 2009). Franzluebbers et al., 2000a found that in Georgia, minimally disturbed grassland had the greatest POC levels in the top 5 cm compared to forested, hayed pasture, cropland, and grazed pasture. Cambardella et al., 1992 also found that no-till cropland had greater POC levels in the top 20 cm compared to conventionally tilled cropland, although native grassland had higher POC levels than both cropland treatments. Both studies indicate that disturbance level is a major influence on POC levels.

Microbial Biomass Carbon

Microbial biomass carbon (MBC) is the living component of soil organic matter. MBC can occupy as much as 5% of the total soil organic carbon pool, with the remaining 95% consisting largely of stable, passive forms of carbon (Paul, 1984; Doran et al., 1996; Franzluebbers et al., 2009). As a highly active form of SOC, MBC has a turnover rate of less than one year. MBC is commonly measured through chloroform fumigation of soil, after which the sample is either incubated for CO_2 extraction or carbon is extracted with a K_2SO_4 solution (Doran et al., 1996). Because of the rapid of turnover MBC, it can serve as an indicator of soil biological activity and changes in SOM. As a highly reactive soil carbon fraction, MBC is sensitive to differences in land management, climate, and soil moisture (Insam et al., 1989; Doran et al., 1996). Insam et al., 1996 found that soil water was one of the greatest sources of MBC variance between different locations and that MBC levels were higher under treatments including crop rotations compared to monocultures. Nakamoto et al., 2012 found that MBC levels correspond with soil disturbance and the distribution of plant residues, with MBC levels higher under no-till treatments compared to plowed treatments (Nakamoto et al., 2012).

Permanganate Oxidizable Carbon

Permanganate oxidizable carbon (POXC), often referred to as active C, is a labile SOC fraction that is readily available as a microbial food source. POXC is frequently measured colometrically with the oxidation of 0.2 M potassium permanganate, $KMnO_4$ (Weil et al., 2003). The POXC fraction of C can serve as an effective soil health parameter due to its quick turnover, with decomposition taking a few weeks or months (Tirol-Padre and Ladha, 2004; Stott, 2019). POXC has been found to be higher under no-till systems, with greater differences found near the soil surface when compared to conventionally tilled soils (Franzluebbers et al., 2009). The accumulation of crop residue at the soil surface under conservation tillage promotes the development of habitats for soil organisms that contribute to the cycling of SOM into biologically active fractions. Conversely, tillage stimulates microbial activity, which leads to the exhaustion organic substrates that could contribute to the biologically active carbon pool.

Permanganate oxidizable carbon is positively correlated with other properties including soil organic carbon, particulate organic carbon, soluble carbohydrates, organic substrate-induced respiration, and microbial biomass carbon. A study by Culman et al., 2012 found that POXC was more closely related to POC than MBC. While POXC was related to POC as a whole, POXC was more closely related to smaller (53–250 µm) particle fractions of POC than larger fractions. The relationship between POXC and MBC was more variable between study locations. However, Weil et al., 2003b observed a strong relationship between MBC and POXC that was also more pronounced than the relationship between MBC and total SOC. The direct relationships between POXC and such soil properties, along with the relatively low cost of measurement, make POXC a standard SOC parameter (Tirol-Padre and Ladha, 2004; Franzluebbers et al., 2009; Culman et al., 2012; Stott, 2019).

SOIL PHYSICAL PROPERTIES

Crop productivity can be greatly influenced by soil physical properties, including the management-sensitive physical properties such as water-holding capacity, porosity, and soil strength. Like many chemical soil health indicators, SOM levels can influence these soil physical properties and can be associated with improved soil health (Benjamin et al., 2007). The implementation of conservation tillage and cover crops has been shown to improve management-sensitive soil physical properties over time (Villamil et al., 2006). For example, Villamil et al. (2006) found that rye and hairy vetch (*Vicia villosa*) cover crops promoted soil aggregation and reduced bulk density and improved porosity along with increased SOM.

Management-sensitive soil physical properties are also greatly influenced by inherent soil texture and climate. Management changes have been found to prompt changes in such soil properties more rapidly under water-stressed conditions relative to non-drought conditions due to the known water-retention aid of high biomass crops

(Williams and Weil, 2004; Benjamin et al., 2007). However, the properties of the crops themselves can also have distinct impacts on below-ground soil physical properties. For example, the robust taproots of brassica cover crops are known to be effective as biological tillage tools with the ability to alleviate soil compaction (Clark, 2007; Blanco-Canqui et al., 2015).

Soil Structure and Aggregation

Soil structure is primarily affected by soil physical properties such as texture, moisture retention, and bulk density (Simoes et al., 2009; Blanco-Canqui et al., 2011). However, soil structure is also influenced by a combination of chemical and biological factors (Franzluebbers et al., 2000b; Hubbard et al., 2013). Crop productivity can be improved by the binding of soil components into macroaggregates by plant roots and hyphae or biochemicals. Similar to SOM, soil structure influences the availability and turnover soil carbon and nitrogen due to the impact of habitable pore space on soil microbial communities. Jensen et al., 1996 found that compaction indirectly influenced microbial activity due to changes in aeration (Jensen et al., 1996). The formation of such soil aggregates affects pore size distribution and soil moisture retention (Hubbard et al., 2013).

Aggregate stability is a measure of how cohered soil particles withstand uniform disruption through erosive forces (Kemper and Rosenau, 1986). The development of stable aggregates and increased porosity contributes to increased water holding capacity of the soil, and the accumulation of SOM near the soil surface protects against raindrop impact, preventing erosion. Shaver et al. (2003) described soil aggregation as the primary determinant of water infiltration at the soil surface based on results of a study in which increased surface residue resulted in increased aggregation, infiltration, and porosity at the soil surface. As relative humidity in soil decreases, soil structure becomes more brittle and susceptible to erosive forces (Kemper and Rosenau, 1986; Weil and Magdoff, 2004; Lal, 2016). SOM accumulation near the soil surface in conjunction with surface residue also moderates soil temperature fluctuations by insulating and shading the soil surface (Weil and Magdoff, 2004).

Soil physical properties are largely affected by tillage due to changes in soil structure and porosity that accompany conventional tillage (Blanco-Canqui et al., 2004; Hubbard et al., 2013). Blanco-Canqui et al. (2004) found that continuously fallow soils under conventional tillage had lower bulk density, SOM, and saturated hydraulic conductivity (K_{sat}) than vegetated soils under various tillage treatments on a silt loam soil. However, the study also found that there were no differences between tillage treatments (Blanco-Canqui et al., 2004). Aggregate stability has been shown to increase in as little as three years under cover cropping, even under conventional tillage indicating that aggregate stability can serve as a dynamic soil health indicator (McVay et al., 1989; Blanco-Canqui et al., 2015).

Blanco-Canqui et al. (2011) found that SOC was positively correlated with wet aggregate stability and influenced by the use of cover crops. Other studies have also noted that organic forms of soil C and N influence on variety of chemical, biological, and physical properties differently under various management systems (Blanco-Canqui et al., 2011; Hubbard et al., 2013).

Soil Compaction

Southeastern Ultisols are highly prone to compaction and the development of hardpans due to the coarse topsoil and comparatively clay-heavy subsoil in the Coastal Plain region (Simoes et al., 2009). The Southeast typically experiences short-term droughts during cash crop growing seasons. The wetting and drying cycles caused by short-term droughts and standard vehicle traffic contribute to increased bulk density and the development of hardpans (Mapa et al., 1986; Williams and Weil, 2004; Blanco-Canqui et al., 2015). Soil compaction can limit crop yield potential by restricting root growth and elongation (Raper et al., 2000; Simoes et al., 2009). Tillage breaks apart compacted soil layers to promote root penetration, so compaction-prone soils have occasionally experienced yield losses under no-till management. In-row subsoiling is frequently utilized in conservation tillage systems, but some studies have found that cover crop implementation can alleviate soil compaction, and deep-rooted Daikon radishes are marketed in part based on potential compaction alleviation (Raper et al., 2000; Blanco-Canqui et al., 2015). Raper et al. (2000) found that spring soil strength and bulk density in a silt loam soil was reduced under shallow in-row tillage and cover crop systems, with similar cotton yields between conventionally tilled and reduced tillage with cover crops.

RESEARCH OBJECTIVES

There have been numerous studies conducted in the Southeast and globally on the effects of cover crops in monoculture and small grain-legume mixtures on various soil health indicators. However, brassica cover crops have rapidly grown in popularity in the

Southeast in recent years. No studies in the Southeast have yet evaluated brassica-containing mixtures alongside monocultures in terms of soil health. The objective of this study was to evaluate soil health changes under treatments of cover crop monocultures alongside two-species and three-species mixtures in conservation tillage systems.

Similarly, multiple studies have been previously conducted to evaluate various herbicide regimes under cover crop monocultures and two-species mixtures, while few have utilized more diverse cover crop mixtures or evaluated the potential benefit of strategic cover crop placement, especially in the Southeast. Therefore, two trials were established in Shorter, AL to evaluate the effect of herbicide regime, cover crop monocultures, cover crop mixtures, and strategic seed placement on weed control and cotton yield.

II. EVALUATION OF COVER CROP MIXTURES FOR IMPROVING SOIL HEALTH IN ULTISOLS

Abstract

The restoration of soil health is a crucial step to maximize productivity in the historically-eroded Ultisols of the Southeast. The utilization of winter cover crops in Southeastern row-crop production systems can potentially improve soil health by increasing soil organic matter, improving soil structure, and enhancing nutrient-use efficiency. Studies were established at the Tennessee Valley Research and Extension Center (TVREC) and Wiregrass Research and Extension Center (WREC) to examine the impact of cover crop monocultures and mixtures on dynamic soil health indicators in cotton (*Gossypium hirsutum*) production systems. Eight treatments including monocultures and combinations of cereal rye (*Secale cereale*), crimson clover (*Trifolium incarnatum*), and Daikon radish (*Raphanus sativus*) were arranged in a randomized complete block design with winter fallow controls. Cover crop biomass was collected at termination, and soil samples were collected two weeks following termination. Measured soil health indicators included permanganate oxidizable carbon (POXC), total carbon (TC), water stable aggregates (WSA), and soil strength ($AUC_{C.I.}$). Stratification of TC with depth occurred at TVREC, and TC under crimson clover, rye-clover, and rye-radish was higher than TC under the winter fallow control. In both 2018 and 2019, POXC at TVREC was not different between treatments at the 0-5 cm and 5-10 cm depths, while POXC was higher under crimson clover compared to the rye-crimson clover mixture at 10-15 cm. There were no differences in TC and POXC between treatments at WREC, but POXC was

higher in 2019 than in 2018. WSA values from both locations were not different between treatments within the same depth class in both 2018 and 2019. No differences in $AUC_{C.I.}$ occurred between treatments at TVREC, while the rye monoculture was less compacted than crimson clover monoculture or the crimson clover/radish mixture at WREC. Additional years under these cover crop treatments may be required to detect changes in soil health.

INTRODUCTION

Agricultural land management consisting of conventional tillage has historically led to the depletion of productive topsoil and soil organic matter across an estimated 38% of global agricultural land (Reeves, 1997; Simoes et al., 2009). Southeastern Ultisols are particularly prone to degradation due to the warm, humid climate of the region (Schomberg et al., 2006; Causarano et al., 2008; Franzluebbers and Stuedemann, 2010). In addition to being highly degraded, sandier Southeastern soils often have poor water holding-capacity and are highly compacted below the plow layer (Schomberg et al., 2006). In recent decades, conservation agriculture has been promoted as a key to improving agricultural productivity and remediating soil degradation. Management under conservation systems consists of regular crop rotation, reduced tillage, and implementation of cover crops.

In reduced tillage systems, cover crops provide a mulch layer that protects the soil surface against erosive forces and promotes soil moisture retention. Small grain cover crops produce high biomass levels and have high C:N ratios, which leads to slower degradation of residue and more extensive soil protection (Clark, 2007). However, cover crop mixtures are frequently promoted for diversified benefits to soil and the proceeding cash crop. Cover crop mixtures often include legumes, for nitrogen additions, and deep-rooted brassica cover crops have also become popular in cover crop mixtures due to compaction-alleviation potential (Clark, 2007; Lawley et al., 2011)

In addition to enhanced productivity, conservation agriculture can build and restore soil health. Soil health refers to the continued ability of soil to function as part of a living ecosystem (Stott, 2019; Fine et al., 2017). Overall soil health changes gradually, but some dynamic soil properties have been known to predict long-term changes in soil health (Causarano et al., 2008; Bünemann et al., 2018). Few studies in the Southeast have evaluated soil health impacts of cover crop monocultures compared to mixtures, particularly those which include brassica cover crops. Given the increased popularity of brassica cover crops in the Southeast and the promotion of cover crop mixtures, a series of trials were established to evaluate dynamic soil health indicators under cover crop monoculture and mixture treatments.

There have been numerous studies conducted in the Southeast and globally on the effects of cover crops in monoculture and small grain-legume mixtures on various soil health indicators. However, brassica cover crops have rapidly grown in popularity in the Southeast in recent years. No studies in the Southeast have yet evaluated brassica-containing mixtures alongside monocultures in terms of soil health. Therefore, the objective of this study was to evaluate soil health changes under treatments of cover crop monocultures alongside two-species and three-species mixtures in conservation tillage systems.

MATERIALS AND METHODS

Experimental Design

Trials were established in the fall of 2017 at the Wiregrass Research and Extension Center (WREC) in Headland, AL (31°30'N, 85°17'W) on a Lucy loamy sand (loamy, kaolinitic, thermic Arenic Kandiudults) and the Tennessee Valley Research and Extension Center (TVREC) in Belle Mina, AL (34°41'22.4"N 86°53'01.7"W) on an Ooltewah silt loam (fine-silty, mixed, active, thermic Fluvaquentic Dystrudepts) and Dewey silt loam (fine, kaolinitic, thermic Typic Paleudults). At WREC, the land had been under conventional tillage prior to trial inception while TVREC was under no-till management for 20 years prior.

Trials were organized in randomized complete block designs with four replications and eight cover crop treatments. Cover crop treatments included winter fallow (no cover), ‘Wrens Abruzzi’ cereal rye, ‘Dixie’ crimson clover, and ‘Sodbuster’ Daikon radish monocultures, along with mixture treatments of any combinations of the three cover crop species. Seeding rates for various cover crop monocultures and mixtures are provided in Table 2.1. Plots were 10.67 m long and 7.32 wide at WREC. Plots were 10.67 m long and 8.16 m wide at TVREC. Cover crops were planted with a Great Plains 1205NT drill with 19 cm row spacing at WREC and a Great Plains 3P606NT with 19 cm row spacing at TVREC. At TVREC, cover crops were planted on October 5 in 2017 and on October 19 in 2018. At WREC, cover crops were planted on November 7 in 2017 and on November 19 in 2018.

Cover crops were chemically terminated with glyphosate two weeks before cash crop planting in the spring of 2018 and 2019, followed by rolling of the cover crops at

WREC only. Cotton, ‘Deltapine 1646 B2XF,’ was planted at both locations in 2018. In 2019 peanut, ‘FloRun 331,’ was planted at WREC and soybean, ‘Asgrow 55X7,’ planted at TVREC. Row spacing for cotton and peanut was 91 cm at WREC. At TVREC, row spacing was 102 cm for cotton and 76 cm for soybean. At WREC, plots were in-row subsoiled prior to planting of cash crops while TVREC was no-till.

Cover Crop and Soil Sampling

All cover crops were sampled for biomass from two randomly selected 0.25 m^2 areas of each plot prior to termination. Cover crop samples were then oven-dried for at least 48 h at 60°C and weighed to obtain dry cover crop biomass. Soil samples were collected following cover crop termination in the spring of 2018 and 2019 using bucket augers. At WREC, soil samples were composited from ten cores by plot at four depths: 0-5 cm, 5-10 cm, 10-15 cm, and 15-30 cm. Due to the high clay content and difficulty sampling, only 0-5, 5-10, and 10-15cm depths were collected at TVREC with 10 cores per plot at each depth.

Soil Chemical Analyses

All soils were sieved to 2 mm, and a portion was ground further using a coffee grinder in preparation for total C and N analysis by dry combustion with a CN LECO 2000 analyzer (Leco Corp., St. Joseph, MI). POXC was analyzed following the method established by Weil et al. (2003). Air-dried soil (2.5 g) was placed in a 50 mL centrifuge tube with 18 mL of deionized water and 2 mL of 0.2 M $KMnO_4$ stock solution. Centrifuge tubes were then shaken at 240 oscillations per minute for 2 min. Once removed from the shaker, centrifuge tubes were placed in a dark area for 10 min to settle. The oxidation of active carbon resulted in the conversion of Mn (VII) to Mn (II), with lighter purple solutions corresponding with higher POXC levels. After 10 min, 0.5 mL of supernatant from each centrifuge tube was added to a new centrifuge tube with 49.5 mL of dionized water for a 100-fold dilution. A 0.2 mL aliquot was then transferred to a 96-well microplate with a single replication. Standards of 0.05, 0.1, 0.15, and 0.2 M $KMnO_4$ were prepared for the standard curve. The absorbance of samples was read at 550 nm on a spectrophotometric microplate reader (Biotek MQX200, Winooski, Vermont). POXC levels 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:

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

Soil Physical Analyses

Water stable aggregates (WSA) were measured by wet sieving (Kemper and Rosenau, 1986). A subset of each air-dried soil sample was sieved to include only 1 to 2 mm sized aggregates, and 4 g were measured into cup-like sieves of 24 mesh/cm wire. Aggregates were slowly rewetted to near field capacity using a household humidifier. Rewetted samples were then uniformly raised and lowered into individual metal containers of distilled water at a rate of 35 times/min for 3 min. After 3 min, new containers were filled with a diluted sodium hexametaphosphate $(NaPO_3)_6$ and sodium hydroxide (NaOH) dispersal solution. The sieves were lowered into this solution at 35 times/min until all remaining aggregates were broken up. All metal containers were then dried over night at 105°C or until all water had evaporated. The weight of the soil remaining in the containers was compared, with corrections for the dispersal solution, to determine the proportion of stable aggregates, not including sand particles.

Soil strength was measured in June of 2018 and 2019 using a tractor-mounted hydraulic, five-probe penetrometer to obtain cone-index values for each plot at multiple depths as described by Balkcom et al. (2016). The tractor was positioned so the center penetrometer rod was positioned in the cash crop row with two penetrometer rods on either side (22.5 and 45 cm away from the cash crop row) to include trafficked and non-trafficked rows. Cone-index values were recorded every up to 50 cm into the soil profile. In order to simplify the analysis of soil strength data, area under the curve for cone index ($AUC_{C.I}$) was calculated for analysis. Calculated $AUC_{C.I}$ values utilize average cone index values to encompass soil strength across all row positions and depths. Area under the curve for cone index was calculated following Equation 2 (Balkcom et al., 2016). Soil moisture samples were obtained from five locations in each plot to correspond with soil strength data. Samples were collected using push probes and separated into 0-15 cm and 15-30 cm depth classes. Soil was weighed, dried for 48 h at 105°C and weighed again to obtain gravimetric water content (θg) (Balkcom et al., 2016).

Equation 2:

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

Data Analysis

Data were analyzed using mixed models in SAS® PROC GLIMMIX. Data were log transformed to achieve normality. Treatment, year, and depth, along with the interactions of these factors were considered fixed effects. Cover crop biomass was analyzed separately by year. Location was always significant for soil health indicators, so data were analyzed separately by location. Replication was always considered a random effect. 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.

RESULTS AND DISCUSSION

Cover Crop Biomass

A three-way interaction of treatment, year, and location was observed for cover crop biomass ($P<0.0001$; Table 2.2). When comparing differences between treatments within a site-year, all cover crop treatments contained more above-ground biomass than the Daikon radish monoculture at TVREC in 2018 and 2019 (Table 2.3). In 2018, radish winterkilled at TVREC, and no biomass remained at the time of sampling. In 2019, radish monoculture treatments contained only 1030 kg ha^{-1} of biomass, while other cover crop treatments contained 3750 to 6050 kg ha^{-1} of biomass at TVREC. At WREC, no differences were observed in biomass production in 2018. However, in 2019 the rye-clover mixture developed higher biomass than the Daikon radish and rye-radish treatments. The rye-radish mixture also contained less biomass than clover-radish and rye-clover-radish treatments

In general, cover crop mixtures did not produce more biomass than rye or crimson clover treatments alone. At WREC, no differences between cover crop mixtures and Daikon radish were observed, while Daikon radish always contained less biomass than cover crop mixtures at TVREC. Inclusion of crimson clover in a cover crop mixture did not affect biomass compared to rye and crimson clover monocultures within the same location and year. In a study conducted by Ranells and Wagger (1997), legume monocultures do not always contain different biomass from grass-legume cover crop mixtures or legumes within a mixture. Finney et al. (2016) found that winter hardy, non-legume cover crops, including cereal rye, developed the most biomass and that no cover crop mixture contained more biomass than a rye monoculture. Similarly, cover crop mixtures at WREC and TVREC did not differ from rye monocultures within the same year in terms of biomass, with the exception of the rye-radish mixture at WREC in 2019.

Few differences were observed when comparing biomass according to year within a location and treatment. At TVREC, Biomass was greater for crimson clover and Daikon radish in 2019 compared to 2018. Differences between the two years are likely due to a milder winter in 2019 and cooler winter at TVREC in 2018, which left no Daikon radish biomass at the time of sampling (AWIS, 2019). Daikon radish is less winter hardy than rye and crimson clover and has been previously observed to winterkill when temperatures drop below 4°C for multiple days (Chen et al, 2014). At WREC, no differences were observed in biomass according to year. However, all clover-containing treatments had numerically higher biomass in 2019 compared to 2018, while rye and radish treatments had numerically lower biomass in 2019 compared to 2018.

Trends for numerically lower biomass of rye and radish in 2019 may be attributed to a delay in cover crop planting due to Hurricane Michael. Winters are generally mild at WREC, but cover crop planting was approximately two weeks later in November in 2019 compared to 2018. In a review, Ruis et al. (2019) found that longer cover crop growing seasons often lead to higher biomass production in spring-terminated cover crops, which can contribute to the high variability of biomass production in the warm, humid climate of the Southeast. Additionally, Bauer and Reeves (1999) observed substantial biomass reduction under November and December planting dates of small-grain cover crops compared to October-planted cover crops on a South Carolina loamy sand. Timely cover crop planting heavily influences cover crop biomass production and the level of potential benefits that cover crops may provide, particularly in cover crops as cold-sensitive as Daikon radish (Clark, 2007; Finney et al., 2016; Ruis et al., 2019).

Water Stable Aggregates

There was a treatment by year interaction at TVREC (P=0.0587), but treatments were not different within the same year (Table 2.4). WSA under the winter fallow, crimson clover, and rye-radish treatments was lower in 2019 compared to 2018. However, previous studies have shown that seasonal differences in soil moisture and microbial activity can influence aggregate stability more than residue additions (Cosentino et al., 2006; Steele et al., 2012). A study by Cosentino et al. (2006) observed that soil moisture changes and increased fungal biomass had pronounced effects on aggregate stability that were comparable to mulch additions. It was hypothesized that differences in WSA for these treatments between 2018 and 2019 were caused by climatic differences between the times soil samples were collected for the two years. The high clay content of the soil and prolonged no-till management likely contributed to the consistency of WSA across all treatments within the same year at TVREC. Soil clay content and organic matter are known to positively relate to soil aggregate stability (McVay et al., 1989; Nimmo and Perkins, 2002). Conversely, base WSA values were lower at WREC, likely due to lower clay content, lack of prolonged no-till management, and warmer climate of the region, which promotes SOM mineralization. In Georgia, McVay et al. (1989) observed higher WSA in the less weathered, finer soils of the Limestone Valley compared to Coastal Plain soils, with differences between cover crops emerging only at the Coastal Plain site. Differences in WSA also did not correlate to POXC at both TVREC (r=0.09) and WREC (r=-0.02), while a study conducted by Steele et al. (2012) observed a strong relationship between the same two soil health parameters.

The treatment by year interaction was not significant at WREC (P=0.1969); however, an overall treatment effect was significant (P=0.0065; Table 2.2). At WREC, WSA was higher in the rye-clover-radish mixture than it was in any other clover-containing treatment (Figure 2.1). WSA was also higher for rye treatments compared to clover and rye-clover treatments. Lower WSA percentages in most clover-containing treatments may be due to the lower C:N ratio of crimson clover relative to rye, which can accelerate organic matter decomposition. However, in a three-year study, McVay et al. (1989) observed generally higher aggregate stability following crimson clover compared to wheat treatments. Water stable aggregates for winter fallow was not different from any other treatment at WREC. Depth did not affect WSA at WREC or TVREC.

No differences in WSA were observed between fallow treatments compared to any other treatment. A previous study by Steele et al. (2012) found that cover crop treatments increased WSA compared to fallow treatments after 13 years of cover cropping in Maryland Coastal Plain and Piedmont soils. An additional study by McVay et al. (1989) observed an increase in WSA for aggregates >0.25mm in size with the inclusion of cover crops compared to winter fallow in a Coastal Plain sandy clay loam. Results from these studies provide precedence for short-term improvements of soil physical properties under cover crops in the Coastal Plain; however, they also indicate that aggregate stability may change at a slower rate in finer, Piedmont soils. Additional years under cover crop treatments at WREC may be necessary to observe differences in WSA between cover crop and fallow treatments.

Total Carbon

Cover crop influenced soil TC across years at TVREC (P=0.0002), TC ranged from 1.1% to 1.29%. Total carbon was lower under Daikon radish treatments compared to crimson clover, rye-clover, rye-radish, and rye-clover-radish. TC was also lower under the winter fallow treatment than under crimson clover, rye-clover, and rye-radish treatments at TVREC (Table 2.5). Cover crop did not affect TC at WREC (P=0.4209; Table 2.2), ranging from 0.54% to 0.59% total carbon. However, the comparatively coarse texture of WREC soils, combined with the warmer climate of the region, likely attributed to the lack of treatment differences (Cosentino et al., 2006; Steele et al., 2012). Hassink and Whitmore (1997) developed a model that found, under the same surface residue additions, coarser soils tended to accumulate SOM at a slower rate than finetextured soils and that higher initial SOM levels contribute to more rapid SOM accumulation. Balkcom et al. (2013) also observed that in a fine sandy loam Coastal Plain soil TC did not significantly differ between cover crop treatments after six years and was more affected by the implementation of conservation tillage. The lack of differences between cover crop treatments was attributed in part to the coarse texture and initially low SOC content at the WREC location.

Depth had an overall effect on TC. Carbon was stratified by depth class at both locations (Figure 2.2a). At TVREC, TC decreased with each depth class ($P<0.0001$). At TVREC, TC in the top 5 cm averaged 2.06%, while the 5-10 cm and 10-15 cm depths averaged at 1.06% and 0.81% carbon respectively. Based on the assumption that 58% of SOM mass is carbon, these values equate to 3.55%, 1.82%, and 1.39% SOM by depth at TVREC. At WREC, TC was higher in the 0-5 cm depth class, at 0.61% C, compared to the 10-15 cm and 15-30 cm depths, which each averaged 0.55% ($P=0.0006$). Such stratification is well documented, with soil C decreasing with depth and distance from surface residue additions, particularly in warm, humid regions (Hassink and Whitmore, 1997; Balkcom et al., 2013). Causarano et al., (2008) observed that changes in management largely affected the top 5 cm of soil, with greater stratification of SOC under conservation tillage compared to conventional tillage. However, Balkcom et al. (2013) observed no impact of tillage system and cover crop treatment on carbon sequestration rates, although numeric changes in SOC were primarily near the soil surface.

Permanganate Oxidizable Carbon

Permanganate oxidizable carbon exhibited a treatment by depth interaction at TVREC (P=0.0021, Table 2.6). In the 0-5 cm and 5-10 cm depth classes at this location, no differences were observed between treatments. However, for the 10-15 cm depth class, POXC was higher under crimson clover than under winter fallow, rye, rye-clover, rye-radish, clover-radish, and rye-clover-radish treatments (Figure 2.3). The lower C:N ratio of crimson clover relative to other treatments may have contributed to higher POXC values in the clover monoculture, since residues with a lower C:N ratio are more readily decomposed. There was not a treatment by depth interaction at WREC (P=0.8951). Trends for POXC stratification with depth were similar to those observed for TC. In Figures 2.2a and 2.2b, a decrease in TC and POXC respectively were observed as depth increased at TVREC, while TC and POXC levels remain relatively consistent throughout the soil profile at WREC. Plots at WREC were conventionally tilled prior to implementing this experiment in 2017. The long-term no-till management of TVREC likely contributed to enhanced stratification of TC and POXC, since the accumulation of crop residue at the soil surface promotes the development of habitats for soil organisms that contribute to the cycling of SOM into biologically active fractions. Previous studies have observed stratification of SOC under conservation tillage, compared with a more homogeneous distribution of SOC under conventional tillage (Causarano et al., 2008; Gamble et al., 2014). Additionally, based on findings from Hassink and Whitmore (1997), the finer texture of TVREC soils contribute to the accumulation of soil C, including POXC, at a faster rate than the coarser soils at WREC. Such management and environmental factors likely contributed to the differences in POXC stratification between the two locations. Similar to POXC results at WREC, Sainju et al. (2007) found that no differences in labile carbon pools occurred after the first two years of cover cropping in coarse Coastal Plain soils. POXC is often recommended as a highly dynamic soil health indicator that is more management-sensitive than larger soil C fractions (Causarano et al., 2008; Gamble et al., 2014). However POXC at TVREC exhibited fewer differences between treatments than TC, which takes into account all carbon fractions, including passive forms of soil C. At WREC, the only significant effect was year, with POXC higher in 2018 (259 mg kg^{-1}) than in 2019 (230 mg kg^{-1}; P=0.0014). Year was not significant at TVREC (P=0.5088). Additional years under cover crop treatments may be necessary to observe differences in POXC.

POXC was evaluated for correlations with TC. POXC was not strongly correlated with TC at WREC (r=0.04). There was, however, a strong POXC and TC correlation at TVREC (r=0.84). A similar correlation between labile carbon forms and larger carbon pools was observed in previous studies (Weil et al., 2003).

Soil Strength – Area Under the Curve for Cone Index

Area under the curve for cone index ($AUC_{C.I.}$) was used to represent soil strength across all depths and row positions. $AUC_{C.I}$ decreased in 2019 compared to 2018 at both TVREC (P<0.0001) and WREC (P<0.0001), indicating reduced soil strength and compaction (Table 2.7). Soil strength is known to decrease with increased soil moisture (Sainju et al., 2007; Lucas and Weil, 2012; Wang et al., 2017). For example, Williams and Weil (2004) observed more prominent differences in soil compaction under drought conditions. Soil moisture levels at TVREC and WREC at the time of sampling were higher in 2019 compared to the sampling date in 2018, which may have contributed to overall reductions in $AUC_{C.I}$ in 2019 (Table 2.8).

Cover crop did not have a significant effect on $AUC_{C.I}$ at TVREC (P=0.4443; Table 2.2). This could be due to prolonged no-till management and inherent soil properties having a greater effect on soil strength than cover crop treatments(Williams and Weil, 2004; Balkcom et al., 2016). Lal et al. (1994) also observed no effect of cover crops on penetration resistance after 28 years in a crop rotation which included alfalfa or grass species as cover crops. Tillage has been shown to impact penetration resistance. For example, Mahboubi et al. (1993) found that after 28 years, bulk density of a silt loam soil in the crop row was highest under no-till management compared to moldboard plow and chisel plowed treatments. However, a study conducted by Ismail et al. (1994) observed no differences in bulk density between 20-year no-till and conventionally tilled silt loam soils.

Cover crop was significant at WREC (P=0.0018; Table 2.2) with $AUC_{C.I}$ lower under rye compared to crimson clover and clover-radish treatments. Clover-radish plots also exhibited greater soil strength than winter fallow and rye-radish treatments (Table 2.7). These results differed from other studies which found that crops with robust taproots develop more root mass in compacted soil layers (Williams and Weil, 2004; Chen and Weil, 2010). Williams and Weil (2004) observed a positive soybean yield response to the implementation of cover crops, particularly in areas of drought and high soil strength (Williams and Weil, 2004; Chen and Weil, 2010). Chen and Weil (2010) also observed that radish penetrated deeper into compacted soils compared to rye monocultures.

Treatment differences in $AUC_{C.I}$ did not coincide with treatment differences in soil moisture (Table 2.7).

CONCLUSIONS

After the first two years under cover crop treatments, selected soil health indicators did not behave consistently between locations and were heavily influenced by climate, soil type, and historic management. There were no differences in WSA between treatments at TVREC. However, WSA values were consistently above 90% at TVREC due to the high clay content and prolonged no-till management of the location. At WREC, differences between treatments were present, but cover crops did not have higher or lower WSA than the winter fallow control. TC and POXC did not differ between treatments at WREC, likely due to the coarse soil texture and warm, humid climate of the region. However, at TVREC, differences in TC and POXC occurred between treatments, with TC exhibiting more distinct changes after two years compared to POXC. Crimson clover, rye-clover, and rye-radish treatments had higher TC than the winter fallow control while only the crimson clover monoculture had higher POXC than winter fallow. Although soil strength was lower in 2019 compared to 2018 at both WREC and TVREC, $AUC_{C.I}$ did not differ between treatments at TVREC while $AUC_{C.I}$ was higher under clover-radish and rye-radish mixtures compared to winter fallow at WREC.

Overall, soil health indicators were not consistently affected after two years of cover crop implementation. The behavior of selected soil health indicators varied with location and long-term management practices. Such results indicate that prolonged cover crop utilization will likely be necessary for consistent, detectable changes from cover crop treatments to emerge.

Table 2.1 Species composition and seeding rates of cover crop monocultures and mixtures planted following cash crop harvest in 2017 and 2018 at Tennessee Valley Research and Extension Center (TVREC) and the Wiregrass Research and Extension Center (WREC).

Treatment	Seeding Rate		
	Rye	Crimson Clover	Daikon Radish
	—— kg ha^{-1} ——		
Rye	100	-	-
Crimson Clover	-	22	-
Daikon Radish	-	-	9
Rye-Clover	50	22	-
Rye-Radish	50	-	9
Clover-Radish	-	22	9
Rye-Clover-Radish	34	11	4

Table 2.2 Summary of analysis of variance (ANOVA) for water stable aggregates (WSA), permanganate oxidizable carbon (POXC), total carbon (TC), and area under the curve for cone index ($AUC_{C.I.}$), in response to cover crop treatment, depth, year and their interactions according to location.

Source of Variance	ANOVA, Pr > F				
	df	WSA	POXC	TC	$AUC_{C.I.}$
			TVREC		
Treatment (T)	7	0.7095	0.0177	0.0002	0.4443
Depth (D)	2	0.1618	<0.0001	<0.0001	-
Year (Y)	1	<0.0001	0.5088	0.4949	<0.0001
T x D	14	0.6917	0.0021	0.1935	-
T x Y	7	0.0587	0.6594	0.3766	0.449
D x Y	2	0.7816	0.0258	0.8919	-
T x D x Y	14	0.6286	0.5192	0.7656	-
			WREC		
Treatment (T)	7	0.0065	0.233	0.4209	0.0018
Depth (D)	2	0.869	0.1282	0.0006	-
Year (Y)	1	0.9707	0.0014	<0.0001	<0.0001
T x D	14	0.7025	0.8951	0.7005	-
T x Y	7	0.1969	0.195	0.4403	0.1633
D x Y	2	0.29	0.8934	0.4185	-
T x D x Y	14	0.1659	0.9615	0.9965	-

Table 2.3 Cover crop biomass according to a treatment by location by year interaction measured in the spring of 2018 and 2019 at the Tennessee Valley Research and Extension Center (TVREC) and the Wiregrass Research and Extension Center (WREC).

	Cover Crop Biomass			
	TVREC		WREC	
Treatment	2018	2019	2018	2019
	(*P<0.0001*)			
	kg ha^{-1}			
Winter Fallow	-	-	-	-
Rye	2580 a†	4080 a	5340 a	3820 abc
Crimson Clover	1520a	5570* a	3830 a	4250 abc
Daikon Radish	0 b	1030* b	5930 a	2880 bc
Rye-Clover	3210 a	6050 a	3420 a	9330 a
Rye-Radish	2790 a	4220 a	3700 a	2010 c
Clover-Radish	1660 a	3750 a	5700 a	6600 ab
Rye-Clover-Radish	3690 a	5220 a	3920 a	6280 ab

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

*Indicates significantly higher biomass within a given cover crop treatment and location according to Tukey's HSD at α= 0.1

Table 2.4 Impact of the interaction between treatment and year on percentage water stable aggregates (WSA) at Tennessee Valley Research and Extension Center (TVREC) and Wiregrass Research and Extension Center (WREC).

Treatment	Water Stable Aggregates	
	2018	
	TVREC (*P = 0.0587*)	WREC (*P*=0.1969)
	%	
Winter Fallow	97.34 abc†	84.62
Rye	97.38 abc	88.33
Crimson Clover	97.58 abc	85.92
Daikon Radish	97.30 abc	83.69
Rye-Clover	97.77 ab	83.8
Rye-Radish	98.06 a	84.51
Clover-Radish	97.40 abc	83.58
Rye-Clover-Radish	96.07 abcd	89.04
	2019	
	TVREC (*P = 0.0587*)	WREC (*P*=0.1969)
Winter Fallow	94.51 d	84.72
Rye	95.02 cd	87.21
Crimson Clover	95.46 d	81.99
Daikon Radish	96.05 abcd	86.83
Rye-Clover	95.60 abcd	83.90
Rye-Radish	95.41 bcd	86.83
Clover-Radish	95.75 abcd	84.58
Rye-Clover-Radish	96.46 abcd	87.18

† Locations analyzed separately. Values followed by the same letter are not significantly different within a location according to Tukey’s HSD at α= 0.1.

Table 2.5 Impact of cover crop treatment on total soil carbon (TC) percentage across years at Tennessee Valley Research and Extension Center (TVREC) and Wiregrass Research and Extension Center (WREC) .

Treatment	Total Carbon	
	TVREC (*P=0.0002*)	WREC (*P=0.4209*)
	%	%
Winter Fallow	1.12 bc†	0.54
Rye	1.17 abc	0.58
Crimson Clover	1.28 a	0.55
Daikon Radish	1.1 c	0.59
Rye-Clover	1.25 a	0.56
Rye-Radish	1.29 a	0.59
Clover-Radish	1.22 abc	0.56
Rye-Clover-Radish	1.24 ab	0.59

† Values followed by the same letter are not significantly different within a location according to Tukey's HSD at α= 0.1.

Table 2.6 Influence of cover crop treatment by depth interaction of permanganate oxidizable carbon (POXC) levels across years at Tennessee Valley Research and Extension Center (TVREC) and Wiregrass Research and Extension Center (WREC).

Treatment	POXC–TVREC (*P=0.0021*)			
	0-5 cm	5-10 cm	10-15 cm	15-30 cm
	mg kg^{-1}			
Winter Fallow	682 a†	456 a	297 b	-
Rye	728 a	403 a	327 b	-
Crimson Clover	833 a	422 a	477 a	-
Daikon Radish	678 a	477 a	331 ab	-
Rye-Clover	784 a	449 a	243 b	-
Rye-Radish	761 a	456 a	330 b	-
Clover-Radish	757 a	441 a	312 b	-
Rye-Clover-Radish	776 a	411 a	311 b	-
Treatment	**POXC–WREC (*P=0.8951*)**			
	0-5 cm	5-10 cm	10-15 cm	15-30 cm
	mg kg^{-1}			
Winter Fallow	236	249	266	217
Rye	257	255	254	247
Crimson Clover	251	234	244	251
Daikon Radish	238	220	190	233
Rye-Clover	284	261	254	239
Rye-Radish	263	241	257	186
Clover-Radish	267	262	224	206
Rye-Clover-Radish	271	269	257	261

† Values followed by the same letter are not significantly different within a location and depth class according to Tukey’s HSD different at α= 0.1.

Table 2.7 Influence of cover crop treatment and year on soil strength, specifically area under the curve for cone index ($AUC_{C.I.}$) at Tennessee Valley Research and Extension Center (TVREC) and Wiregrass Research and Extension Center (WREC).

Treatments	$AUC_{C.I.}$			
	TVREC		WREC	
	2018	2019	2018	2019
	(P=0.9964)	*(P=0.0116)*	*(P=0.2604)*	*(P=0.0004)*
	MPa cm^{-1}			
Winter Fallow	261	175 a†	355	210 c
Rye	268	152 c	344	2015 c
Crimson Clover	261	167 abc	375	270 ab
Daikon Radish	274	173 ab	397	227 abc
Rye-Clover	265	158 abc	356	263 ab
Rye-Radish	268	152 c	365	225 bc
Clover-Radish	267	162 abc	414	275 a
Rye-Clover-Radish	261	155 bc	364	256 ab

Year	TVREC	WREC
	(P<0.0001)	*(P<0.0001)*
2018	266 a	371 a
2019	162 b	240 b

† Locations and years analyzed separately. Values followed by the same letter are not significantly different according to Tukey's HSD at α= 0.1.

Table 2.8 Difference in soil moisture at the time of area under the curve for cone index ($AUC_{C.I.}$) measurements between cover crop treatments and years at Tennessee Valley Research and Extension Center (TVREC) and Wiregrass Research and Extension Center (WREC).

Treatments	Soil Moisture			
	TVREC		WREC	
	2018	2019	2018	2019
	(P=0.0413)	*(P=0.01309)*	*(P=0.2611)*	*(P=0.1979)*
	%			
Winter Fallow	15.77 a†	18.22	5.13	8.20
Rye	15.76 a	18.13	5.12	7.92
Crimson Clover	15.71 a	18.05	5.05	7.76
Daikon Radish	15.61 a	18.02	4.99	7.67
Rye-Clover	15.44 a	17.88	4.84	7.62
Rye-Radish	15.12 a	17.70	4.75	7.60
Clover-Radish	14.99 a	17.27	4.60	7.56
Rye-Clover-Radish	14.84 a	17.22	4.45	7.49

Year	TVREC *(P<0.0001)*	WREC *(P<0.0001)*
2018	266 a	371 a
2019	162 b	240 b

† Locations analyzed separately. Values followed by the same letter are not significantly different according to Tukey's HSD at α= 0.1.

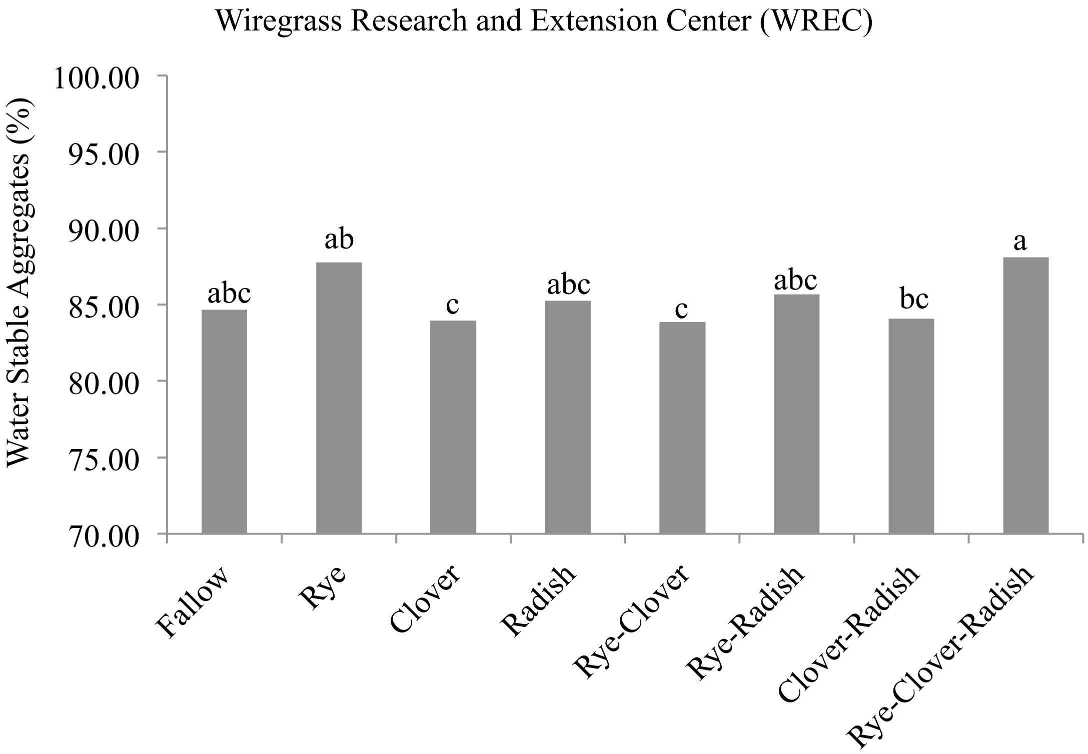

Figure 2.1 Differences between treatments in water stable aggregates (WSA) at Wiregrass Research and Extension Center (WREC) (P=0.0065). Columns with the same letter do not differ between cover crop treatments (α = 0.1). Treatment did not have a significant effect on WSA at Tennessee Valley Research and Extension Center (TVREC) (P=0.7095).

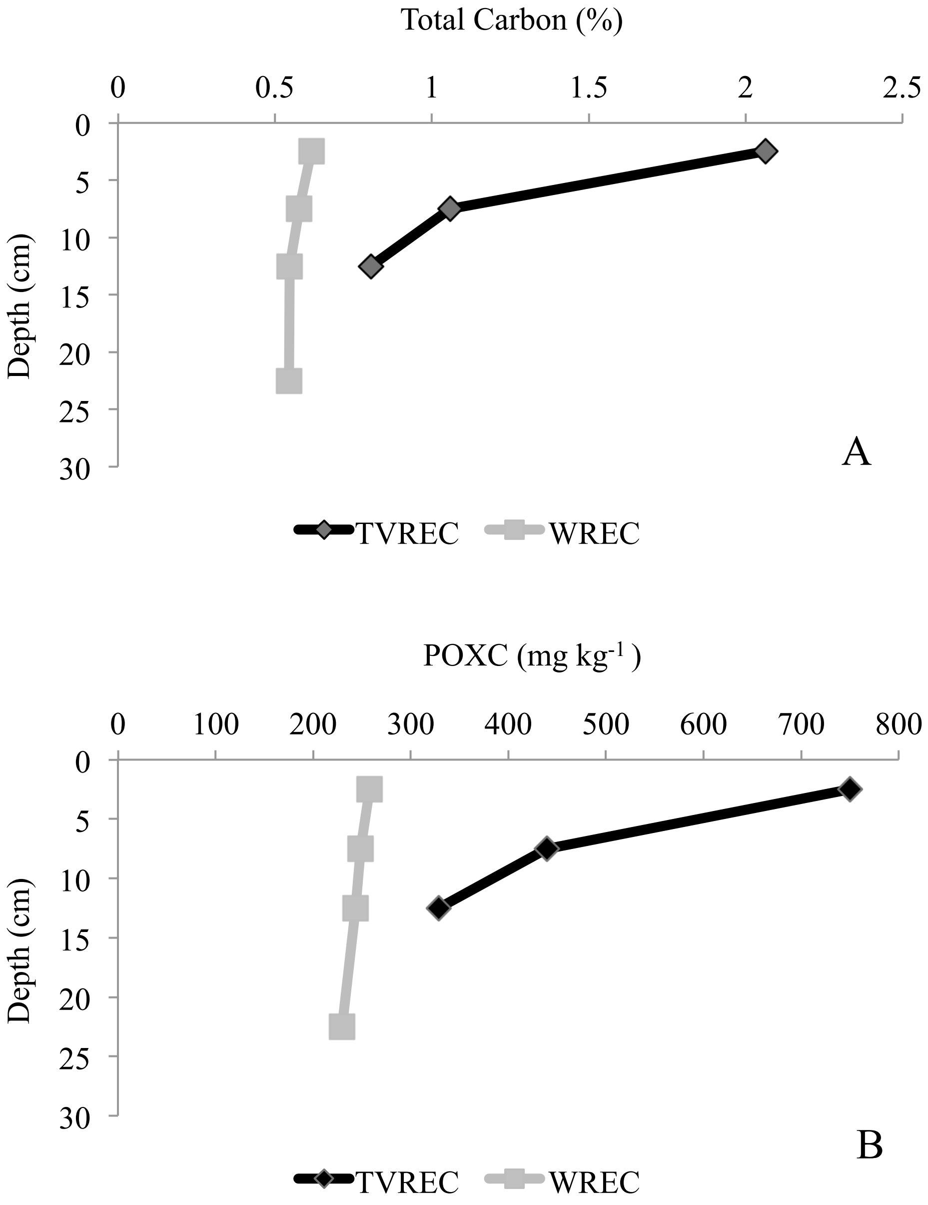

Figure 2.2 a) Total carbon (TC) and b) Permanganate Oxidizable Carbon (POXC) by depth at Tennessee Valley Research and Extension Center (TVREC) and Wiregrass Research and Extension Center (WREC). Locations were analyzed separately. Depth had a significant effect on TC at both TVREC ($P<0.0001$) and WREC ($P=0.0006$). Depth did not have a significant effect on POXC at Wiregrass Research and Extension Center (WREC) ($P=0.1282$) while depth was a significant factor at TVREC ($P<0.0001$).

III. IMPACT OF HERBICIDE, COVER CROP PLACEMENT, AND HIGH RESIDUE COVER CROP MIXTURES ON WEED CONTROL IN COTTON

Abstract

Winter cover crops are also a common tool for integrated weed management in both conventional and conservation agricultural systems. Two trials were established at E.V. Smith Research Center in Shorter, AL in November 2016 to evaluate the efficacy of several cover crop systems as a supplemental form of weed control in cotton production systems. The first trial evaluated the effects of cover crops and herbicide regime on weed control in cotton (*Gossypium hirsutum*). Cover crop treatments included a rye monoculture, a mixture of rye (*Secale cereale*), oats (*Avena sativa*), wheat (*Triticum aestivum*), crimson clover (*Trifolium incarnatum*), and Daikon radish (*Raphanus sativus*), and winter fallow. Each cover crop system was evaluated under four herbicide regimes including PRE only (pendimethalin and fomesafen), POST only (dicamba fb glyphosate), PRE+POST, and herbicide-free. *Amaranthus* control was lower in all herbicide-free treatments compared to all PRE+POST, and cotton lint yield was lower in the herbicide-free treatments compared to treatments with herbicide applications. Lint yield was higher in PRE+POST treatments compared to herbicide-free treatments, regardless of cover crop. Lint yield under PRE only treatments were not different from PRE+POST while POST only treatments had lower lint yield than PRE+POST treatments in 2018. The second trial included rye monocultures as whole-plot and precision placements between intended cotton rows onl, a clover-radish mixture in the whole plot and within the cotton row only, and three-species mixtures as whole-plot treatments and with precision placements; all treatments managed with a PRE+POST herbicide regime. In the cover

crop placement trial, weed control was often similar between treatments in the same year, and cotton yield was only influenced by year. Results indicate that cover crops alone will not eliminate the need for chemical weed control.

INTRODUCTION

Conservation agriculture has become increasingly popular in recent decades. Conservation practices, including reduced tillage and cover crop implementation, primarily address soil fertility and health. However, the adoption of conservation agriculture has largely coincided with the development of herbicide-resistant crops, which has led to reliance on consistent herbicide efficacy for productivity in conservation agriculture systems (Williams and Weil, 2004). The adoption of herbicide resistant crop varieties has contributed to the overuse of some herbicides and the development of herbicide resistant weeds, notably glyphosate-resistant palmer amaranth (*Amaranthus palmeri)* (Price et al., 2016). Challenges associated with resistance management have reduced the reliability of standard chemical weed control in conservation systems, which have led to expanded interest in integrated weed control. High biomass cover crops have been known to potentially supplement chemical weed control through competition, physical suppression, and the release of allelopathic chemicals (Webster et al., 2013; Price et al., 2016).

Terminated cover crops in reduced tillage systems provide a mulch layer over the soil surface. This mulch layer can provide early season weed control by shading the soil surface, which can make conditions unsuitable for weed germination. Mulch layers and actively growing cover crops cool the soil surface relative to bare ground and interfere with light quality (Price et al., 2011; Mirsky et al., 2013). Cooler soil surfaces, in addition to cool-season weed competition and the inhibition of germination-inducing red light can reduce weed germination rates, with the potential to reduce herbicide requirements (Teasdale and Mohler, 1993; Mirsky et al., 2011).

Small grain cover crop monocultures, cereal rye (*Secale cereale*) in particular, are widely utilized between warm season cash crops in the Southeast. Small grain cover crops are known to develop high biomass and are slow to decompose after termination, due to high C:N ratios, which further contributes to weed suppression potential (Teasdale and Mohler, 1993; Reberg-Horton et al., 2011; Lawley et al., 2012; MacLaren et al., 2019). Small grain cover crop monocultures provide a variety of benefits in addition to potential weed suppression, including soil moisture retention and nutrient scavenging, and cover crop mixtures may provide additional benefits. Cover crop mixtures have been promoted in recent years for diversified benefits and the enhancement of various ecosystem services, including nitrogen fixation by legumes and compaction alleviation by brassicas (Reeves et al., 2005; Clark, 2007). However, overall cover crop biomass and proportion of small grain biomass has been previously reported as influencing weed suppression more than diversity of a cover crop mixture (Blanco-Canqui et al., 2015; Murrell et al., 2017). Cover crop biomass accumulation and, by extension, weed suppression potential, are highly variable based on climate, management, and the inherent complexity of agroecosystems (Price et al., 2008; MacLaren et al., 2019). A number of trials have been conducted to evaluate various herbicide regimes under cover crop monocultures and two-species mixtures, but few have utilized more diverse cover crop mixtures, especially in the Southeast. Therefore, two trials were established in Shorter, AL, to evaluate the effect of herbicide regime, cover crop monocultures, cover crop mixtures, and strategic seed placement on weed control and cotton yield.

MATERIALS AND METHODS

Site Description

Trials were conducted at the E.V. Smith Research Center Field Crops Unit (32°26'32.1"N 85°53'51.5"W) in Shorter, AL on a Compass loamy sand (Coarse-loamy, siliceous, subactive, thermic Plinthic Paleudults). The study was initiated in November of 2016 and was conducted through 2019. Dates of trial operations are listed in Table 3.1. Both trials were managed with reduced tillage, with all plots in-row subsoiled following cover crop termination. The area was irrigated as needed throughout the cash crop season.

Cover Crop Mixture and Herbicide Timing –Design and Data Analysis

This trial was established to compare weed control and subsequent cotton yield under rye monoculture, multi-species cover crop mixture, and winter fallow systems under different herbicide regimes. The trial was designed in a factorial arrangement of three cover crop systems and four herbicide treatments replicated four times.

Cover crops were planted following cotton harvest each year with the Great Plains 3.7-m 1205NT grain drill, with clover and radish seed mixed in the smaller box and small grains in the larger box. Cover crop systems included winter fallow, a ‘Wrens Abruzzi’ cereal rye (*Secale cereale*) monoculture, and a five-species mixture of cereal rye, ‘Coker’ oats (*Avena sativa*), ‘Pioneer 26R61’ wheat (*Triticum aestivum*), ‘Dixie’ crimson clover

(*Trifolium incarnatum*), and Daikon radish (*Raphanus sativus*). The rye monoculture was planted at 100 kg ha^{-1}; the mixture was planted with small grains at 17 kg ha^{-1}, crimson clover at 11 kg ha^{-1}, and radish at 4 kg ha^{-1} . Each Cover crop treatment was fertilized once each spring at 34 kg N ha^{-1}. Cover crops were terminated three weeks prior to planting the cash crop by rolling using a 3-section straight crimping bar roller along with a broadcast tank mix of glyphosate at 1.0 kg ae ha^{-1} and glufosinate at 0.5 kg ai ha^{-1}. Herbicide treatments included a preemergent herbicide-only (PRE) treatment of pendimethalin at 1.1 kg ai ha^{-1} and fomesafen at 0.28 kg ai ha^{-1}, a postemergent herbicide-only (POST) treatment of dicamba at 0.56 kg ae ha^{-1} followed by glyphosate at 1.0 kg ae ha^{-1}, a PRE+POST herbicide regime, and herbicide-free treatments. Cotton, DeltaPine 1538 B2XF, was planted in the spring each year with a row spacing of 91 cm and four cotton rows per plot (Table 3.1).

Cover crops were sampled prior to termination using two randomly placed 0.25 m^2 areas in each plot. All cover crop samples were oven-dried at 60°C for at least 48 h to obtain dry cover crop biomass. Weed samples were taken in the summers of 2017, 2018, and 2019. Samples were collected from two 0.25 m^2 squares both inside the cotton row and between cotton rows. Visual control ratings of prominent weed species were also collected on a 0-100% scale each summer. Cotton was harvested from the two middle rows of the plots after defoliation. Cotton lint yield was calculated from seed cotton harvested from the middle two rows at 40% lint. Plots with no yield potential due to weed infestation were mowed prior to harvest.

Data were analyzed using mixed models in SAS® PROC GLIMMIX. Data were log transformed to achieve normality. Cover crop, year, and herbicide treatment, along with the interactions of these factors were considered fixed effects. Replication was considered a random effect. Row positions for weed biomass were analyzed separately. Mean separations were performed using Tukey's honestly significant difference (HSD) test ($\alpha = 0.1$).

Cover Crop Placement –Design and Data Analysis

This trial was established for the evaluation of cotton yield and weed control under rye monocultures and cover crop mixtures with and without strategic, precision cover crop placements. This study was organized in a factorial arrangement of three cover crop systems as whole-plot or precision seed placements with four replications.

Cover crops included 'Wrens Abruzzi' cereal rye, 'Dixie' crimson clover, and Daikon radish. Treatments included a winter fallow control (WF) and whole-plot treatments of rye (R-W), clover-radish mixture (CR-W), and a rye-clover-radish mixture (RCR-W). Precision seed-placement treatments included rye in between planned cotton rows (R-BR), the clover-radish mixture inside the planned cotton rows (CR-IR), and rye-clover-radish with precision placements (R-BR/CR-IR). Seeding rates are listed in Table 3.2.

Cover crops were planted following cotton harvest each year with the Great Plains 3.7-m 1205NT grain drill, with rye in the larger box and the clover-radish mixture in the smaller box. Precision seed-placement treatments were established by blocking 2 large box units above planned cotton rows and 3 small box units above row-middles. Cover crops were fertilized once each spring at 34 kg N ha^{-1}, and cover crops were terminated three weeks prior to planting the cash crop by rolling using a 3-section straight crimping bar roller and with glyphosate at 1.0 kg ae ha^{-1} and glufosinate at 0.5 kg ai ha^{-1}. DeltaPine 1538 B2XF cotton was planted in the spring of each year with a row spacing of 91 cm and four cotton rows per plot. All treatments included preemergent (PRE) and postemergent (POST) herbicide treatments. The PRE herbicides included pendimethalin at 1.1 kg ai ha^{-1} and fomesafen at 0.28 kg ai ha^{-1}, and POST herbicides included dicamba at 0.56 kg ae ha^{-1} followed by glyphosate at 1.0 kg ae ha^{-1}.

Cover crops were sampled prior to termination using two randomly placed 0.25 m^2 areas in each plot. Precision treatments were sampled for both in-row and row-middle cover crop biomass as needed. All cover crop samples were oven-dried at 60°C for at least 48 hours to obtain dry cover crop biomass. Visual weed control ratings, on a 0-100% scale, were taken each summer. Cotton was harvested from the two middle rows of the plots after defoliation. Cotton lint yield was calculated with seed cotton harvested from the middle two rows at 40% lint.

Data were analyzed using mixed models in SAS® PROC GLIMMIX. Data were log transformed to achieve normality. Cover crop treatment and year, along with the interactions of these factors were considered fixed effects. Replication was considered a random effect. Mean separations were performed using Tukey's honestly significant difference (HSD) test ($\alpha = 0.1$).

RESULTS AND DISCUSSION

Cover Crop Mixture and Herbicide Timing - Cover Crop Biomass

Year was the only significant factor for cover crop biomass ($P<0.0001$). The cover crop mixture and rye monoculture did not have different biomass within the same year

(P=0.7319). Mean cover crop biomass was highest in 2017, at 6510 kg ha^{-1}, while biomass was lowest in 2018 at 2060 kg ha^{-1} (Table 3.5). Cover crop planting date and winter temperatures may have contributed to biomass differences between years. Cover crops were planted in late November of 2017 and 2018 while cover crops were planted about two weeks earlier in 2016. The importance of timely cover crop establishment for maximum benefit is well documented (Blanco-Canqui et al., 2015). Bauer and Reeves (1999) found that, in South Carolina, mid-April small grain biomass decreased substantially with later fall planting dates, with biomass highest in October-planted treatments and lowest in December-planted treatments.

Cover Crop Placement – Cover Crop Biomass

Both cover crop treatment (P<0.0001) and year (P<0.0001) had an effect on cover crop biomass (Table 3.4). Cover crop biomass was highest in 2017, averaging 7250 kg ha^{-1} while biomass was lower in both 2018 and 2019, averaging 2090 and 3200 kg ha^{-1}, respectively (Table 3.6). The differences in cover crop biomass between years are likely due in part to planting dates for the second and third years of the trial that were a few weeks later than the first year planting date. This reduced the length of the growing season and limited biomass production (Bauer and Reeves, 1999; Clark, 2007; Balkcom et al., 2013). Ruis et al. (2019) found that longer cover crop growing seasons lead to higher biomass production in cool-season cover crops. This contributes to high variability of biomass production, particularly in the warm, humid climate of the Southeast. Another study observed substantial biomass reduction under November and December planted small-grain cover crops compared to October-planted cover crops in South Carolina

(Bauer and Reeves, 1999). The shorter cover crop growing seasons during the second and third years of the study The 2016-2017 cover crop season was also milder compared to both of the following cover crop seasons, which potentially affected cover crop growth as well

Treatments without rye and the R-BR/CR-IR treatment had less biomass than all other treatments. All whole-plot treatments had similar biomass (Table 3.6). Small grain cover crops are known for high biomass accumulation that is not necessarily impacted by the addition of other species in the mixture; however, small grains are often more winter-hardy than crimson clover and radish (Clark, 2007; Lawley et al., 2011; Webster et al., 2013; Duzy et al., 2016; Murrell et al., 2017). A study conducted by Murrell et al. (2017) found that delayed cover crop planting led to small grain cover crops outperforming other cover crops in mixture due to reduced legume biomass and reduced growth of brassica cover crops in mixtures compared to monocultures. Considering the relative cold-sensitivity of clover and radish and the effect of mixture on biomass accumulation, winter freezing and competition within mixtures may have contributed to numerically lower biomass in clover-radish mixture treatments compared to rye-monoculture and rye-clover-radish whole plot treatments.

Cover Crop Mixture and Herbicide Timing - Weed Biomass

A herbicide treatment by year interaction was observed for both in-row ($P<0.0001$) and between-row weed biomass ($P<0.0001$; Table 3.3). Weed biomass numerically increased with year in herbicide-free treatments, with 2019 between-row biomass significantly higher than 2017 and 2018 biomass. In-row weed biomass for herbicide-free treatments in 2019 was only significantly different from 2017. Biomass in both row positions also decreased between 2017 and 2019 under PRE+POST treatments. No differences in between-row weed biomass occurred between herbicide treatments within the same year in 2017 and 2018, although PRE+POST only weed biomass was always numerically lower than herbicide-free weed biomass (Table 3.7).

PRE and POST only treatments did not differ within the same year and row-position while PRE+POST treatments also exhibited consistently lower weed biomass from other herbicide-containing treatments within the same year. In 2017, all treatments that included herbicides had similar between-row and in-row biomass, while 2018 PRE+POST treatments exhibited in-row weed biomass that was only lower than the POST only treatment (Table 3.7).

Increased differentiation between herbicide treatments as the trial progressed is perhaps due to expansion of the weed seedbank that has been known to occur when weeds are not sufficiently managed. Buhler et al. (1997) reported on the rapid expansion of the weed seedbank when weeds are left unmanaged and that substantial changes in seedbank density affect the efficacy of weed control systems. Teasdale et al. (2004) observed a trend of seedbank expansion following periods of high weed pressure accompanied by the rapid reduction of the seedbank following seasons of sufficient weed control. The same study also found that diversified crop rotations and weed management reduced the seedbank while MacLaren et al. (2019) found that cover crop mixture diversity had a less significant effect on weed control than overall biomass (Clark, 2007; Lawley et al., 2011; Webster et al., 2013; Duzy et al., 2016; Murrell et al., 2017).

A herbicide by cover crop interaction was observed within the cotton row

(P=0.0977; Table 3.8). Under the rye monoculture, the POST only treatments had higher weed biomass than other herbicide-containing treatments and similar weed biomass to the herbicide-free treatment. Weed biomass in herbicide-containing treatments was similar under both the mixture and winter fallow cover crop treatments. However, different cover crop treatments with the same herbicide regime did not have different in-row weed biomass (Table 3.8).

Although large changes in weed seedbanks have been previously shown to impact weed control efficacy, other studies have also shown that a substantial mulch layer can provide supplemental weed control (Teasdale et al., 2004). Teasdale and Mirsky et al. (2013) observed optimum weed suppression at 8000 kg ha^{-1} while soybean yield also declined under substantially high mulch biomass. However, because cover crop biomass did not exceed 7000 kg ha^{-1} throughout the duration of this study and weed pressure increased as plots remained untreated, insufficient biomass may have contributed to the lack of cover crop effect on weed control. Weed biomass was also affected by year for both in-row (P=0.0001) and between-row (P<0.0001) positions, with weed biomass highest in 2018, when cover crop biomass averaged only 2060 kg ha^{-1}, which may have contributed to an increase in overall weed pressure (Table 3.9).

Cover Crop Mixture and Herbicide Timing - Weed Control Ratings

A herbicide by year interaction was observed for *Amaranthus* control ratings, while no interaction was observed for annual grass control (P=0.1233; Table 3.3). No differences in annual grass control between herbicide-containing treatments were observed within the same year, and no differences in annual grass control occurred between years within the same herbicide treatment. *Amaranthus* control significantly declined in nontreated plots after the first year without herbicide applications while POST only *Amaranthus* control increased in 2019 compared to 2017 and 2018. The POST only herbicide treatment also exhibited less *Amaranthus* control than other herbicide-containing treatments in 2017, with similar control ratings as the herbicide-free treatments. In 2017 and 2019, the PRE only herbicide treatments controlled *Amaranthus* species at a similar rate as PRE+POST treatments (Figure 3.1). These results differ from those of a study by Lawley et al. (2011), which found that residue from a September-planted radish cover crop provided sufficient early season weed control for early-planted corn in the absence of a preemergent herbicide, with no decline in yield so long as a postemergent herbicide was applied.

Amaranthus control also exhibited a herbicide by cover crop interaction (P= 0.0003), while annual grass control did not (P= 0.3587; Table 3.3). When no herbicides were applied, winter fallow treatments controlled *Amaranthus* more than the rye and mixture treatments. All herbicide-containing treatments had similar *Amaranthus* control, with the exception of the winter fallow POST only treatment, which controlled *Amaranthus* less effectively than the winter fallow PRE+POST treatment (Table 3.10). A study conducted in Alabama by Aulakh et al. (2012) observed inconsistent *Amaranthus* control under a POST only herbicide regime, while PRE only and PRE+POST treatments provided sufficient *Amaranthus* control, whether or not cover crops were utilized. Another study conducted by Price et al. (2016) observed that winter fallow treatments resulted in reduced palmer amaranth control compared to cover crop treatments, with palmer amaranth control influenced by cover crop biomass. Additionally, Reeves et al.

(2005) found that small grain cover crops in conjunction with PRE herbicides provided similar weed control to PRE+POST herbicide regimes. Such results indicate that cover crops alone will not eliminate the need for weed control, especially when weed pressure is high and cover crop planting is delayed.

Cover Crop Placement - Weed Control Ratings

Overall weed pressure was low throughout the entirety of the cover crop placement trial due to a thorough herbicide regime, which is often necessary under heavy weed pressure (Buhler et al., 1997; Teasdale and Mohler, 2000; Webster et al., 2013). *Amaranthus* control was only influenced by year, with *Amaranthus* control in 2018 lower than both 2017 and 2019 *Amaranthus* control ratings. Control of annual grasses was also numerically lowest in 2018, and 2019 annual grass control was higher in 2017 compared to proceeding years (Table 3.11). Although *Amaranthus* control was significantly lower in 2018, cotton yield was not negatively affected.

There was also an interaction of cover crop treatment and year on annual grass control (Table 3.4). No differences occurred between treatments in 2017 and 2019. In 2018, annual grass control under the WF treatment was lower than that of the RCE-W and R-BR treatments. However, annual grass control was always above 85%. Annual grass control was usually similar between years of the same cover crop treatment. The exception is that annual grass control was lower under WF and CR-W treatments in 2018 compared to 2017 (Table 3.12).

Cover Crop Mixture and Herbicide Timing - Cotton Yield

Cotton lint yield was affected by both year ($P<0.0001$) and herbicide ($P<0.0001$) treatment, with a herbicide by year interaction ($P=0.0195$; Table 3.3). Herbicide-free treatments had significantly lower cotton yield compared to all herbicide-containing treatments, with several individual plots exhibiting no yield potential due to weed pressure. Notably, weed pressure left no yield potential in every herbicide-free plot in 2018. PRE only and POST only treatments were not different from each other and had lower yield than the PRE+POST treatments (Table 3.13). Cotton yield was also higher in 2018 (961 kg ha^{-1}) compared to 2017 (705 kg ha^{-1}) and 2019 (618 kg ha^{-1}).

When evaluating the herbicide by year interaction, cotton yield within the same herbicide treatment did not differ between years. Although not significantly different, there was a yield reduction of more than 250 kg ha^{-1} in the PRE only treatment in 2019 compared to yield in 2017 and 2018. Yield in the PRE+POST treatment was also more than 200 kg ha^{-1} higher in 2018 compared to the other two years. In 2018, the PRE and POST only treatments had similar yields, while POST only treatments had lower yield than the PRE+POST treatments. In-row weed biomass under POST only treatments was also higher than PRE+POST in-row weed biomass in 2018, so early season weed pressure may have inhibited yield potential in POST only treatments, similarly to herbicide-free treatments. In 2017 and 2019, yield did not differ between herbicide-containing treatments, even though PRE+POST treatments always had numerically higher yields.

Findings from previous studies complement these results. A study conducted in Alabama by Duzy et al. (2016) found that in high *Amaranthus* weed pressure, net economic returns were highest under PRE+POST and PRE only herbicide applications when managed with reduced tillage. Such findings indicate that thorough weed management, particularly early-season weed management, is crucial in cotton production systems with substantial weed pressure. A similar study by Aulakh et al. (2012) found that under reduced tillage, PRE+POST herbicide applications were necessary under heavy *Amaranthus* pressure while herbicide-free treatments had cotton yield one-third that of herbicide-containing treatments.

Cover crop treatment did not have an effect on yield (P=0.4495; Table 3.3). However, a study conducted by Reeves et al. (2005) observed reduced cotton yield under a winter fallow PRE+POST herbicide treatment compared to cover crop-containing treatments with the same herbicide regime. Although previous studies have observed yield and weed suppressive benefits from cover crops, results are largely variable and inconsistent, indicating that chemical weed control will not necessarily eliminate the need for preemergent or postemergent herbicides, especially under extensive weed pressure (Webster and Nichols, 2012; Aulakh et al., 2012; Duzy et al., 2016).

Cover Crop Placement - Cotton Yield

Year was the only significant factor to affect cotton lint yield (P<0.0001; Table 3.4). Average cotton yield was highest in 2018 at 1831 kg lint ha^{-1}, which fell well above the Alabama state average of 962 kg ha^{-1} (Webster et al., 2013; Blanco-Canqui et al., 2015; USDA-NASS, 2018; MacLaren et al., 2019). Lint yield was significantly lower in both 2017 and 2019 (Table 3.14). Cover crop treatment did not have a significant effect on cotton yield (P= 0.7009).

CONCLUSIONS

The inclusion of cover crops did not affect weed control or cotton lint yield positively or negatively. Cover crop biomass was generally consistent within the same year with rye-containing cover crop mixtures almost always producing similar biomass to rye monocultures.

In the cover crop mixture and herbicide timing trial, the PRE only herbicide regime controlled *Amaranthus* at a similar rate as the PRE+POST treatments in 2017 and 2019, and weed pressure increased with year when left untreated, in terms of between-row weed biomass. Annual grass control also tended to be similar within the same year under all herbicide-containing treatments, while *Amaranthus* control was more numerically variable. In the cover crop placement trial, cover crop biomass was reduced under non-rye containing treatments and the precision three-species treatment compared to most rye-containing treatments. Weed pressure was also quite low due to the thorough herbicide regime. However, in 2018 annual grass control was reduced under the WF treatment compared to the RCR-W and R-BR cover crop treatments. In the cover crop placement trial, cotton lint yield did not differ between treatments, so differences in weed control were not yield limiting.

Cover crop implementation did not affect cotton lint yield in either trial. Herbicide-free treatments always led to reduced cotton lint yield compared to herbicide-containing treatments, regardless of cover crop treatment. PRE+POST treatments always had numerically the highest cotton lint yields, while PRE only and POST only treatments always had similar yield. Overall, cover crop implementation did not eliminate the need for either PRE or POST herbicides under heavy weed pressure.

Table 3.1 Dates of field operations for the cover crop mixture and herbicide timing trial and the cover crop placement trial.

Operation	2016	2017	2018	2019
Cover crop planting	8-Nov	20-Nov	27-Nov	-
Cover crop termination	-	19-Apr	18-Apr	24-Apr
Cotton planting	-	16-May	10-May	8-May
Cotton harvest	-	14-Nov	4-Oct	7-Oct

Table 3.2 Species composition and seeding rates of cover crop monocultures and mixtures planted in 2017, 2018, and 2019 for the cover crop placement study.

Treatment	Seeding Rate		
	Rye	Crimson Clover	Radish
	Whole-plot		
	——— kg ha^{-1} ———		
Rye (R-W)	100	-	-
Clover-Radish (CR-W)	-	22	9
Rye-Clover-Radish (RCR-W)	50	11	4
	Precision Placements		
	——— kg ha^{-1} ———		
Rye, row-middles (R-BR)	100	-	-
Clover-Radish, in-row (CR-IR)	-	22	9
Rye-Clover-Radish (R-BR/CR-IR)	50	11	4

Table 3.3 Summary of analysis of variance (ANOVA) in the cover crop mixture and herbicide timing trial for In-row weed biomass (Biomass – R), between-row weed biomass (Biomass-RM), *Amaranthus* control, annual grass control, and cotton yield in response to cover crop treatment, herbicide treatment, year, and their interactions.

		ANOVA, Pr > F				
Source of Variance	df	Biomass R	Biomass RM	*Amaranthus control*	Annual grass control	Cotton yield
Cover Crop (C)	2	0.3044	0.3415	0.0978	0.1359	0.4495
Herbicide (H)	3	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Year (Y)	2	0.0001	<0.0001	<0.0001	0.7736	0.0040
C x H	6	0.0977	0.3475	0.0003	0.3587	0.4104
C x Y	4	0.1290	0.1656	0.4971	0.3420	0.2235
H x Y	6	<0.0001	<0.0001	<0.0001	0.1233	0.0195
C x H x Y	12	0.1761	0.9017	0.5585	0.8125	0.1595

Table 3.4 Summary of analysis of variance (ANOVA) in the cover crop placement trials for cover crop biomass (Biomass), *Amaranthus* control, annual grass control, and cotton yield in response to cover crop treatment, year, and their interactions.

Source of Variance	ANOVA, Pr > F				
	df	Cover Crop Biomass	*Amaranthus control*	Annual grass control	Cotton yield
Cover Crop (C)	5	<0.0001	0.8364	0.0899	0.7009
Year (Y)	2	<0.0001	<0.0001	<0.0001	<0.0001
C x Y	10	0.3706	0.9966	0.0833	0.8107

Table 3.5 Effect of year on cover crop biomass measured in the spring of 2017, 2018, and 2019 for the cover crop mixture and herbicide timing trial.

Year	Cover Crop Biomass (*P<0.0001*)
	—— kg ha^{-1} ——
2017	6510 a†
2018	2060 c
2019	4620 b

† Values followed by the same letter are not significantly different according to Tukey's HSD at α= 0.1.

Table 3.6 Effect of cover crop treatment and year on cover crop biomass in the spring of 2017, 2018, and 2019 for the cover crop placement study.

Treatments	Cover Crop Biomass ($P<0.0001$)
	kg ha^{-1}
Rye, whole plot (R-W)	4430 ab†
Clover-Radish, whole plot (CR-W)	3020 bc
Rye-Clover-Radish, whole plot (RCR-W)	4470 ab
Rye, row-middles (R-BR)	5560 a
Clover-Radish, in-row (CR-IR)	2670 c
Rye-Clover-Radish, precision (R-BR/CR-IR)	2650 c
Year	**Cover Crop Biomass ($P<0.0001$)**
	kg ha^{-1}
2017	7250 a
2018	2090 c
2019	3200 b

† Values followed by the same letter are not significantly different according to Tukey's HSD at α= 0.1.

Table 3.7 Effect of herbicide treatment by year on in-row and between-row above-ground weed biomass measured in June of 2017, 2018, and 2019 for the cover crop mixture and herbicide timing trial.

Herbicide Treatment	Weed Biomass					
	Between-row ($P<0.0001$)			In-row ($P<0.0001$)		
	2017	2018	2019	2017	2018	2019
	——— kg ha^{-1} ———					
Nontreated	170 bcd†	340 bc	1490 a	220 bcde	540 abc	1410 a
PRE only	100 d	400 bc	400 bc	120 de	250 bcde	160 cde
POST only	240 bcd	500 ab	120 cd	460 abcd	690 ab	90 ef
PRE+POST	140 cd	190 bcd	20 e	140 cde	130 cde	10 f

† Values followed by the same letter are not significantly different according to Tukey's HSD at α= 0.1. Row positions were analyzed separately.

Table 3.8 Effect of herbicide treatment by cover crop treatment on in-row above-ground weed biomass for the cover crop mixture and herbicide timing trial.

Herbicide Treatment	In-row Weed Biomass ($P=0.0977$)		
	Mixture	Rye	Winter Fallow
	——— kg ha^{-1} ———		
Nontreated	540 ab†	734 a	415 abc
PRE only	247 abcd	113 cd	175 bcd
POST only	196 abcd	519 ab	221 abcd
PRE+POST	82 d	63 d	46 d

† Values followed by the same letter are not significantly different according to Tukey's HSD at α= 0.1.

Table 3.9 Effect of year on between-row and in-row above-ground weed biomass measured in June of 2017, 2018, and 2019 for the cover crop mixture and herbicide timing trial.

Year	Weed Biomass	
	Between-row (*P<0.0001*)	In-row (*P=0.0001*)
	kg ha^{-1}	
2017	160 b†	200 b
2018	350 a	330 a
2019	180 b	120 b

† Values followed by the same letter are not significantly different according to Tukey's HSD at α= 0.1. Row positions were analyzed separately.

Table 3.10 Effect of herbicide by cover crop treatment on percent *Amaranthus* control for the cover crop mixture and herbicide timing trial.

Herbicide Treatment	*Amaranthus* Control (*P=0.0003*)		
	Mix	Rye	Winter Fallow
		%	
Nontreated	20.96 d†	16.54 d	43.60 c
PRE	67.24 abc	80.71 ab	72.53 abc
POST	69.86 abc	59.95 abc	53.42 bc
PRE+POST	91.51 ab	92.17 ab	93.98 a

† Values followed by the same letter are not significantly different according to Tukey's HSD at α= 0.1.

Table 3.11 Effect of year on percent control of *Amaranthus* and annual grasses for the cover crop placement study.

Year	*Amaranthus* control (*P<0.0001*)	Annual grass control (*P<0.0001*)
	%	
2017	96.40 a†	98.70 a
2018	76.30 b	95. 50 b
2019	99.23 a	93. 40 b

† Values followed by the same letter are not significantly different according to Tukey's HSD at α= 0.1. Weed categories were analyzed separately.

Table 3.12 Effect of year by treatment on percent control annual grasses for the cover crop placement study.

Treatments	Annual Grass Control (*P=0. 0833*)		
	2017	2018	2019
	%		
Rye, whole plot (R-W)	97.40 ab†	87.10 c	96.23 abc
Clover-Radish, whole plot (CR-W)	99.88 a	94.94 abc	96.23 abc
Rye-Clover-Radish, whole plot (RCR-W)	100 a	88.58 bc	95.00 abc
Rye, row-middles (R-BR) Clover-Radish, in-row (CR-IR)	97.40 ab	97.47 ab	93.73 abc
Rye, row-middles (D)	100 a	97.47 ab	96.23 abc
Rye-Clover-Radish, precision (R-BR/CR-IR)	98.72 a	96.23 abc	96.23 abc
Rye, whole plot (R-W)	97.47 ab	92.33 abc	95.00 abc

† Values followed by the same letter are not significantly different according to Tukey's HSD at α= 0.1.

Table 3.13 Effect of herbicide by year on cotton lint yield for the cover crop mixture and herbicide timing trial.

Herbicide Treatment	Cotton Lint Yield (*P=0.0195*)		
	2017	2018	2019
	kg ha^{-1}		
Nontreated	342 d†	-	201 d
PRE only	890 abc	914 abc	633 c
POST only	822 bc	791 bc	881 abc
PRE+POST	989 ab	1228 a	895 abc

† Values followed by the same letter are not significantly different according to Tukey's HSD at α= 0.1. Lint yield was calculated at 40% of seed cotton weight.

No yield potential in nontreated plots in 2018.

Table 3.14 Effect of year on cotton lint yield for the cover crop placement trial.

Year	Cotton Lint Yield ($P<0.0001$)
	——— kg ha^{-1} ———
2017	1356 b†
2018	1831 a
2019	1287 b

† Values followed by the same letter are not significantly different according to Tukey's HSD at α= 0.1. Lint yield was calculated at 40% of seed cotton yield.

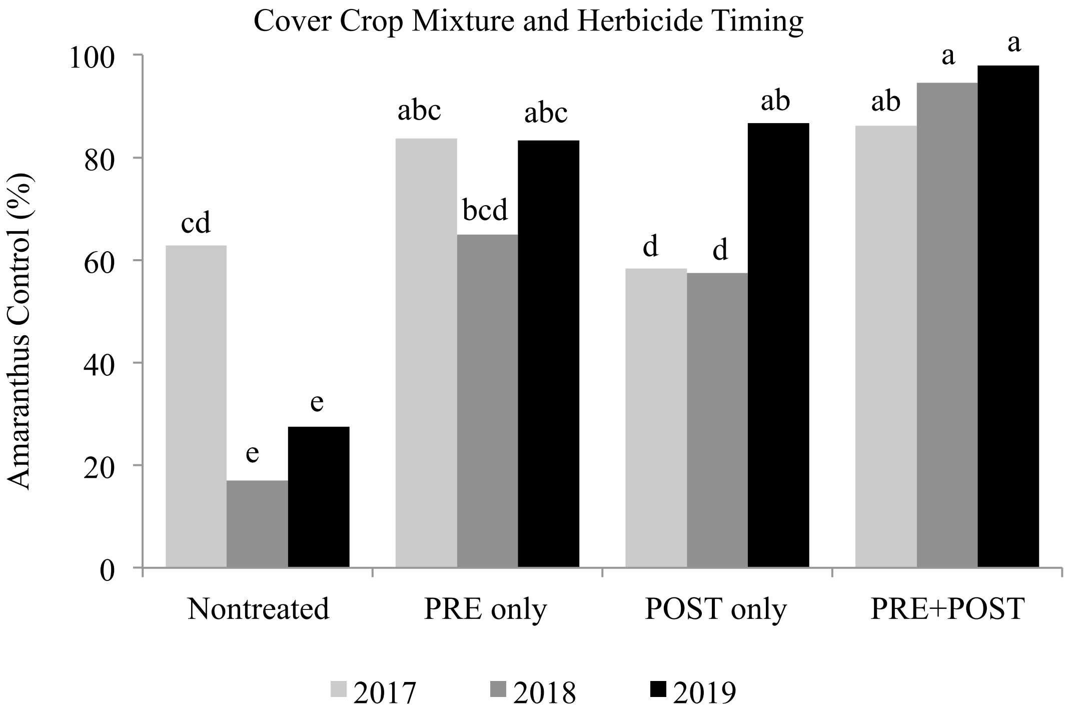

Figure 3.1 Effect of herbicide treatment and year on *Amaranthus* control for the cover crop mixture and herbicide timing trial (P<0.0001).

Note: Values followed by the same letter are not significantly different according to Tukey's HSD at α= 0.1.

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