

WHITE CLOVER LIVING MULCH FOR COTTON: EFFECTS OF VEGETATION-FREE  
STRIP WIDTH ON SOIL HYDROLOGY AND COTTON PRODUCTION

by

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(Under the Direction of Nicholas T. Basinger and Nandita Gaur)

ABSTRACT

The US cotton belt had intensive cotton cultivation leading to destruction of fertile topsoil. Living mulches (LM) are being recommended to sustainably produce cotton. LM-es are cover crops grown within the interrow with cash crops. The objectives of these studies were to optimize LM vegetation-free strip (VFS) width in cotton and measure its short-term impacts on soil physical properties and moisture dynamics. The study was done at two locations; J. Phil Campbell Research and Education Center and Southeast Georgia Research and Education Center, comprising two different soil types and climatic conditions. Results showed 30 cm is optimal VFS width which maximizes cotton yield and LM benefits. LM had limited influence on soil moisture dynamics. LM did not affect soil moisture management zones and physical properties except for bulk density in lighter soils. Thus, a long-term study is required to understand difference between soil health parameters before and after establishing LM.

INDEX WORDS: living-mulch, cotton, vegetation-free strip width, soil moisture management zones.

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## DEDICATION

To Shelly Shome (mother), and Dr. Shreya Some (elder sister), thanks for all the support and being my backbone for this crazy journey. To Stephan Alexander Holzner and Gabriella Holzner, thank you for helping me with housing when I was homeless in Italy for a short period of time. I would have never completed my dual masters without the immense support from you guys.

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“Believe in yourself, you know the art of letting go, just let it go, you will survive and come out victorious.”

- Sambit Shome

I did it- One pandemic, 2 years 8 months of hard work, 2 accidents, 3 continents and Lebenslanger Schicksalsschatz (lifelong worth of memories)!!!!

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## CHAPTER 1

### INTRODUCTION

#### 1.1 Cotton and the Global world: An overview

Cotton is the most important fiber crop in the world. It has nearly 50 species and belongs to the family Malvaceae and genus *Gossypium* (Cotton Acres 2023). Out of the 50 species only four are cultivated around the world. They are *Gossypium hirsutum* L., *Gossypium barbadense* L., *Gossypium herbaceum* L., and *Gossypium arboreum* L. Cotton is grown in 75 countries across the world and provides consumable products like fiber, oil, animal feed, etc. (USDA-NASS 2023). *Gossypium hirsutum* L (Upland Cotton) and *Gossypium barbadense* L. (Pima Cotton) are the two main species cultivated in the United States and around the world due to their high yield and are grown as an annual plant despite being perennial in nature.

The top four global cotton producers China, India, United States, and Brazil contribute to 70-75% of global cotton production, with China and India contributing to approximately 45-50% of the global production (World Population Review 2023). Global cotton production in 2022-2023 is projected at 115.7 million bales, 700,000 bales below the November projection (World Population Review 2023). The United States is the third largest producer of cotton globally and is the world's leading cotton exporter providing approximately 35% of global cotton (USDA-NASS 2023).

## **1.2 Cotton Production in the United States**

Cotton production in the United States fluctuates significantly from previous years to 2022 due to significantly less area harvested in Texas (USDA-Cotton Outlook 2023). In 2022, 14.68 million bales of cotton were produced in the United States, a decrease from 17.5 million bales in 2021. Upland and Pima cotton are predominantly grown in 17 southern US states known as the cotton belt (USDA-NASS 2023). The cotton belt extends from Virginia to California with Texas as the largest producer followed by Georgia, Mississippi, and Arkansas. The estimated harvest area in 2022 was predicted at 7.9 million acres- the lowest since 2013, also 19 % below the 2021 crop (USDA-NASS 2023). The US cotton production in 2023-2024 is projected to be around 15.8 million bales. A higher production is expected from 2023-2024 due to a 20% increase in harvested area (USDA-Cotton Outlook 2023).

## **1.3 Cover Cropping: a potential tool for sustainable cotton production.**

Historically, cotton was cultivated intensively in the US cotton belt leading to the destruction of the fertile topsoil. This resulted in the exploration of innovative management practices to sustainably produce cotton. Of the management practices that have been explored, cover cropping has proven to be an effective method for sustainable production in the US (Kumar et al. 2020). According to the USDA a cover crop is defined as “Crops, including grasses, legumes, and forbs, for seasonal cover and other conservation purposes. Cover crops are primarily used for erosion control, soil health improvement, and water quality improvement. A cover crop managed and terminated according to these guidelines is not considered a ‘crop’ for crop insurance purposes. The cover crop may be terminated by natural causes such as frost, or intentionally

terminated through chemical application, crimping, rolling, tillage, or cutting” (USDA-NCRS 2014). Cover crops can be forages, legumes, cereals, etc. grown asynchronously to cash crops to provide agroecosystem services such as preventing soil erosion (Sarrantonio and Gallandt 2003), minimizing nutrient leaching (Ochsner et al. 2010), increasing water holding capacity (Sanders et al. 2018), increasing soil organic matter (Ginakes et al. 2020), facilitating water infiltration (Chalise et al. 2018), supporting wildlife (Shirley and Janke 2022), promoting beneficial insects (Irvin et al. 2016), and carbon sequestration (Blanco-Canqui 2022).

Most cover crops are grown opposite to cash crops as annuals and are terminated before planting cash crops, are used as green manure, and need reseeding year to year. However, unlike annual cover crops perennial cover crops can persist for multiple years without reseeding. In the US, farmers use a variety of cover crops of which cereal ryegrass (*Secale cereale* L.) and winter wheat (*Triticum aestivum* L.) are the most common cover crops used (USDA-ERS 2022).

Although annual cover crops have numerous benefits, they often do not last the entirety of the season, and if N is fixed it is often released during the initial breakdown of the cover crop.

Living mulches that grow with the cash crop could overcome some of these challenges and require further investigation.

#### **1.4 Living mulch Tradeoffs**

A living mulch (LM) is defined as a cover crop grown within and in between planted cash crops (Freyman 1989). One perennial LM that has been previously investigated for use in corn is white clover (*Trifolium repens* L.) (Andrews et al. 2020; Basinger and Hill 2021; Sanders et al. 2017).

White clover is an ideal perennial LM because it is a low-growing stoloniferous plant, that is fecund and persistent enough to proliferate from year to year, fixing atmospheric nitrogen into

the soil at 56-168 kg ha<sup>-1</sup> (Sanders et al. 2017). It also suppresses weed growth (Weisberger 2022) thereby reducing competition with cash crops (Bhaskar et al. 2021; Ciaccia et al. 2016). LMs also provide other agroecosystem services by reducing herbicide inputs (Sanders et al. 2017), improving water holding capacity (Ochsner et al. 2010), and soil organic matter (Hiltbrunner et al. 2007).

Despite many of the benefits of LMs, there are some tradeoffs associated with their use. LMs can compete with cash crops for resources including water, and nutrients. However, to maximize both yield and ecosystem services provided LM, that establishing a vegetation-free strip (VFS) within the planted row using herbicides would minimize the competition during cash crop establishment (Alexander et al. 2023; Sanders et al. 2018). Due to the variability in competitiveness of crops, VFS widths are not necessarily transferable between cash crops. For corn, the optimal VFS width is 20 cm (Sanders et al. 2017), but the optimal VFS width in cotton, where both cotton yield and LM ecosystem services can be maximized, has not been established.

### **1.5 Impact of living mulch on soil hydrology**

Soil hydrology is quantified using soil hydraulic and physical properties like soil texture, water holding capacity of soil and bulk density (Wang et al. 2022). The effect of soil hydraulic properties manifests in the form of soil moisture dynamics in the field which can be highly variable across scale (Gaur and Mohanty, 2013). Soil moisture is an important factor to be considered when opting for a LM cash crop system. Given that a white clover LM is prostrate and able to fix its own N, competition for light and fertility are often not an issue (Hill et al. 2021). Competition for water tends to be a major aspect to consider when implementing a white clover LM system (Sanders et al. 2018). Research suggests LM displays a varied response to soil

moisture on cash crops than clean tillage by 3.88 % and 5.55 % for the study years of 2013 and 2014 (Tang et al. 2022). A study by the effects of soil moisture and weed suppression over a three-year period when rye is used as a LM suggests that rye cover crops can partially suppress weeds but compete with asparagus for soil moisture in dry years unless irrigation is used (Brainard et al. 2012). Byod et al. 2001 studied the effects of LM on tuber yield of potato with three different types of LM. They were hairy vetch (*Vicia Villosa* L.), ‘Marino’ red clover (*Trifolium pratense* L.), and Kentucky bluegrass (*Poa pratensis* L.). There was no effect of soil moisture in the LM plots as compared to the control. Since, the response of LM in a cash crop LM system is variable with no definite results. Therefore establishing management zones seems to be a viable option to study the relationship between LM and cash crop moisture requirements. Developing soil moisture management zones in a cash crop LM system will integrate site-specific irrigation in such systems. Previous studies in soil moisture management zones by Reyes et al. 2019 suggest soil moisture has spatial and temporal variability over soil health physical properties. Moisture need not be uniform for a homogenous yield. Thus, to have better yield soil moisture management zones should be considered. This opens the opportunity for farmers for site specific management in the peach orchard, aiming at precision agriculture.

This study on cotton addresses the impact of LM on soil physical properties and its consequent effect on the soil moisture dynamics as a function of LM density in the field. These impacts have been assessed in two hydrologically diverse soil types found in Georgia and the southeastern U.S.

## **1.6 Project Scope**

Studies have been done in VFS widths in some row crops like corn (Sanders et al. 2017) and horticultural crops like blackberry (Basinger 2018a) and grape (Basinger 2018b). An optimal VFS width and its impact on soil hydrology and cotton yield has not been established. Previous studies done in corn (Alexander et al. 2023; Ginakes et al. 2018; Sanders et al. 2017) are not transferable to cotton due to the differences in plant physiology and leaf area indexes of both plants. Thus, this study was conducted to determine the optimal VFS width and cotton planting density on soil hydrological factors and cotton yield across two growing regions in Georgia.

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**CHAPTER 2**

**OPTIMIZING LIVING MULCH VEGETATION-FREE STRIP WIDTH IN**

**SOUTHEASTERN US COTTON<sup>1</sup>**

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<sup>1</sup> Shome, S; Gaur, N; and Basinger, NT. To be submitted to [Weed Technology]

## 2.0 Abstract

Cover crop adoption has been increasing nationally due to short-term benefits during the growing season and long-term contributions to soil health. Annual cover crops are often terminated before planting a cash crop, while living mulches grow synchronously with the cash crop during the season's production. White clover (*Trifolium repens L.*), a stoloniferous low-growing perennial living mulch, has successfully been used in corn, but has not been optimized for use in cotton production systems. A study to determine the optimal vegetation-free strip (VFS) width and planting density for cotton production was conducted in 2021 and 2022 in two growing regions of the Georgia. Prior to planting VFS widths of 0, 15, 30, 60, and 90 cm were established. Cotton was planted at either 6.6 or 13.2 seeds  $m^{-1}$  for each VFS width resulting in 10 treatments. Data were collected on cotton plant height, nodes, leaf area index and phenology. Before harvest, 10 cotton plants per plot were selected randomly to evaluate cotton yield parameters (boll  $plant^{-1}$ , lint boll $^{-1}$ , seed cotton yield, lint yield, and HVI index). A 0 cm VFS reduced stand establishment and all growth and yield parameters measured, indicating a VFS must be established and maintained for living mulch use in cotton. Plant height, nodes  $plant^{-1}$ , canopy coverage, leaf area index, and lower light transmittance were observed when a VFS was established. Cotton yield and cotton growth parameters were similar in all VFS widths, except for 0 cm VFS in three of the four site years. Therefore, to maximize yield and living mulch benefits a VFS width of 30 cm VFS width will be optimal for cotton production as it maximizes both agroecosystem benefits imparted by LM and cotton yields.

Nomenclature: White Clover, *Trifolium repens L.*; Cotton, *Gossypium hirsutum L.*

Keywords: cover crop, height, nodes, Leaf-Area-Index, High-Volume-Intensity

## 2.1 Introduction

Cotton (*Gossypium* spp.) is a major global textile commodity. Upland (*Gossypium hirsutum* L.) and Pima (*Gossypium barbadense* L.) cotton are the two main species cultivated in the US and around the world for commercial use. Their principal difference is fiber length, growing conditions, and post-harvest uses. In the US, cotton is predominantly grown in the Cotton Belt which stretches across 17 southern US states, from Virginia to California. The harvested area in 2022 was estimated to be 7.9 million acres, the lowest since 2013, producing 14.2 million bales, 19% below the 2021 crop (USDA-NASS-2023). Efforts to make cotton production more sustainable have included cover cropping which is becoming increasingly popular amongst farmers (USDA-ERS 2022).

According to the USDA a cover crop is defined as “Crops, including grasses, legumes, and forbs, for seasonal cover and other conservation purposes. Cover crops are primarily used for erosion control, soil health improvement, and water quality improvement. A cover crop managed and terminated according to these guidelines is not considered a ‘crop’ for crop insurance purposes. The cover crop may be terminated by natural causes such as frost, or intentionally terminated through chemical application, crimping, rolling, tillage, or cutting” (USDA-NCRS 2014). Cover crops are often grown asynchronously to cash crops to provide agroecosystem services such as preventing soil erosion (Sarrantino and Gallandt 2003), minimizing nutrient leaching (Ochsner et al. 2010), increasing water holding capacity (Sanders et al. 2018), increasing soil organic matter (Ginakes et al. 2020), facilitating water infiltration (Chalise et al. 2018), supporting wildlife (Shirley and Janke 2022), promoting beneficial insects (Irvin et al. 2016), and carbon

sequestration (Blanco-Canqui 2022). Most cover crops are grown as annuals with US farmers using a variety of cover crops species (Kumar et al. 2020). Cereal ryegrass (*Secale cereale* L.) and winter wheat (*Triticum aestivum* L.) are the most common cover crops used for winter hardiness and biomass potential (USDA-ERS 2022). Cover crops are used more frequently in cotton and corn silage compared to corn for grain or soybeans (USDA-ERS 2022). Many federal programs support the adoption of cover crops including the Pandemic Cover Crop Program (PCCP) (USDA-RMA 2022), Conservation Stewardship Program (CSP) (USDA-NRCS 2023a), Environmental Quality Incentives Program (EQIP) (USDA-NRCS 2023b), and state government programs like Cover Crop Initiative (CCI) (USDA-NRCS 2023c) have helped increase awareness about cover crops among farmers, providing financial and technical assistance to adopt them (USDA-ERS 2022). While the adoption rate of cover crops has increased by over 50% from 2012 (10.3 million acres planted) to 2017 (15.4 million acres), cover crops are still not a widely adopted practice across all states (USDA-ERS 2022). Studies suggest that the eastern US has the highest cover crop adoption due to state incentive programs and favorable growing conditions (USDA-ERS 2022). Maryland has the highest cover crop adoption and promotion rate in the eastern US, with other states such as Pennsylvania, Virginia, and Georgia having relatively high levels of adoption as well.

Farmers adopt cover crops depending on their farming goals and cover crop life cycle, habit, growth parameters, and function. Although cover crops can be categorized according to type (legume, grass, forage), active growth period, function, and environmental tolerances such as cold, heat and drought, they are more broadly classified by lifecycle into annual and perennial cover crops. Annual species are planted and terminated before planting a cash crop and need reseeding each year, which can be a financial and time burden each year. Perennial cover crops,

sometimes referred to as perennial living mulches (LM) (Freyman 1989), are persistent from year to year and do not require reseeding. Perennial LMs can be beneficial to producers by reducing seed, planting, and labor costs (Alexander et al. 2019; Sarrantonio and Gallandt 2003).

Both annual and perennial cover crops provide agroecosystem services, but the establishment, maintenance, and in-season crop management present different challenges.

The idea of LMs is not new (Elkins et al. 1983; Enache and Iniki 1990). However, increasing challenges surrounding fertilizer prices and weed resistance has caused a resurgence in interest in implementing these practices on farms. One perennial LM that has been recently investigated for use in summer cash crops is white clover (*Trifolium repens* L. 'Durana') (Andrews et al. 2020; Basinger and Hill 2021; Sanders et al. 2017). White clover is an ideal perennial living mulch because it is a low-growing stoloniferous plant, that has high levels of fecundity, fixes atmospheric nitrogen, suppresses weed growth, and is persistent enough to proliferate from year to year (Weisberger 2022). Another type of clover, kura clover is grown in colder climates.

Previous research has demonstrated that legume LM systems can sequester atmospheric nitrogen at a rate of nearly 56 to 168 kg ha<sup>-1</sup> into the soil depending on the edaphic factors and the environmental conditions (Andrews et al. 2018; Mulongoy and Akobundu 1990). This ability to sequester nitrogen can reduce N fertilizer inputs, while LM biomass reduces weed seed germination and weed competition with the cash crop (Bhaskar et al. 2021; Ciaccia et al. 2016; Weisberger 2022). Furthermore, LM provides additional benefits by reducing herbicide inputs (Sanders et al. 2017), improving water holding capacity (Ochsner et al. 2010), and soil organic matter (Hiltbrunner et al. 2007).

Despite the many benefits of living mulch, there are trade-offs associated with its use; mainly that it can compete with the cash crop for resources, specifically moisture (Brainard et al. 2012;

Sanders et al. 2018). To reduce interspecific competition between the LM and the cash crop, a vegetation-free strip (VFS) is established prior to planting using herbicides (Table 2.1), into which the crops can be planted. The width of this VFS can alter interspecific competition between the ground cover and cash crop and affect plant growth and yield and has been studied in perennial crops such as grapes (Basinger et al. 2018a), blackberries (Basinger et al. 2018b), and peaches (Buckelew et al. 2018). However, limited research has been conducted on VFS width in annual row crops. Research using white clover LM in corn in Georgia determined that a VFS width of 20 cm and between row spacing of 90 cm suppressed clover enough for the corn to establish, while interrow widths were wide enough to optimize clover growth and productivity (Sanders et al. 2017). Other studies in Midwestern row crop systems have integrated kura clover as a LM in corn cultivation while establishing 10 cm or 30 cm VFS widths in combination with banded N fertilizer applications. The 10 cm VFS width when combined with any form of banded N fertilizer successfully managed to integrate kura clover in the midwestern corn system (Alexander et al. 2023).

Soil has been an important factor to study a cash crop LM system. Previous research conducted in the southeastern US has primarily occurred on heavier clay soils. (Sanders et al. 2017; Hill et al. 2021) However, in the southeastern US much of the agricultural production is conducted on lighter sandy loam or loamy sand soil types. Thus, further research needs not only determine VFS widths for cotton but also explore the feasibility of the white clover LM system in production areas where lighter soils are commonplace. The objective of this study is to examine combinations of VFS widths and cotton seeding rates to optimize cotton production in a perennial white clover LM system across two different edaphic regions in Georgia.

## 2.2 Material and Methods

The study was conducted from November 2020 to November 2022 at the J. Phil Campbell Research and Education Center (JPCREC) near Watkinsville, GA (33°52'3" N 83° 26'58" W, 239 m elevation), and at the Southeast Georgia Research and Education Center (SEGREC) near Midville, GA (33°87'4" N, 82°21'4" W, 79 m elevation). The temperature variation of both the study locations are described in the appendix and the end of this chapter. The soil at the JPCREC is Cecil sandy loam (Fine, kaolinitic, thermic Typic Kanhapludults), whereas the soil at the SEGREC is a Dothan sandy loam (Fine-loamy, kaolinitic, thermic Plinthic Kandiudults). These two soils are representative of the major crop production regions of the southern piedmont and southern coastal plain regions of the state. The details of field operations for both years and locations are stated in Table 2.1. To initially establish the LM, the area was first disked and cultipacked, followed by sowing of white clover 'Durana' at 9 kg ha<sup>-1</sup> using the small seed-box on a Great Plains no-till drill, with 19 cm between planting coulters (1525 E. North Street Salina, KS 6740). After seeding, the planted area was cultipacked to ensure seed soil contact following methods by Andrews et al. (2018). The clover was allowed to be established under natural environmental conditions until plot and VFS establishment. UGA extension recommendation for fertilizer application was followed at both locations and years except the application of nitrogen (Hand et al. 2022). Only potassium and phosphorous amendments were applied using Shindaiwa RS76 walk-behind rotatory spreader (400 Oakwood Road Lake Zurich, IL 60047) to each plot before cotton planting at 67.25 kg ha<sup>-1</sup> and 22.4 kg ha<sup>-1</sup> respectively. Previous research suggests that nitrogen fixed by a white clover LM under similar conditions is 43-105 kg ha<sup>-1</sup> (Andrews et al. 2018). This meets cotton's requirement of nitrogen at the study locations (Hand et al. 2022). Therefore, no external N fertilizer was applied at either study location.

The experimental design was a randomized complete block with a factorial arrangement of treatments having four replicates at each location. The first factor of VFS width included widths of 0, 15, 30, 60, and 90 cm, where 0 cm represented planting directly into growing clover and 90 cm represented current no-till bareground production. The second factor were two seeding rates (6.6 seeds  $m^{-1}$  (73,333 seeds  $ha^{-1}$ ) and 13.2 seeds  $m^{-1}$  (146,666 seeds  $ha^{-1}$ )) which correspond to the upper and lower limits of seeding rates for the region (Hand et al. 2022). Plots had four rows with the center two serving as the data collection rows. The study site at SEGREC remained in the same location over both years with plot sizes of 9.9 m x 3.6 m. Plot dimensions at JPCREC were 10.39 m x 3.74 m in 2021 and 7.79 m and 3.62 m in 2022. In the Fall of 2022, a center pivot irrigation system was installed at the JPCREC location, which would no longer irrigate plots established in 2021. Therefore, plots were re-established in 2022 in the same field, 30 m west of the original plots. As a result, on the 24 November 2021, white clover was planted in a new location, and plots were established using the rates and methods previously described. Bareground plots were established shortly after clover establishment (Table 2.1) using a 4-nozzle  $CO_2$  powered backpack sprayer with Teejet Technologies 8002VS nozzles (1801 Business Park Drive, Springfield, IL 62703) apply glyphosate at 1.26 a.e.  $kg\ ha^{-1}$  at 289 kPa and a spray volume of 280 L  $ha^{-1}$  to emerged clover seedlings. Two to three weeks prior to planting, VFS treatments were established using a tank mix of glyphosate (1.26 a.e.  $kg\ ha^{-1}$ ) and diglycoamine salt of dicamba (0.81 a.e.  $kg\ ha^{-1}$ ) at 289.58 kPa and a spray volume of 280 L  $ha^{-1}$ . All herbicide applications were made with a tractor mounted 3-point hitch sprayer outfitted with QJ365C nozzle bodies to allow for ease of switching between nozzles (Figure 2.1; Table 2.1). VFS treatments were established by rotating every other nozzle body to the off position and applying herbicides only in-row where the cotton would be planted. A 6504EVS on 45 cm aluminium

drop pipes was used to establish the 15, 30 cm VFS widths and a 6504 EVS attached directly to the boom for 60 cm VFS width treatments. Broadcast applications using all nozzle bodies and 8004 VS nozzles within the planted area were used in the 90 cm VFS. No herbicide was applied in the 0 cm VFS width.

DP1646B2XF was planted using a John Deere MAXEMERGE 2 (John Deere, One John Deere Place, Moline, IL61265) at both locations and years (Table 2.1). The planters were set up for no-till planting with row cleaners, ahead of the disk openers, to assist with residue in the planted row, and cast-iron press wheels to ensure seed-soil contact. Plots designated for one seeding rate were planted, the planter adjusted, and then the remaining plots were planted in a separate pass. There was poor emergence at SEGREC in 2021 in some plots as there was a 7.6 mm rainfall event immediately after planting which caused field flooding and some seeds to be washed out of the furrow and herbicide injury to some emerging cotton seeds. Therefore, seeds were replanted manually in plots with poor emergence (Table 2.1). Cotton at JPCREC was re-planted in 2022 due to extreme dry field conditions at planting in 2022 (Table 2.1) resulting in a poor stand.

Irrigation was applied according to the UGA extension recommendations using the cotton irrigation checkbook method (Hand et al. 2022) which is calibrated for bareground cotton production. Reel irrigation was used for JPCREC 2021 and center pivot for SEGREC in 2021 and 2022. In the Fall of 2021, a center pivot irrigation system was installed at JPCREC and was used for irrigation during the 2022 season.

Herbicides were applied three times during the growing season. At planting as a preemergence application (PRE), at the three-leaf cotton stage early postemergence (POST1) and second post

emergent application (POST2) applied two weeks after POST1. The list of herbicides used in this study and their respective timing of application are shown in Table 2.1 and 2.2.

Stand counts (Equation 1) were done on three leaf cotton to determine the effect of VFS width and seeding rate on stand establishment. Plants from the center two data rows of the four-row plots were counted using clicker counter. Stand establishment was calculated by the following formula:

$$\text{Stand establishment} = \frac{\text{Emerged Cotton Plants}}{\text{Number of Seeds Planted}} \times 100\% \quad [\text{Equation 1}]$$

To ensure uniform measurements for data collected bi-weekly, a one-meter section of row within the data row was flagged for each plot. Ten plants were randomly selected from within this 1 m to collect plant heights and count node number. Soil Plant Analysis Development (SPAD) (Apogee® Instruments, 721 West 1800 North Logan, UT 84321, United States of America) readings from the first true leaf were taken from five plants randomly within the data rows and then the average value was reported. It measures the chlorophyll concentration of plants in absolute units ( $\mu\text{mol m}^{-2}$ ). Leaf area index (LAI) was collected using Accupar LP-80 (Meter® Environment, 2365 NE Hopkins CT., Pullman, WA 99163). LP-80 baseline readings were taken above the cotton canopy to establish ambient PAR. Eight readings below the cotton canopy (below PAR) were taken perpendicular to planted cotton row to determine LAI and below canopy PAR. Data for PAR and LAI were collected at each timing between the hours of 10 am and 2 pm when the sun was at its maximum height and minimize variability between measurement timings. Finally, Transmittance (%) (Equation 2) was calculated to estimate the amount of light penetrating through the cotton canopy to the clover canopy. Transmittance (%) was calculated using the following formula:

$$\text{Transmittance (\%)} = \frac{\text{Below PAR}}{\text{Ambient PAR}} \times 100 \quad [\text{Equation 2}]$$

Before harvest, 10 plants were chosen randomly within the data rows to evaluate cotton yield parameters. Cotton plants were cut at the soil surface using a set of hand bypass pruners (A.M. Leonard, Inc., 241 Fox Drive, Piqu, Ohio 45356). Bolls on each plant were counted and seed lint was removed by hand and used to calculate yield parameters on a per boll basis (total number of bolls plant<sup>-1</sup> seed lint boll<sup>-1</sup> (Equation 3), seed boll<sup>-1</sup> (Equation 4), lint per boll<sup>-1</sup> (Equation 5). Hand harvested seed lint was weighed and then ginned using a 12-saw table-top gin. Seeds were retained to determine seed number boll<sup>-1</sup> and turnout. Turnout (%) was calculated using Equation 6. Lint from ginned samples were weighed to determine lint yield boll<sup>-1</sup> for each treatment. A 10 g lint sample treatment<sup>-1</sup>, and location was sent to the Cotton Inc. Product Evaluation Lab (Cotton Incorporated, 6399 Weston Pkwy, Cary, NC 27513) for High Volume Instrument Testing (HVI) analysis.

Seed boll<sup>-1</sup> was calculated by the following formula:

$$\text{Seed boll}^{-1} = \frac{\text{Seed number (10 plants)}}{\text{Number of bolls harvested (10 plants)}} \quad [\text{Equation 3}]$$

Seed lint boll<sup>-1</sup> was calculated by the following formula:

$$\text{Seed lint boll}^{-1} = \frac{\text{Seed lint (10 plants)}}{\text{Number of bolls (10 plants)}} \quad [\text{Equation 4}]$$

Lint boll<sup>-1</sup> was calculated by the following formula:

$$\text{Lint boll}^{-1} = \frac{\text{Ginned lint (10 plants)}}{\text{Number of bolls (10 plants)}} \quad [\text{Equation 5}]$$

Turnout was calculated by the following

$$\text{Turnout (\%)} = \frac{\text{Ginned lint yield}}{\text{Seed cotton yield}} \quad [\text{Equation 6}]$$

Cotton was picked the same day as the hand harvest using a Case 1822 two-row spindle picker (Case IH, 766 Pine Street, Pinehurst, GA-31070) equipped with bagging attachments for small

plot harvest. Hand-harvested seed lint yield amounts were added to the machine-harvested yield data to calculate yield ha<sup>-1</sup>.

Statistical analysis and graphs were conducted using JMP® Pro 16.0.0 (100 SAS Campus Drive Cary, NC 27513). To determine how to treat VFS width, data were subjected to linear and non-linear Analysis of Variance (ANOVA). Data were determined to be not normally distributed using Levine's test and were square-root and log-transformed. Even after applying transformations data did not fit linear or non-linear models and is therefore treated as a categorical variable. ANOVA was performed on biweekly data and harvest parameters using the mixed procedure with VFS width, seeding rate and their interaction, as fixed effects and location, year, and their interaction as random effects. Means of biweekly and harvest parameters were separated using the Tukey-Honestly Significant Difference (HSD) post-hoc test at  $\alpha \leq 0.05$ .

## **2.3 Results and Discussion**

VFS width and seeding rate for biweekly measurements were significant for height, and nodes plant<sup>-1</sup>. VFS width and seed rate interaction for harvest parameters were significant only for seed lint boll<sup>-1</sup>. Location and year interaction was non-significant for both biweekly measurements and harvest parameters.

### **2.3.1 Establishment and growth parameters**

At JPCREC 2021 (Figure 2.2a) and SEGREC 2022 (Figure 2.2d), stand counts were similar across all VFS widths. At JPCREC 2022 (Figure 2.2b), 0 cm VFS had the lowest stand count, compared to 15, 30, 60, and 90 cm which had similar stand counts. At SEGREC 2021 (Figure 2.2c), 0 cm had the lowest stand count and 90 cm highest, while 15, 30, and 60 cm VFS were reduced compared to the 90 cm width. A third of the plots at SEGREC 2021 were hand planted

due to heavy rainfall after planting. This had no effect on stand establishment as stand reductions for 0 cm VFS width were the result of clover stolons being hair-pinned in the furrow preventing the press wheels from properly closing the furrow and reducing seed to soil contact. Dead stolons where a VFS was established using herbicide allowed for furrow closure resulting in a stand similar to that in the bareground plots. Therefore, the stand counts were reduced when no VFS was established, indicating the need to establish a VFS prior to planting. Another study on soybean reported that living mulch reduces cooler soil temperatures, cause excessively dry or wet soils, cause poor seed to soil contact, and difficulty in applying starter fertilizers (Zinati et al. 2017).

Cotton plant height (Figures 2.3 and 2.4) increased throughout the season for all treatments and was affected by VFS width and seeding rate for each location and year. The 0 cm VFS width had the lowest height for both locations, years, and seeding rates compared to the other studied VFS width (Figures 2.3 and 2.4). The height of plants at 90 cm VFS width was the highest for both locations, years, and seeding rates from 4 to 15 WAP, compared to 15, 30, and 60 cm VFS width which had similar heights (Figure 2.3 and 2.4.4). However, from 16 to 21 WAP there was no difference in height amongst the VFS width except 0 cm VFS which was the lowest compared to the other VFS widths. In addition to poor stand establishment, plants emerging in the 0 cm VFS width were subjected to interspecific competition with clover as opposed to 15, 30, and 60 cm VFS width where cotton was planted in the VFS, and competition was limited during cotton establishment. However, starting 15 WAP as the cotton canopy becomes established and the clover becomes less competitive later in the season, cotton in the 15, 30, and 60 cm VFS catches up to cotton grown without LM in the 0 cm VFS. The interspecific competition between the

cotton and clover is most likely responsible for the height difference between the studied VFS widths.

Similar cash crop responses to competition with LMs have been observed. Corn grown in a white clover LM system in Georgia was shorter in height than corn grown in the conventional bareground system (Sanders et al. 2017). A similar study on intercropping green bean (*Phaseolus vulgaris* L.) with cotton in conventional and no-till straw mulch conditions reported that cotton plant height increased significantly by 18.4% across mulching treatments under no-till and conventional tillage (Adil et al. 2023). However, the use of Barbados nut (*Jatropha curcas* L.) as an organic mulch in wheat growth under water-stressed conditions resulted in a decrease in wheat height due to water stress as Barbados nut competed with wheat for water (Irshad et al. 2021).

Corresponding to increase in plant height nodes plant<sup>-1</sup> (Figure 2.5 and Figure 2.6) increased throughout the season for all treatments but was affected by VFS width and seeding rate for both locations and years. The nodes plant<sup>-1</sup> at 0 cm VFS width were reduced from 4-15 WAP at both locations, years, and seeding rates compared to the other VFS widths studied. At SEGREC 2022 13.2 seeds m<sup>-1</sup>, 0 cm VFS width was lowest throughout the season. However, from 16 to 21 WAP the number of nodes for cotton in the 0 cm VFS width was similar to other VFS widths in the study. The 90 cm VFS width had the greatest number of nodes plant<sup>-1</sup> when compared to 15, 30, and 60 cm VFS from 4 to 8 WAP for both seeding rates, year, and location, except SEGREC 2021 6.6 seeds m<sup>-1</sup>. The 15, 30, and 60 cm VFS width comparatively had similar number of nodes throughout the season for both location and seeding rate with some decreasing differences in nodes plant<sup>-1</sup> at SEGREC 2022 for 6.6 seeds m<sup>-1</sup> from 9 to 15 WAP. Cotton being a perennial plant will have indeterminate growth which will result in more vegetative growth and less

reproductive growth. LM will reduce the plant height as a result of competition and will help to transition into reproductive growth phase faster. Studies have suggested that a link between cotton height on nodes plant<sup>-1</sup> (Kerby et al. 1997). This is also known as height to node ratio (HNR) which can be affected by factors like environmental stress. Other studies on cotton growth parameters in conventional and ultra-low spacings of 101.6 cm, 76.2 cm, 38.1 cm, and 19 cm, where cotton was under more competition, reported a positive linear difference between height and nodes plant<sup>-1</sup>. The higher the plant spacing the greater the nodes plant<sup>-1</sup> (Jost and Cothern 2000). However, towards the end of season, nodes plant<sup>-1</sup> were significantly similar across all studied spacing except the ultra-narrow-row spacing of 19 cm. In present study, the relationship between height and nodes were similar in the present study where plants under the most competition were shorter and had fewer nodes. This effect could be beneficial to producers by controlling some rank growth during the season and result needing fewer growth regulator applications in-season without affecting yield (Weisberger 2022).

In addition to crop growth parameters, LAI (Figure 2.7) can often be tied to plant size and development. Non-destructive LAI values increased across the season in a manner similar to height and nodes plant<sup>-1</sup>, and in all treatments, were affected by VFS width, year, and location. At both locations, the 0 cm VFS width had the lowest LAI throughout the season due to low plant establishment in VFS widths. By 4 to 8 WAP the LAI index of 0 cm VFS width was similar to other VFS widths due to overall plant size and phenology. The 90 cm VFS had the greatest LAI compared with other VFS widths throughout the season for both locations and years. Comparatively it was also the treatment that had the greatest height and nodes plant<sup>-1</sup> and hence the highest canopy coverage. At JPCREC 2021 (Figure 2.7a), 15, 30, and 60 cm VFS had similar LAI values from 6 to 10 WAP. However, the LAI values of 15, 30, and 60 cm VFS width

were similar to 90 cm VFS width from 12 WAP to 21 WAP. At JPCREC 2022 (Figure 2.7b), at 7 WAP VFS has similar LAI values however, from 10 to 13 WAP 90 cm had the greatest LAI followed by 60, 30, 15 and then 0 cm VFS. At the end of season all VFS widths had similar LAI except for 0 cm which had the lowest LAI. At SEGREC 2021 (Figure 2.7c) and 2022 (Figure 2.7d), all VFS widths had similar LAI values throughout the season except for the 0 cm VFS width. In corn grown in LM it was reported that LAI was not different between corn seeding rates and VFS width. LAI is a salient parameter in plant growth and development. It estimates the amount of photosynthetically active leaf area as well as the leaf area subjected to transpiration. Greater LAI will have a greater canopy coverage and greater LAI index.

In a LM system, LAI is not only important for cash crop growth and reproduction but also affects the growth of the clover. When a crop canopy is dense, and light does not make it to the ground clover persistence and growth can be reduced (Sanders et al. 2018). Transmittance (%) (Figure 2.8) was the significantly greatest for 0 cm VFS width across all locations and years compared to other treatments. Two exceptions were JPCREC 2022 and SEGREC 2022 from 6 to 9 WAP where the transmittance values were similar to other VFS widths. At JPCREC 2021, 90 cm had the lowest transmittance value followed by 15, 30, and 60 cm. The 90 cm had the greatest height and larger canopy cover when compared to other VFS widths. This data corresponds with data for plant heights, nodes plant<sup>-1</sup>, and LAI. At JPCREC 2021, 90 cm VFS had the lowest transmittance from 4 to 11 WAP, however, 12 to 20 WAP the transmittance values were similar across 15, 30 and 60 cm VFS widths. At SEGREC 2021, 15, 30, 60 and 90 cm VFS widths had similar transmittance values across the season. At JPCREC 2022, at the beginning of the season all the VFS widths had similar transmittance values, but from 9 to 11 WAP 90 and 60 cm had the lowest transmittance values. From 15 to 21 WAP 15, 30, 60 and 90

cm VFS had similar transmittance values as the cotton canopies became more established. Similar studies in corn report no effect on canopy light interception on row spacing and population density but shading on LM occurred at 40 % of ambient light intensity i.e., when corn plants were 100 cm tall (Sanders et al. 2017). In the same study, as increased shading throughout the season reduced clover biomass. As the clover dies back over the season, N is released from the degrading biomass making it available for plant uptake (Andrews et al. 2018). This may explain why cotton growth parameters lag during the season but eventually become similar later in the season. In this study, the crop nitrogen status was evaluated with the help of SPAD. SPAD values were assessed to determine the effects of the VFS widths and seeding rate effects on cotton. SPAD (Figure 2.9) values increased for all treatments throughout the season, and were affected by VFS width, year, and location but not the seeding rate. The 0 cm VFS width had the reduced SPAD value throughout the season for SEGREC 2021 (Figure 2.9c) compared to treatments with an established VFS, except at 4 to 6 WAP where it was similar with other VFS widths. For JPCREC 2022 (Figure 2.9b), 0 cm VFS width had similar SPAD values when compared to other VFS widths at 4 to 11 WAP but from 12 to 21 WAP, 0 cm had the lowest SPAD values amongst other VFS widths. SPAD values of 15, 30, 60, and 90 cm were similar throughout the season for JPCREC 2021 (Figure 2.9a) and 2022 (Figure 2.9b) and SEGREC 2021 (Figure 2.9c) and 2022 (Figure 2.9d) and were lowest for the 0 cm VFS width. However, higher SPAD values are not always correlated with higher yield since a SPAD meter measure the relative greenness at a specific time point (Kerby et al. 1997). The readings can therefore be used in detecting N deficiencies rather than making accurate predictions about the quantity of N fertilizer needed by a crop during a growing season. Therefore, 0 cm VFS width during the 4 to 8

WAP had similar SPAD values compared to other VFS widths which indicates that LM was sufficient in providing with required N.

### ***2.3.2 Yield and yield parameters***

VFS width positively affected yield parameters (number of bolls plant<sup>-1</sup>, seed cotton yield plant<sup>-1</sup>, cotton lint, seed boll<sup>-1</sup>, seed lint boll<sup>-1</sup>, turnout, fiber upper mean length, and fiber elongation) for all treatments by year and location but not seeding rate. Data are therefore reported by VFS width and year combined over seeding rate. Seed lint boll<sup>-1</sup> was affected by VFS width, seeding rate, location, and year. At JPCREC 2022 there was an early frost in the month of October which resulted in earlier application of defoliator. This resulted in lesser yield at JPCREC 2022 when compared to 2021.

Seed cotton yield (Table 2.3) was similar across all VFS widths for JPCREC 2022 and SEGREC 2022. At SEGREC 2021, 90 cm VFS had the greatest seed cotton yields compared to other VFS widths, while yields were similar across all VFS widths JPCREC 2021 excluding 0 cm. In three of the four site-years seed cotton yield in the 15, 30, and 60 cm VFS yielded similar to the bareground, 90 cm VFS, treatments.

To better understand the effects of VFS width on yield and its parameters, data were examined on a per-boll basis. The number of bolls plant<sup>-1</sup> were similar across all VFS widths at SEGREC 2022 (Table 2.3). At JPCREC 2021 and SEGREC 2021, 0 cm VFS had the lowest number of bolls plant<sup>-1</sup>, compared to other VFS widths which had a similar number of bolls plant<sup>-1</sup>. This reduction in boll number corresponds to the reduced overall plant size seen in the 0 cm VFS (Figure 3.1). At JPCREC 2022, 0 cm VFSW has the lowest number of bolls plant<sup>-1</sup>. The 15 cm and 30 cm had the highest bolls plant<sup>-1</sup> when compared to the 90 cm and 0 cm treatments. Other

studies examining ultra-narrow widths of reports that yield in narrow row widths of 19 and 38.1 cm were higher than the wide rows of 76.2 and 101.6 cm (Jost and Cothorn 2000).

Seed lint boll<sup>-1</sup> (Table 2.4) was similar for all VFS widths for JPCREC 2021 and 2022 and SEGREC 2022. At SEGREC 2021 for 6.6 seeds m<sup>-1</sup>, 0 cm VFS had the lowest seed lint boll<sup>-1</sup> as compared to other VFSs' which had similar seed lint boll<sup>-1</sup>. However, for 13.2 seeds m<sup>-1</sup>, all the VFS widths had similar seed lint boll<sup>-1</sup>. This can be attributed to no difference among studied VFS widths across both locations and years except 0 cm VFS width at SEGREC 2021 for 6.6 seeds m<sup>-1</sup>. No distinct advantage was seen overall by increasing the seeding rate to 13.2 seeds m<sup>-1</sup>. Therefore, it is recommended the lower seeding rate of 6.6 seed m<sup>-1</sup> be used in a LM cotton production system in southeastern USA as it will lower the seed input cost (USDA-AMS 2022). For cotton lint and seeds boll<sup>-1</sup>, JPCREC 2021, 2022 and SEGREC 2022 all the VFS width have similar lint boll<sup>-1</sup> (Table 2.3). At SEGREC 2021, 0 cm VFS had the lowest lint boll<sup>-1</sup> and seeds boll<sup>-1</sup> while the other VFS width had a similar lint boll<sup>-1</sup> and seeds boll<sup>-1</sup>. In addition to lower yields, smaller plant sizes, and lower lint boll<sup>-1</sup> in the 0 cm VFS width, seeds boll<sup>-1</sup> (Table 3) for 0 cm VFS width was lowest only at SEGREC 2021. Seeds boll<sup>-1</sup> remained unaffected for JPCREC 2021 and 2022 and SEGREC 2022. Therefore, a 30 cm VFS width LM cotton system yields similar yield when compared to conventional cotton yields.

In addition to other cotton yield parameters turnout (%) was affected by VFS width (Table 2.3) at JPCREC 2021 and SEGREC 2022, all the VFS widths had similar turn out as there was no difference in seed cotton yield and lint boll<sup>-1</sup> amongst the studied VFS widths at JPCREC 2021 and SEGREC 2022. The average turnout (%) for JPCREC 2021 was 55.60% while for SEGREC it was 45.00%. The 0 cm VFS width had the lowest turnout for 0 cm for SEGREC 2021 (14.53%) and JPCREC 2022 (19.09 %), whereas 15, 30, 60, and 90 cm VFS width had similar

turnout of 40.78 % (JPCREC 2022) and 47.46 % (SEGREC 2022) as it had the lowest seed cotton yield and lint yield. At JPCREC 2022, 15 cm had the highest turnout when compared to 30, 60, and 90 cm VFS. The average turnout that a farmer should expect is between 35-40%. However, turn out in tabletop gin is considerably higher (Cotton INC 2023). Other studies on pima cotton studying the effects of plastic mulching and non-mulching on yields reported a 38% higher turnout than non-mulched plots which helped farmers to increase profits by \$450 per acre (Feres and Goldhamer 1991).

Ginned cotton lint from the 10 hand-harvested plants were subjected to High Value Instrument (HVI) analysis. Short fiber index, micronaire, and uniformity strength were unaffected by any treatment combination. Fiber upper half mean length and elongation (Table 2.5) were different amongst VFS widths for both locations and years indicating that using a LM cotton system have minimal effects on fiber quality. Another study on cotton fiber analysis in a cotton intercropped with a legume crop reported a decrease in lint yield and fiber quality (Hulugalle et al. 2001) whereas in our study we have found no difference in fiber quality amongst the VFS widths and comparatively similar yields. Fiber elongation was similar across all VFS widths for both locations and years except JPCREC 2022. At JPCREC 2022, 0, 15 and 30 cm VFS width had the lowest fiber elongation as compared to 60 and 90 cm VFS width. The fiber elongation between 30 and 60 cm were also comparatively similar as other studied VFS widths. Another study examining combinations of different types of tillage, cover crops, and N fertilizer reported no difference in fiber quality amongst plants grown in different treatments (Bouquet et al. 2004), which is similar to the results of this study which has shown no significant difference in cotton fiber elongation amongst the VFS width at both locations and years except JPCREC 2022 where there were differences between 0, 15, 30 cm VFS and 30 and 90 cm VFS.

This study examined cotton growth and yield parameters to establish an optimal VFS width and seeding rate in a perennial white clover living mulch. Thus, a recommendation of VFS width of 30 cm is recommended. A VFS width of 30 cm allows for cotton to maximize its productivity, as 3 of 4 site years a 30 cm VFS had similar yields to the bareground system. A VFS of 30 cm also allowed for light to be transmitted through the canopy allowing for clover persistence while still developing a robust enough plant to maximize yields. Furthermore, a 30 cm VFS allows for two-thirds of the field to continue to be covered by the clover. Thus, this study recommends an optimal VFS width of 30 cm for cotton grown in south-eastern US, as it will be wide enough for the cotton to grow without being in interspecific competition with the clover, thereby maximizing both yield from cotton and agroecosystem services from LM. The 15 cm VFS, and 60 cm VFS both provided similar yield results but present other trade-offs. The 15 cm VFS if not properly established can result in reduced stands if the establishment of the VFS and the planter pass do not line up due to field variability. This can be addressed with the use of GPS equipment but should be considered. The 60 cm VFS allows for cotton to perform similarly to the bareground (0 cm) control. However, the full benefit of the clover is minimized given that it only occupies one-third of the remaining field space. This could result in decreased clover stand persistence and reduced benefits (N fixation, water infiltration, improving soil organic matter). Therefore, a 30 cm VFS optimizes both the growth and yield of the cotton, and the potential benefit of integrating LM white clover into the system.

Future research in the area of living mulches should investigate additional ways to manage weeds within the interrow area of the field. In areas where the clover stands were thin, some weeds were able to grow through the LM resulting in some plots being relatively weedy. Although the clover was grown in two soil types in this study the clover did not grow and

reduced weeds as well as on the coastal plains soil, leading to late season weed challenges like hand weeding in plots. Investigating early season or layby applications that are safe for the clover and suppress weeds in the interrow would be beneficial to further understanding this system. Furthermore, examining additional benefits of the LM system long-term to determine contributions to soil health measures, and cycling of nutrients would be fruitful areas of research. Overall, the LM system provides benefits to cotton production in the Southeastern US.

## **2.4 Practical implications**

This study was conducted in two diverse soil types and climatic conditions in the southeastern US. The heavier soils of the southeastern Piedmont and the sand to loamy sand soils of the southern coastal plain. In the southeastern US, the majority of row crops are grown in the sandy soils of the coastal plain. Clover grown in sandy loam soils was more difficult to maintain throughout the season compared to clover planted on heavier clay soil. Therefore, white clover living mulches may be easier to maintain long term as part of a production system when on heavier clay or high organic matter soils. LM was able to replace the nitrogen fertilizer need with atmospheric nitrogen fixed by LM. With actively growing plants in the LM at planting soil moisture at this time is critical to ensure furrow closure. Supplemental irrigation of 1cm prior to planting heavier soils is necessary if there has been no rain within 48 hours of cotton planting. LM serves as an excellent ground cover for weeds in clayey soils however in sandy soils it's very hard to maintain a LM ground cover throughout the season. In this research, a 30 cm VFS width is optimal as it maximizes both cotton yield and benefits from clover. Planting directly into clover resulted in significantly reduced stands, smaller cotton plants, and very poor yields, indicating that a VFS width must be established to mitigate stand loss. Additionally, increased

seeding rates did not improve any of the measured data and planting rates of 6.6 seeds m<sup>-1</sup> was sufficient to achieve similar yields to bareground stands. This will help farmers to reduce the cost of production. Cotton yields in a LM system are similar when compared to conventional grown irrigated cotton. Therefore, farmers should consider LM if they are comfortable with cover crops, have irrigation, and are on soil types that have been shown in this study to support the LM clover with cotton.

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## Tables

Table 2.1: Field operations at JPCREC and SEGREC 2020-2022

Field Operations	Location					
	JPCREC			SEGREC		
	2020	2021	2022	2020	2021	2022
Clover Planting	21 <sup>st</sup> October			3 <sup>rd</sup> November		
Bareground Plot Establishment	3 <sup>rd</sup> December		13 <sup>th</sup> April	20 <sup>th</sup> November		29 <sup>th</sup> March
VFSW Establishment		17 <sup>th</sup> May	25 <sup>th</sup> April		16 <sup>th</sup> April	20 <sup>th</sup> April
Cotton planting		9 <sup>th</sup> June	9 <sup>th</sup> May		10 <sup>th</sup> May	10 <sup>th</sup> May
Fertilizer Application		30 <sup>th</sup> June	12 <sup>th</sup> May		14 <sup>th</sup> may	3 <sup>rd</sup> May
Cotton Re-planting		3 <sup>rd</sup> June	6 <sup>th</sup> June		3 <sup>rd</sup> June	
Pre Herbicide Application		9 <sup>th</sup> June	9 <sup>th</sup> May		10 <sup>th</sup> May	10 <sup>th</sup> May
Post Herbicide 1 Application		1 <sup>st</sup> July	31 <sup>st</sup> May		16 <sup>th</sup> June	24 <sup>th</sup> May
Stand Counts		21 <sup>st</sup> June	21 <sup>st</sup> June		24 <sup>th</sup> May	13 <sup>th</sup> June
Post Herbicide 2 Application		15 <sup>th</sup> July	18 <sup>th</sup> July		28 <sup>th</sup> June	7 <sup>th</sup> June
Growth Regulator Application		28 <sup>th</sup> September	24 <sup>th</sup> August		24 <sup>th</sup> August	26 <sup>th</sup> July
Cotton Harvested		17 <sup>th</sup> December	11 <sup>th</sup> November		3 <sup>rd</sup> November	24 <sup>th</sup> October

Table 2.2: Herbicide names and corresponding application times with rates

Herbicide	Trade Name	Information	Application timing	Rate Kg ha <sup>-1</sup>
Glyphosate	Roundup® Powermax II	Bayer Crop Science LP USA, 800N. Lindbergh Blvd. St Louis, Missouri 63167	Establish VFS width, PRE, POST 1, POST 2	1.26 a.e.
Dicamba	Xtendimax®	Bayer Crop Science LP USA, 800N. Lindbergh Blvd. St Louis, Missouri 63167	Establish VFS width	0.81 a.e.
Sodium Salt of Fomesafen	Reflex®	Syngenta Crop Protection LLC USA, P.O. Box 18300, Greensboro, North Carolina 27419-8300	PRE	0.175 a.i.
Diuron	Direx® 4L	Makhteshim Agan of North America Inc d/b/ ADAMA, 3120 High Wood Blvd. Suite 100, Raleigh, NC 27604	PRE	0.35 a.i.
Glufosinate – ammonium	Liberty® 280 SL	Bayer Crop Science LP USA, 800N. Lindbergh Blvd. St Louis, Missouri 63167	POST 1, POST 2	0.656 a.i.
Acetochlor	Warrant	BASF Corporation, 26 Davis Drive, Research Triangle Park, NC 27709	POST1	0.84 a.i.

Table 2.3: Cotton harvest parameters for both years and locations separated out by VFS width

Cotton Harvest Parameter																					
Bolls plant <sup>-1</sup>		Seed Cotton Yield				Lint boll <sup>-1</sup>		Seeds boll <sup>-1</sup>				Turnout									
		kg ha <sup>-1</sup>				g						%									
VF	S	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022						
cm		JPCREC																			
0		2.3	B	5.5	D	451.3	C	734.1	A	10.3	A	4.06	A	13.5	A	3.37	B	54.7	A	19.1	B
15		6.6	A	16.2	A	4162	B	776.1	A	7.5	A	13.3	A	21.1	A	9.62	A	56	A	49.2	A
30		7	A	15.3	AB	5278.4	A B	1000	A	10.8	A	10.4	A	23.1	A	8.25	A	60.6	A	46.1	A B
60		7.2	A	11.6	BC	5245.6	A B	1597.1	A	18.1	A	10.7	A	22.6	A	11.37	A	57	A	44.2	A B
90		6.4	A	10.1	C	7167.1	A B	913.1	A	10	A	4.53	A	22.5	A	3.87	A	50	A	40.2	A B
		SEGREC																			
0		1.7	B	18	A	632.3	C	2732.1	A	8.06	B	26.5	B	6.9	A	15.4	A	14.5	B	42.6	A
15		15.7	A	16.9	A	5508.9	B	4237	A	33	A	24.8	A	26.1	A	15.8	A	54.4	A	44.7	A
30		18.1	A	19.6	A	6237.3	B	4955.6	A	38.1	A	31.4	A	30.6	A	14.9	A	54.5	A	47.6	A
60		16.5.0	A	16.2	A	6302.4	B	3474.8	A	31.9	A	26	A	23.8	A	17.4	A	54	A	45.0	A
90		16.3	A	16.9	A	10348	A	4299.4	A	33.5	A	25.9	A B	18	A	15.3	A	54	A	45.1	A

Table 2.4: Seed Lint Boll<sup>-1</sup> for both years and locations as separated by VFS width and seeding rate

Seed Lint Boll <sup>-1</sup>								
6.6 seeds m <sup>-1</sup>					13.2 seeds m <sup>-1</sup>			
g								
VFS	2021		2022		2021		2022	
cm	JPCREC							
0	4.25	A	10.82	A	28.15	A	7.75	A
15	11	A	32.12	A	14.38	A	14.8	A
30	22.25	A	40.12	A	13.62	A	17.4	A
60	15.25	A	35.6	A	45.87	A	13.2	A
90	31.75	A	15.05	A	32.5	A	5.85	A
SEGREC								
0	3.87	B	41.62	A	25	A	81	A
15	59	A	51.87	A	62.12	A	58.7	A
30	77.75	A	63.45	A	61.37	A	68.5	A
60	60.12	A	59.9	A	58	A	56.9	A
90	68	A	75.22	A	56.25	A	39.7	A

Table 2.5: Fiber quality parameters for both locations and years separated by VFS

VFS cm	Upper Half Mean Length				Elongation				Short Fiber Index				Micronaire				Uniformity Index				Strength			
	2021		2022		2021		2022		2021		2022		2021		2022		2021		2022		2021		2022	
	cm		cm		cm		cm		cm		cm		cm		cm		cm		cm		cm		cm	
	JPCREC																							
0	1.24	A	1.25	A	7.15	A	6.96	A	7.7	A	7.2	A	2.2	A	3.5	A	82.15	A	84.33	A	28.5	A	31.1	A
15	1.22	A	1.23	A	7.27	A	6.94	A	8.17	A	7.4	A	2.3	A	3.35	A	82.15	A	83.87	A	28	A	30.82	A
30	1.23	A	1.25	A	7.31	A	7.1	A	8.21	A	7.33	A	2.32	A	3.06	A	81.41	A	83.87	A	28.1	A	30.81	A
60	1.24	A	1.23	A	7.27	A	7.06	A	7.72	A	7.56	A	2.43	A	3.39	A	81.25	A	83.36	A	27.8	A	30.23	A
90	1.24	A	1.25	A	7.27	A	7.11	A	7.42	A	7.11	A	2.69	A	3.08	A	82	A	83.83	A	27.7	A	30.52	A
	SEGREC																							
0	1.19	A	1.2	A	6.65	B	6.95	A	7.5	A	7.9	A	3.46	A	4.42	A	83.8	A	83.31	A	30.5	A	30.95	A
15	1.21	A	1.22	A	7.02	B	7.15	A	8.14	A	7.86	A	3.98	A	4.15	A	82.21	A	83.26	A	30.2	A	30.88	A
30	1.22	A	1.22	A	7.02	B	7.17	A	7.86	A	7.65	A	3.78	A	4.2	A	82.9	A	83.25	A	30.1	A	30.78	A
60	1.23	A	1.22	A	7.25	AB	7.07	A	7.57	A	7.65	A	3.91	A	4.34	A	82.6	A	83.21	A	29.7	A	30.42	A
90	1.23	A	1.22	A	7.48	A	7.33	A	7.88	A	7.86	A	3.71	A	4.35	A	82.16	A	83.07	A	29.6	A	30.03	A

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Figure 2.1

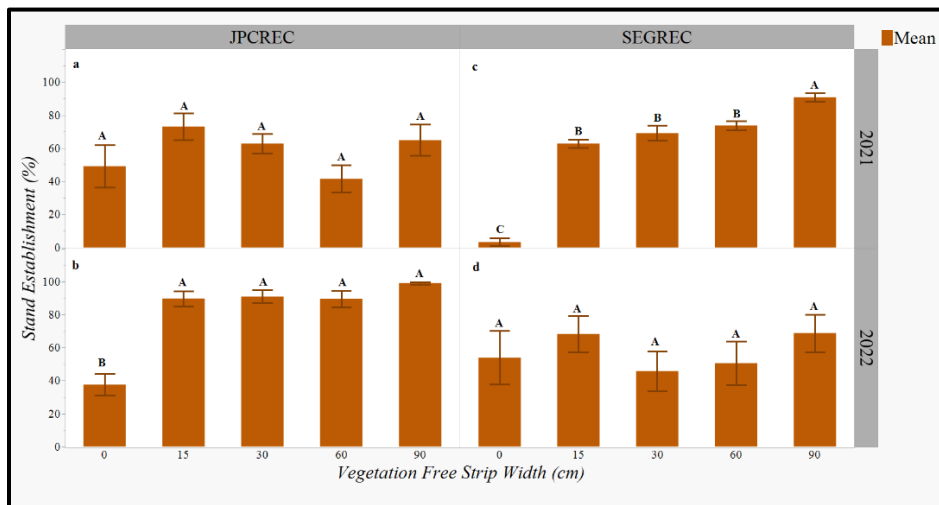


Figure 2.2

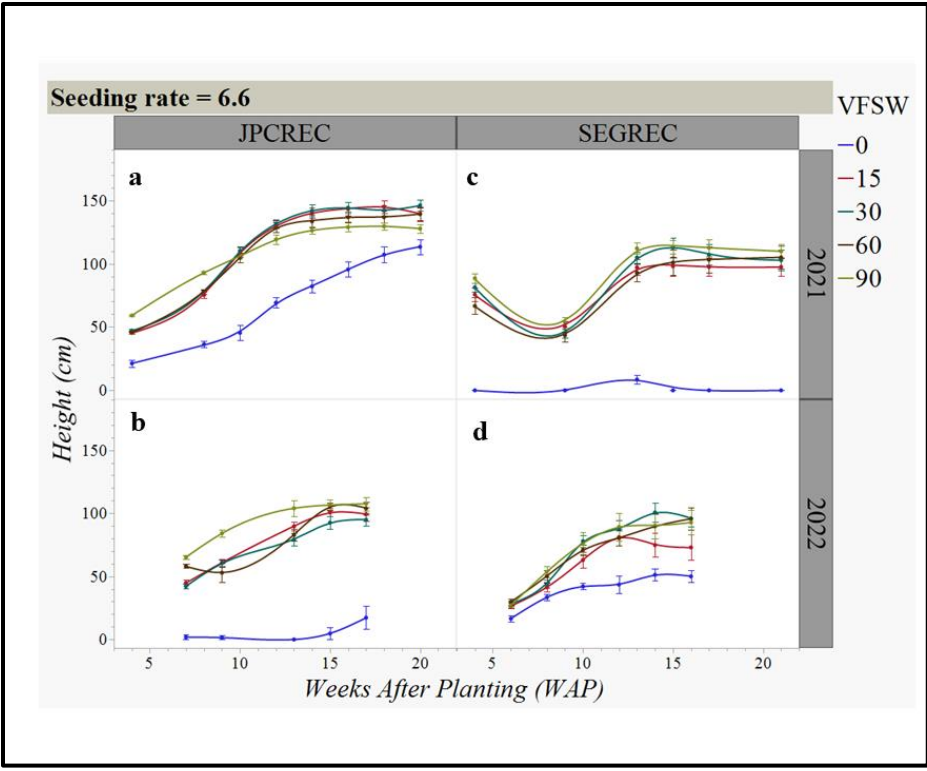


Figure 2.3

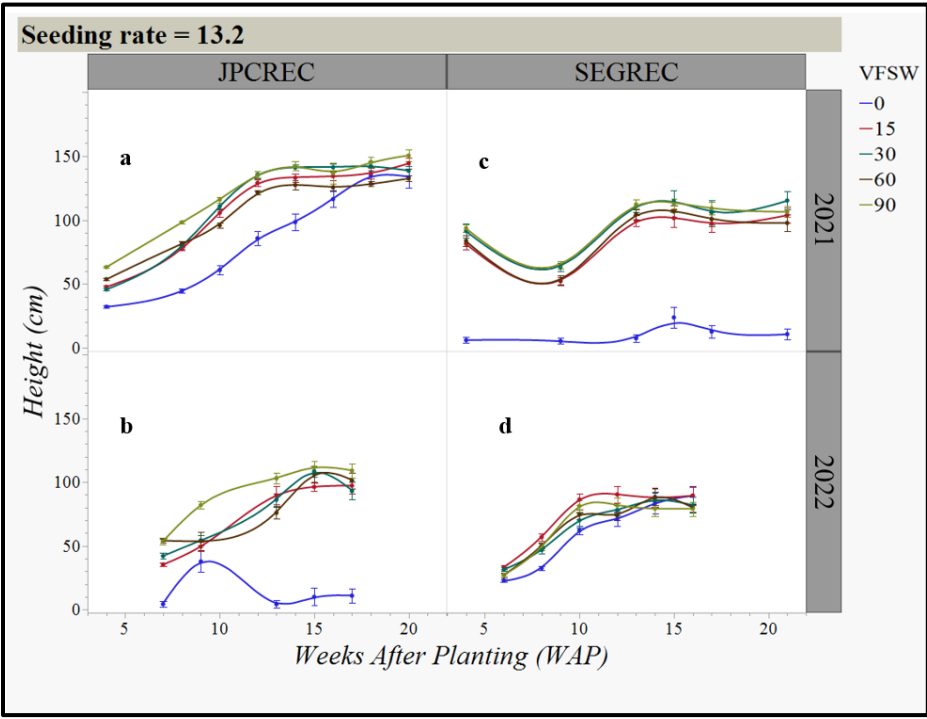


Figure 2.4

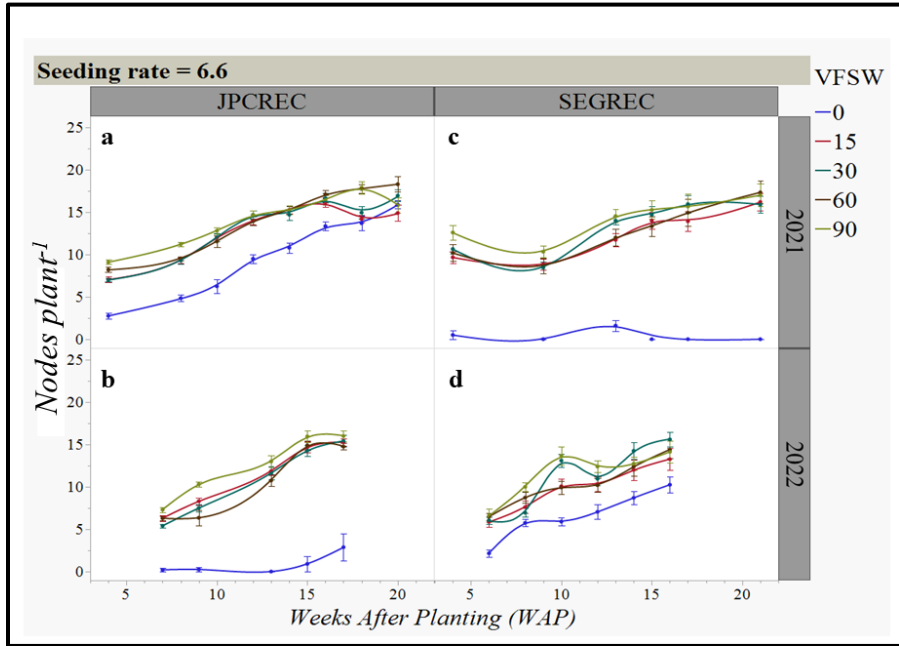


Figure 2.5

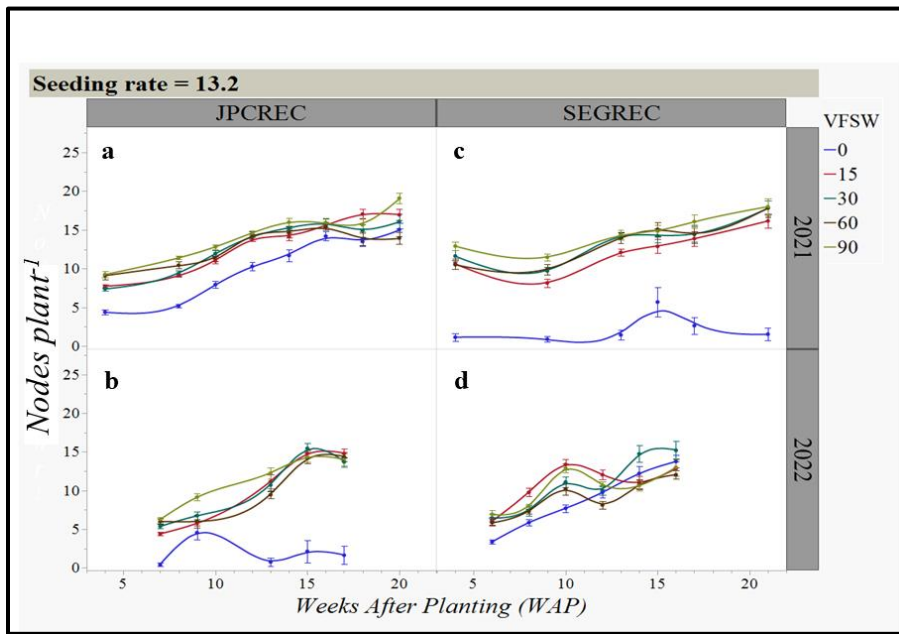


Figure 2.6

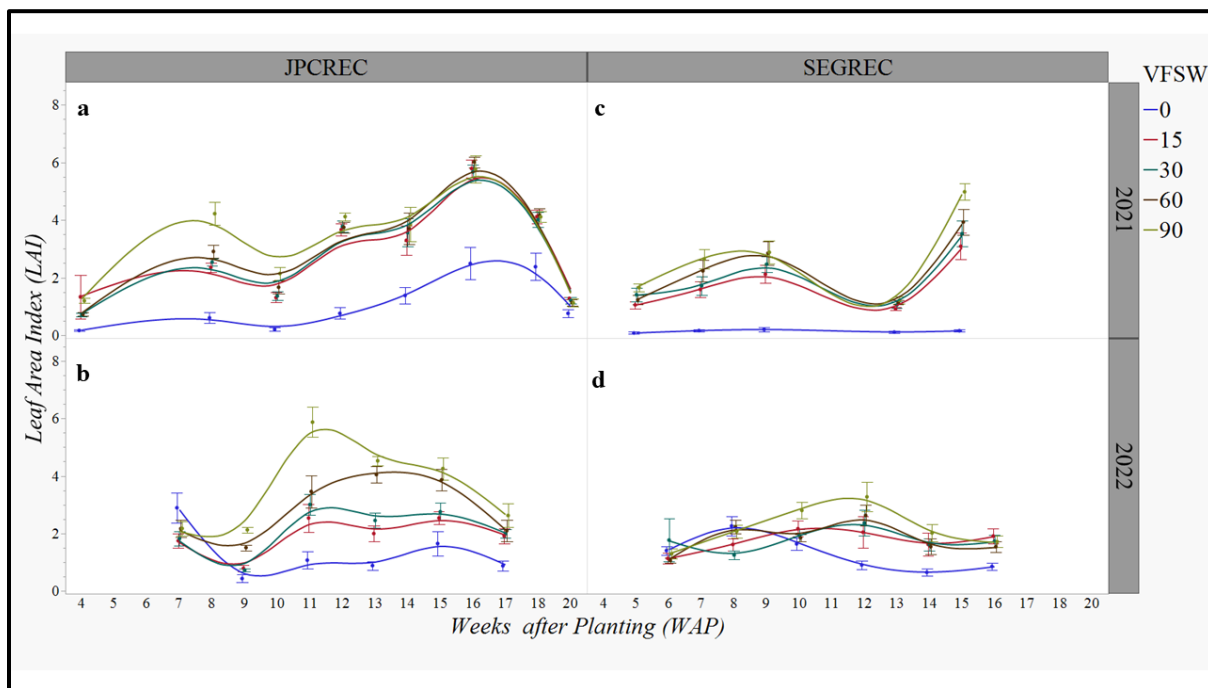


Figure 2.7

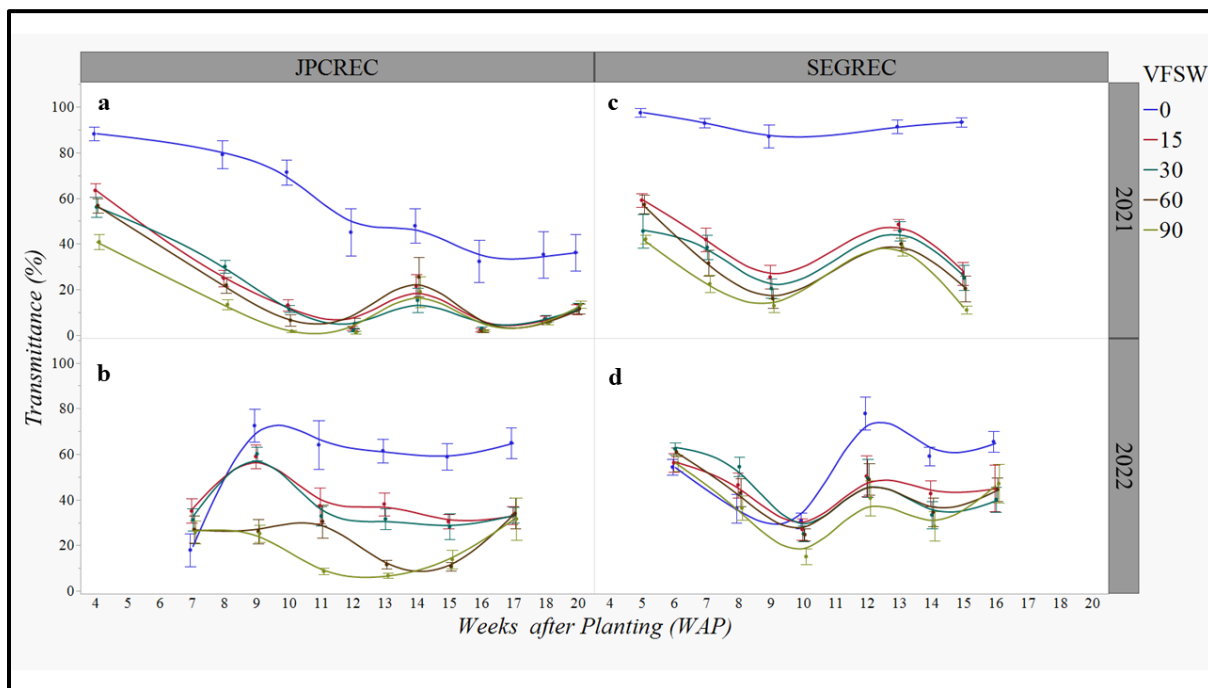


Figure 2.8

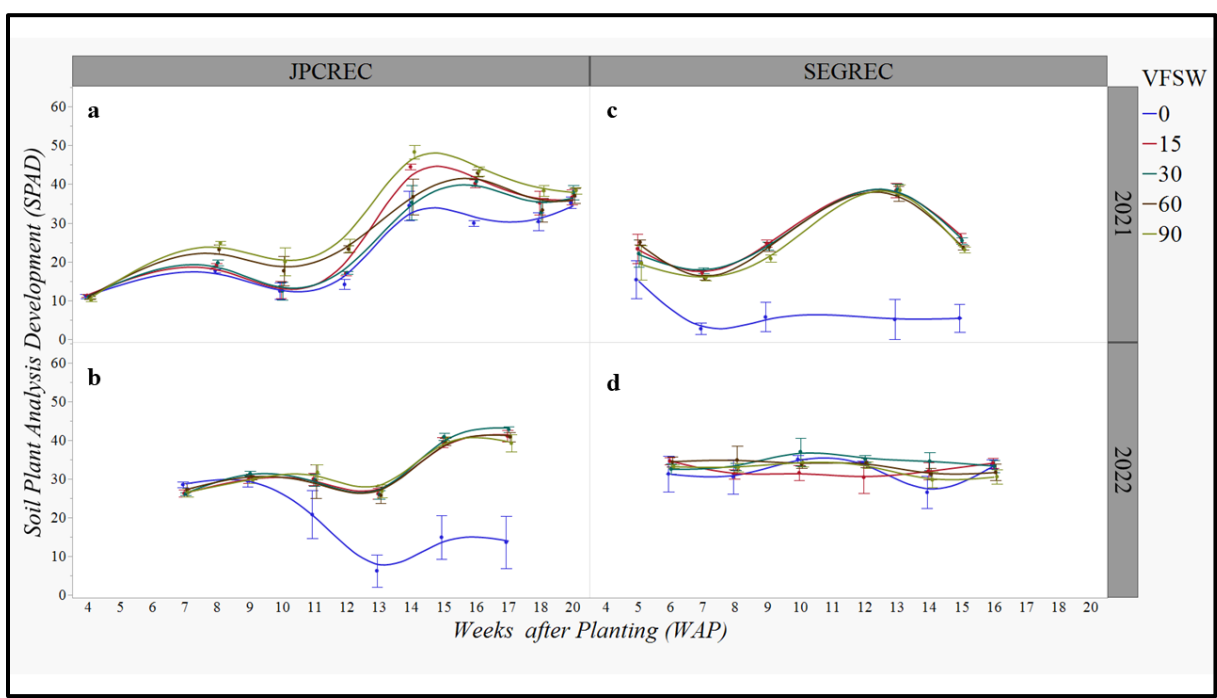


Figure 2.9

### Appendix

The meteorological data at both the study sites describes the trends in temperature (° C) for the study years of 2021 and 2022. JPCREC (Figure 2.10) had a higher summer temperature in 2022 as compared to 2021. During the month of September JPCREC had similar temperature at both years while later at fall JPCREC had a lower temperature in fall 2022 as compared to fall 2021. This lower temperature in 2022 can be attributed to the early frost in October 2022. Similar trends were seen at SEGREC (Figure 2.11), where temperatures in summer were higher in 2022 than 2021 with similar temperatures around September.

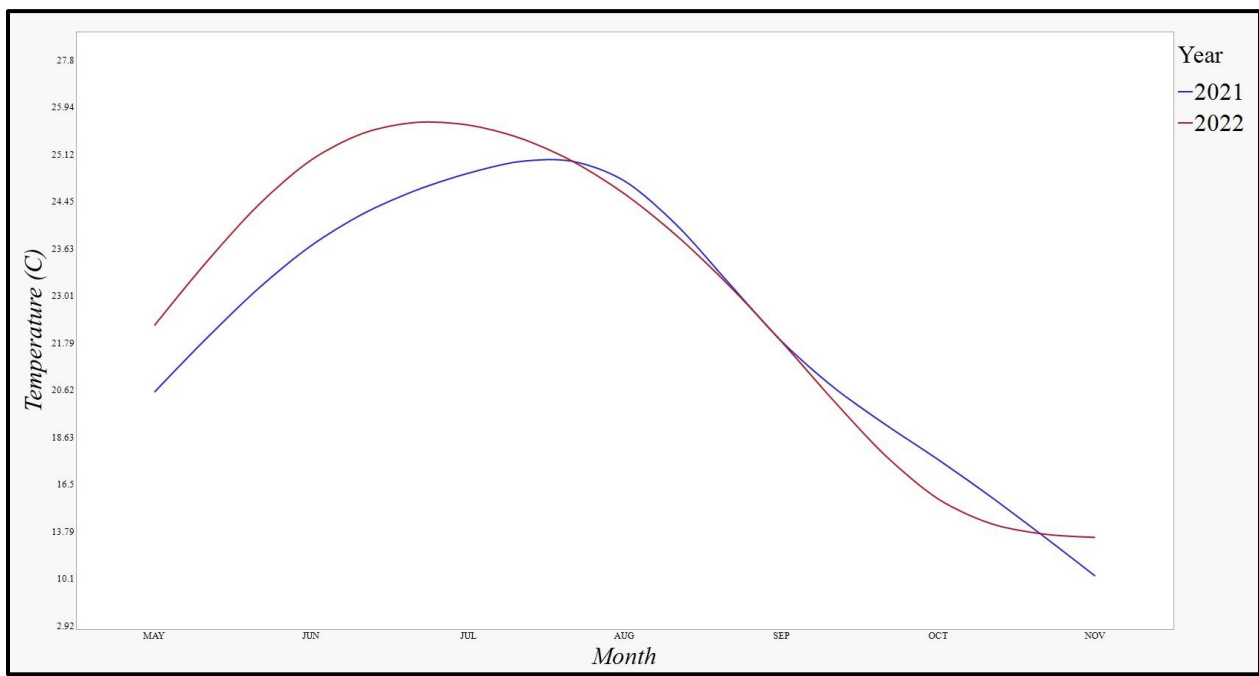


Figure 2.10

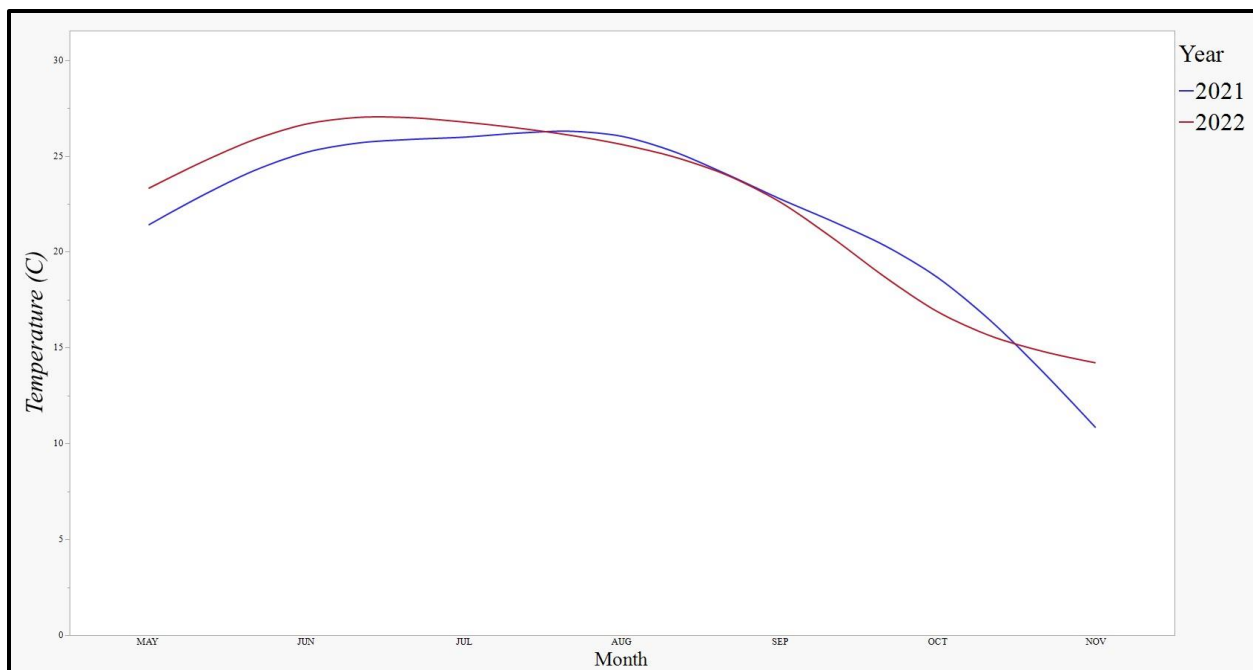


Figure 2.11

### **CHAPTER 3**

## **SHORT-TERM IMPACTS OF LIVING MULCH MANAGEMENT ON SOIL PHYSICAL PROPERTIES AND SOIL MOISTURE DYNAMICS IN DIFFERENT SOIL TYPES<sup>2</sup>**

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<sup>2</sup> Shome, S; Gaur, N; and Basinger, NT. To be submitted to [Precision Agriculture]

### **3.0 Abstract**

Living mulch (LM) defined as cover crops grown with cash crops. LM management can be varied in the form of termination of LM along variable strip widths while planting cash crops. This study evaluates the impact of management of a white clover LM grown in cotton on soil physical properties and soil moisture dynamics for two hydrologically diverse soil types. Five treatments in the form of vegetation free strip (VFS) width were applied. The VFS widths were 0, 15, 30, 60 and 90 cm with 0 cm being the VFS width where cotton is sown within the LM and 90 cm being bare ground as is currently practiced. The study was conducted for one year post establishment of LM at the J. Phil Campbell Research and Education Center (JPCREC) and two years post establishment at the South-East Georgia Research and Education Center (SEGREC). Results suggested that the impact of LM on soil physical properties was soil and length of study specific. While bulk density, available water holding capacity, field capacity and wilting points were evaluated for the two soil types and different treatments, there was no short-term impact of LM on any property except for bulk density in sandy soils. It was also found that while there were changes in some soil physical properties for the sandier soil type, the presence of LM system did not appreciably alter the soil moisture management zones in either soil type. Soil physical health properties that need to be considered while describing soil moisture management zones in a living mulch cotton system also remained unaffected for the duration of the study.

Key words: Soil Moisture management zones, living mulch, cotton, Vegetation free-strip.

### **3.1 Introduction**

USDA defines cover crop as “Crops, including grasses, legumes, and forbs, for seasonal cover and other conservation purposes. Cover crops are primarily used for erosion control, soil health

improvement, and water quality improvement. A cover crop managed and terminated according to these guidelines is not considered a ‘crop’ for crop insurance purposes. The cover crop may be terminated by natural causes such as frost, or intentionally terminated through chemical application, crimping, rolling, tillage, or cutting” (USDA-NCRS 2014). Cover crops that are not terminated at the start of the growing season, also known as living mulch (LM) (Freyman 1989; Basinger and Hill 2021 ) can provide several ecosystem services during the growing season in addition to the non-growing season. These services include weed-suppression (Osipitan et al. 2019), nitrogen fixation in soil (Andrews et al. 2018), prevention of soil erosion (Sarrantonio and Gallandt 2003), minimization of nutrient leaching (Andrews et al. 2020), and increase in soil water holding capacity (Sanders et al. 2018), and soil organic matter (Vukicevich et al. 2016). Several of these ecosystem services result from changes in soil physical properties induced by LM. The LM system develops a synergy in the cropping system by actively growing throughout the year and self-regenerating without reseeding. This synergy translates to improvement in soil hydraulic properties by changing the soil physical parameters like soil aggregation (Salata et al. 2017), soil structure (Nakamoto and Tsukamoto 2006), and water holding capacity (Sanders et al. 2018). The changes induced by LM on soil properties also alter irrigation and fertilization requirements and hence can have environmental and economic consequences for growers which can vary across the years that the LM is allowed to persist on the field. Hence, an in-depth understanding of the short and long-term impact of LM on agricultural systems and resources can aid in designing more efficient precision agriculture strategies and quantify yearly return on investment for growers.

The hydraulic properties of a soil that supports LM are largely altered by the continuous interaction of its rooting system with the soil. The roots of LM have a symbiotic association with

Arbuscular Mycorrhizal (AM) fungi (Dabney et al. 2007) that help the plants uptake water and nutrients. The plants in return provide polysaccharides while the fungus provides a glycoprotein called glomalin which promotes soil aggregation stability and improved soil structure (Dabney et al. 1996, 2007). Hence, the impact of this symbiotic association on soil properties can vary as a function of changes in rooting structure and density across years for the same field as the LM grows. As a consequence of changes in soil structure, the water holding capacity is improved (Dabney et al. 1996, 2007) which helps offset the nitrogenous fertilizer leaching (Vukicevich et al. 2016). The soil water holding capacity also builds up an advantageous soil moisture profile in the soil, (Hill et al. 2021; Sanders et al. 2018) thereby minimizing fluctuating temperatures between night and daytime and resulting in a more stable environment (Dabney et al. 1996, 2007). LM also helps to increase water infiltration by decreasing surface runoff and runoff velocity (Dabney et al. 2007).

While LM provides several hydrological ecosystem services to enhance soil water storage, it also leads to significant loss of soil water through transpiration. Hence, the density of LM in a field can lead to unpredictable soil water dynamics in the fields. Since soil hydrology varies as a function of soil physical properties, these impacts of LM on soil can also vary based on soil type. However, very few studies have been done to determine the effect of LM on soil physical health properties (Salata et al. 2017). Results from previous research suggest that soil water dynamics respond variably to LM systems in different soil types. While a few long-term studies like Hill et al. 2021 and Das et al. 2022 reported enhancement of soil physical properties (Hill et al., 2021) in Cecil sandy loam soils and increased yield in sandy clay soils (Das et al., 2022). Sanders et al. 2018 reported that LM systems are best suited to sandy loam regions with high rainfall, high water holding capacity, and supplemental irrigation. Brainard et al. 2012 also reported that LM

competes with cash crop for moisture in loamy fine sand during dry years unless supplemented by an external irrigation source.

In irrigated systems, even in the absence of LM, soil physical health properties like field capacity, wilting point, available water holding capacity etc. that help determine crop irrigation requirements are highly variable (Gülser et al. 2016, Haruna and Nkongolo 2013) in space (Liu et al. 2020) and necessitate precision irrigation strategies (González Perea et al. 2018; Lozoya et al. 2016) that involve applying variable irrigation amounts to different regions in the field based on soil properties. These regions are referred to as soil moisture management zones (SMNZ). A SMNZ is typically delineated by assessing differences in soil physical health properties like bulk density (BD) (Vrindts et al. 2005), field capacity (FC) (Chen et al. 2019), wilting point (WP) (Jiang et al. 2011), available water content (AWC) (Chen et al. 2019), soil texture (Gili et al. 2017), and apparent electrical conductivity (EC) (Peralta and Costa 2013). Since a LM system impacts the physical properties of soils, we hypothesize that soil moisture management zones can change under LM systems.

While several studies assess the impact of LM on agronomic parameters, results for the studies may not directly be transferable because of differences in soil types. Shome et al., 2023 had conducted an experiment to assess variability in agronomic parameters in two distinct soil types and hydro-climates. They found variable response of LM in the two regions, but the differences could not be attributed to a specific mechanism responsible for variability in response to LM management without isolating the impact of either soils or hydro-climate. Hence in this study, we have evaluated the short-term impact of LM density on soil physical properties in those two hydrologically diverse soil types. For the same soil types, we have also evaluated if the presence of a LM system appreciably alters soil moisture management zones by affecting the spatially

distributed soil moisture dynamics of the field in a living mulch-cotton system. The results from this study can inform growers and scientists about the contribution of different environmental variables on the impact of LM management for fields in different hydro-climates and soil types and make informed decisions while managing LM.

## **3.2. Materials and methods**

### ***3.2.1 Study area***

The study was conducted at the J. Phil Campbell Research and Education Centre (JPCREC) in Oconee County (33°52'3" N, 83° 26'58" W, and 246 m elevation), GA and at the Southeast GA Research Centre near Midville (SEGREC) (33°87'4" N, 82°21'4" W and 59 m elevation), GA (Figure 3.1). The soil at JPCREC is Cecil sandy loam which is (fine, kaolinitic, thermic Typic Kanhapludults), whereas the soil at the SEGREC is a Dothan sandy loam (Fine-loamy, kaolinitic, thermic Plinthic Kandiudults) (SSURGO-NRCS 2023). The VFS widths were established as described in Chapter 2. The experimental design was similar to Chapter 2 except the factors. Two replicates of each VFS width per study location were selected. The effect of seeding rate was not studied as it did not have any significant effect on most cotton yield parameters.

### ***3.2.2 Soil moisture measurements***

Two types of soil moisture measurements were made in the field- 1) continuous and, 2) spatially distributed spot measurements. The continuous measurements were made using Time Domain reflectometer (TDR) sensors (Acclima Inc, 1763 W Marcon Ln #175, Meridian, ID 83642)- . Two sensors were installed at 10 cm depth per VFS width at both JPCREC and SEGREC and data was collected every 30 minutes. The depth of installation was selected based on the rooting

density of cotton and LM rootzone which was found to be maximum at 10 cm (Hill et al. 2021). The sensors were located strategically based on electrical conductivity data to capture variability but more importantly for logistical reasons so that multiple sensors could be plugged into one data logger and assure ease of farming operations.

The spatially distributed measurements of soil moisture were made adjacent to the soil sampling locations as described below in soil moisture data collection.

### ***3.2.3 Sampling design:***

Soil samples were collected from six points in two plots per VFS width at both JPCREC and SEGREC (Figure 3.2). Sampling locations in the field were determined using a stratified random sampling (Wang et al. 2021) in ArcGIS pro (Esri-ARCGIS Pro 3.0.3) and the stratification was done based on topography (slope and aspect) and soil series data. Digital Elevation Model (DEM) data of the study area was downloaded from USGS Earth Explorer with spatial resolution of 30 x 30 m. Slope and aspect were derived from DEM data using ArcGIS Pro (Esri-ARCGIS Pro 3.0.3). Soil series data with 30 m spatial resolution was obtained from a database (Survey Geographic Database (SSURGO)) compiled by the United States Department of Agriculture-Natural resources Conservation Services (USDA-NRCS) (<http://soildatamart.nrcs.usda.gov>, website accessed on 24 February 2022). The stratified random sampling method (Basheer et al. 2022; Thompson and Kolka 2005) was implemented over the study area using fishnet (Redlich et al. 2022) in ArcGIS Pro (Esri-ARCGIS Pro 3.0.3). The plots were divided into 2 x 2 m squares and one point per square was randomly chosen by the algorithm. Finally, six points per plot (two plots per treatment) were chosen randomly and georeferenced using Trimble (Trimble Terrasat GmbH, Haringstrasse 9, 85635, Hohenkirchen, Germany) (Figure 3.3).

### 3.2.4 Collection of soil sample:

Soil samples were collected using a hollow metallic cylinder (5.2 cm diameter). The cores were collected using a wooden block and slide hammer following Bagnall et al. 2022 at 10 cm depth. The cores were wrapped in plastic and preserved during transport to the lab where they were stored at 2.8°C in a fridge until analysis.

### 3.2.5 Soil physical health properties

#### 3.2.5.1 Field capacity

Field capacity, defined as volumetric water content after a prolonged period of gravity drainage (Jang et al. 2002), was estimated using tempe cells (Redlich et al. 2022). It was measured in the lab by setting the tempe cell pressure to 33kPa (USDA-NRCS 2023). The air entry value of the ceramic plates used in this study was 1 bar. The following formulas were used to calculate volumetric soil moisture at field capacity (%).

$$\theta_{g,fc} = \left( \frac{W_{wet\ soil} - W_{eq}}{W_{eq}} \right) \quad [1]$$

where,

$\Theta_{g,fc}$  = Gravimetric soil moisture at field capacity  $g\ g^{-1}$

$W_{wet\ soil}$  = wet weight of soil at 0.33 bar (g)

$W_{eq}$  = equivalent over dry weight of sample (g)

$$W_{eq} = \frac{OD_{sub}}{AD_{sub}} \times AD_s \quad [2]$$

where,

$OD_{sub}$  = Oven dried sub sample (g)

$AD_{sub}$  = Air dried sub sample (g)

$AD_s$  = Air dried sample obtained after tempe cell experiment (g)

$$\theta_{v,fc} = \left( \frac{\theta_{g,fc} \times \rho_b}{\rho_w} \right) * 100\% \quad [3]$$

where,

$\Theta_{v,fc}$  = Volumetric soil moisture at field capacity ( $\text{g g}^{-1}$ )

$\Theta_{g,fc}$  = Gravimetric soil moisture at field capacity ( $\text{g g}^{-1}$ )

$\rho_b$  = Bulk density of soil ( $\text{g cm}^{-3}$ )

$\rho_w$  = Density of water ( $\text{g cm}^{-3}$ )

### 3.2.5.2 Bulk density

Soil bulk density is an indicator of soil compaction. A 20 g sub sample was taken from the resultant air-dry sample from tempe cell experiments. They were kept in a hot air oven (Precision, 170 Marcel Drive, Winchester VA 22602) at  $105^0$  C for 24 hours (Bremner 1986). The resultant weight (g) was weighed, and BD was calculated using equation 4.

$$\text{Bulk density} = \frac{W_{eq}}{V_s} \quad [4]$$

Where,

$V_s$  = Volume of sample collected

### 3.2.5.3 Wilting point

Water content at wilting point (-1500 kPa) was measured using WP4C, a dewpoint potentiometer (WP4C, Decagon; Pullman, WA, USA). The following formula was used to estimate volumetric WP (%) (Equation 5)

$$WP_g = \theta_g^{dry} - \theta_g^{wet} \times \left( \frac{\ln(Mpa_{dry} / -1.5)}{\ln \left( \frac{Mpa_{dry}}{Mpa_{wet}} \right)} \right) \quad [5]$$

where,

$WP_g$  = Gravimetric soil moisture at wilting point

$\theta_g^{dry}$  = Dry gravimetric soil moisture at wilting point

$\theta_g^{wet}$  = Wet gravimetric soil moisture at wilting point

$Mpa_{dry}$  = Water potential<sub>dry</sub>(MPa)

$Mpa_{wet}$  = Water potential<sub>wet</sub>(MPa)

$$Wp_v = WP_g * \frac{\rho_b}{\rho_w} * 100 \% \quad [6]$$

where,

$Wp_v = \text{Volumetric soil moisture at wilting point}$

$WP_g = \text{Gravimetric soil moisture at wilting point}$

#### ***3.2.5.4 Electrical conductivity data collection***

Soil electrical conductivity was collected using an EM 31 (Geonics Limited, 1745 Meyerside Dr., Unit 8, Mississauga, Ontario Canada L5T 1C6) to interpret soil spatial variability. EM 31 was partly collected via a fiber glass cart pulled by an UTV and partly hand-held depending on the height of the cotton stand. Preliminary analysis (not presented in this study) showed a high correlation between the hand-held and cart- driven EC values. A UTV driven fiber glass was only used for data collection when there was no cotton stand present in the study area (Figure 3.4A). Hand-held EM 31 was mostly used to measure data when cotton stands were present in the study area (Figure 3.4B). Data was collected in only odd rows in the cotton stand as it comprised of our data row. Data was collected over 4-6 days that had varying wetness conditions. The sets of data included two wet, two dry, and two normal conditions. However, due to changing weather conditions, only two wet, and two normal conditions data from JPCREC and two wet, two dry, and two normal conditions data from SEGREC were collected.

#### ***3.2.5.5 Spatially distributed soil moisture data***

In addition to the continuous soil moisture data, spatially distributed soil moisture was measured at the 60 sampling locations (Figure 3.3) on each day EM data was measured. This was done during the growing season in 2022. Soil moisture was measured using an impedance probe (i.e. Theta Probe) having a hand held datalogger (HH2, Delta-T Devices, Cambridge, UK). Each data point was collected with three replications and averaged to estimate the soil moisture value at that point. The three data points were georeferenced (Figure 3.3) using Trimble (Trimble terrasat GmbH, Haringstrasse 9, 85635, Hohenkirchen, Germany) to ensure data collection from same location each time.

#### ***3.2.5.6 Soil texture analysis***

Particle size analysis was performed to determine the soil texture of samples from both locations using the LS 13 320 Laser Diffraction Particle Size Analyzer (LDPSA). A 50 g subsample from the air-dry soil after the Tempe cell experiments were taken and sieved through < 2mm sieve in a fume hood. Each sample was replicated twice weighing  $0.6 \pm 0.03$  g. Sodium Hexametaphosphate-a dispersing agent was added to each replicate for each soil to separate the smaller particles suspended in solution. The samples were then shaken for 16 hours at 120 oscillations  $\text{min}^{-1}$  after which the samples were analyzed in LDPSA for texture (Xu, 2002 ; Tancredi et al. 2022).

#### ***3.2.6 Random forest modelling***

Soil parameters are typically non-linearly related with soil moisture (Heng et al. 2015) which linear regression models cannot explain. Hence, they are increasingly being replaced by machine learning algorithms like artificial neural networks, support vector machines, classification &

regression trees and random forests (Hengl et al. 2015). In this study, random forest model was implemented to predict spatially distributed soil moisture from environmental covariates including field capacity, bulk density, wilting point, soil texture and apparent electrical conductivity.

Random forest modelling approach out performs linear regression and can fit complex non-linear relationships in  $p+1$  dimensional space, where  $p$  is the number of covariates (Castro-franco et al. 2015, Hengl et al. 2015, Strobl et al. 2009). Random forest is effective only when the range in covariate values is exhibited by training data. In this study, the model was made using R version 4.2.1 (R Development Core Team, 2021).

The dataset was divided into 80% as training data and 20 % as test data. We used bootstrapping to create 1000 iterations of this process. The RF model was developed using training data and validated using test data sets (Castro-franco et al. 2015) and the model was developed for both locations together. The model used numerous decision trees called  $n_{trees}$ . A variable importance rank of each variable in each iteration was calculated followed by mean rank for 1000 iterations. We used the ranks to determine the number of parameters to retain and create a parsimonious model.

The accuracy of estimates was evaluated using root-mean-square (RMSE) 1985) and R-square or goodness of fit ( $R^2$ ) value (Willmott et al. 1985). RMSE is defined as

$$RMSE = \sqrt{\frac{1}{n} \sum_{i=1}^N \{((x_i) - (y_i))\}^2} \quad [7]$$

where,

$x_i$  = observed values

$y_i$  = predicted values

It helps in predicting accuracy, indicating a better fit model.

### ***3.2.7 Soil Moisture prediction***

Soil moisture was predicted after running the RF model using predict function. The prediction was done individually for the different EM data collection days i.e. wet, dry, and normal conditions. The predicted values were spatially interpolated using ordinary kriging in ArcGIS Pro (Esri-ArcGIS Pro 3.0.3). Finally, individual maps were clustered in ArcGIS pro (Esri-ARCGIS Pro 3.0.3) and a t-test was done in R version 4.2.1 (R Development Core Team, 2021) to compare soil moisture between clusters (described below).

### **3.2.8 Clustering analysis.**

Two K-medoid clustering exercises were performed 1) on the predicted and 2) on the measured soil moisture from the 60 points. Clustering was done to delineate regions with similar soil characteristics and identify specific soil moisture management zones to correlate with the VFS width in the study areas. Studies have shown that dividing fields into soil moisture management zones (SMNZ) via clustering reduces the interzone soil moisture spatial variability by about 50 % against when compared with the entire field (Ciampitti et al. 2021). However, there is no optimal clustering method. Javadi et al. 2022 evaluated five clustering algorithms including k-means, fuzzy C-means, hierarchical, means shift, and density based spatial clustering of applications with noise (DBSCAN) in different scenarios and assessed their impacts. K means

was found to be optimal clustering method for SMNZ delineation. Arora et al. 2016 suggested the use of K-medoids instead of K-means as it is better in terms of execution time, non-sensitive to outliers and reduces noise as compared to K-Means as it minimizes the sum of dissimilarities of data objects. A pairwise comparison test was then performed between clusters for each condition (wet, dry, and normal) within location to assess differences in soil moisture between them.

### **3.3 Results**

#### **3.3.1 Soil textural differences at JPCREC and SEGREC:**

The study was set up to evaluate the impact of LM on two hydrologically diverse soil types. The soil in Georgia, US is predominantly separated by the fall line into the Piedmont region having more clayey soil and the coastal plains having more sandy soils (Hendrix et al. 1992; Sainju et al. 2002). Likewise, our study areas were located across the fall line with JPCREC being in Piedmont region and SEGREC being in coastal plains. The soil texture from the 60 locations is shown in (Figure 3.5). The samples from JPCREC (depicted in red color), were finer textured and their texture varied between clay-to-clay loam with a composition of clay 30-80 %, and silt 20-70% while those from SEGREC (depicted in blue color) were sandy to loamy sand with a composition of clay 0-20%, and sand 70-100%).

#### **3.3.2 Soil physical health properties**

Soil health was evaluated through static physical parameters like FC, BD, WP AWC, and soil texture (Amirinejad et al. 2011; Haruna et al. 2020). Static parameters in this study are defined as parameters that change more slowly than other soil variables like soil moisture. The

measurement of static parameters involves a lower labor requirement and can be more easily implemented by growers. The soil physical health parameters for JPCREC and SEGREC are provided in Figure 3.6. FC (Figure 3.6A), BD (Figure 3.6B), WP (Figure 3.6C), AWC (Figure 3.6D).

A pair wise comparison of soil properties between the two locations showed that not all soil physical properties were significantly different despite the large differences in soil texture. Field capacity was significantly different between the two locations with the clayey JPCREC soils having a higher field capacity as compared to the sandier SEGREC soils. On the other hand, there was no significant difference between BD and WP between the two locations indicating that soil textural differences did not drive these two physical health parameters for these locations. AWC showed a significant difference as a consequence of differences in field capacity. However, the lack of a difference between bulk densities in the two locations but a significant difference in field capacity highlights that changes in bulk density do not imply changes in plant available water content for the crop. Bulk densities are directly impacted by management activities like the amount of compaction in addition to soil textural differences. To understand the effects of soil physical health parameters on LM management within the same soil type, a pairwise comparison test was performed for different VFS widths at each location (Figure 3.7 & 3.8). None of the soil physical health parameters were significantly different between the VFS widths at JPCREC during the period of study. However, in the sandier soils at SEGREC, we observed significant differences in BD (Figure 3.7 B) between the 0 and 60 cm VFS width and 60 and 90 cm VFS width. These results imply that differences in soil physical health parameters may manifest variably in different soil types. These results also support the above observation where the impact of management on BD differences was found to dominate

more than soil textural differences indicating that LM management will more quickly alter some soil physical properties more than the others.

### **3.3.3 Apparent electrical conductivity (EC)**

EC data was collected to assess within field variability. A pairwise test between location for apparent EC data collected at normal soil moisture conditions (10-20%) and wet conditions (>20% moisture) was significant at  $p < 0.00001$  (Figure 9). Despite higher clay contents, JPCREC had much lower EC values as compared to SEGREC under both normal and wet conditions. This is consistent with previous studies that show clayey soil particles especially in fertilized environments have a strong attraction with the cations in the soil sphere which lowers the available charge in the soil, thereby having low EC reading as compared to sandy soils (Auerswald et al. 2001).

### **3.3.4 Ordinary kriging of soil physical health parameters**

Spatially distributed values of soil health physical parameters (FC, BD, WP and AWC) were estimated using ordinary kriging in ArcGIS Pro (Esri-ARCGIS Pro 3.0.3). BD values at SEGREC (Figure 3.11C) varied between 1.52-1.83  $\text{g cm}^{-3}$  and are higher than typical values observed for sandy soils. BD values at JPCREC (Figure 3.110C) varied between 1.41-1.92  $\text{g cm}^{-3}$  and were also higher than typical values for clayey soils (NRCS 2023) likely as a result of the soils being under long term agricultural management. Soils at SEGREC (Figure 3.11A) had FC values varying between 12.8-18.18 %, whereas at JPCREC (Figure 3.10A), values varied between 4.62 - 45.91%. SEGREC (Figure 3.11B) had WP values varying between 0.73-10.5%, while at JPCREC (Figure 3.10B), WP varied between 2.38-9.19%. SEGREC (Figure 3.11D) had

lower AWC with values ranging between 8.64-14.59 % as compared to JPCREC (Figure 3.10D) where the values ranged from 4.88-37.94%.

### **3.3.5 Random forest model and K medoids clustering**

A random forest-based model was developed in order to assess the spatial distribution of soil moisture in the field. The RF model was run with 8 different independent variables namely EC, sand, silt, clay, bulk density, field capacity, wilting point, and location on dependent variable soil moisture. A mean variable importance plot rank showed that EM always ranked the most important variable in explaining the variance in the data (Figure 3.12). Hence, to create a parsimonious yet useful model, the RF model was run with only EM data. The  $R^2$  and RMSE values of the model was 0.7 and 3.9% respectively. Clustering was performed on the output of the modeled soil moisture data for the field to assess the patterns of soil moisture variability in the field and identify soil moisture management zones. The optimal number of clusters were determined using the Pseudo F- Statistic (Figure 3.13 & 3.14). The optimum number of clusters for SEGREC (Figure 3.15) and JPCREC (Figure 3.16) were two and three, respectively. A pairwise comparison between the predicted soil moisture from the RF model was made for each soil moisture condition within clusters of each location. While statistically significant differences (Figure 3.17 and Figure 3.18) between clusters at SEGREC and JPCREC were observed, the differences were practically unimportant and the statistical difference was observed owing to the large number of observations (>70,000 points). A study by shows that which large number of data points will yield practically unimportant but statistically significant results

There was a significant difference between clusters at SEGREC during normal moisture conditions (Figure 3.17 A), wet moisture conditions (Figure 3.17 B and dry (Figure 3.17 C)

while the clusters differed significantly at JPCREC during only normal moisture conditions (Figure 3.18 A) and wet moisture conditions (Figure 3.18 B) conditions at  $p < 0.001$ . At JPCREC during normal moisture (Figure 3.18 A) conditions, there was a significant difference only between cluster ID 1 and 2, and no difference between 1 and 3 and 2 and 3 at  $p < 0.001$ . An overlay of the soil moisture management zones with VFS width did not highlight any relationship between the two indicating that dense LM as compared with no LM did not impact soil moisture dynamics. This is also supported by a lack of difference in water holding capacities between different VFS widths at each location and indicates that across a span of a year, variable VFS widths do not impact soil moisture dynamics appreciably for either soil type. Soil moisture management zones also had no correlation with yield from plots indicating that moisture stress did not account for yield differences in either location.

A second K-medoid clustering was performed with the 60 soil sampling points from each location with their corresponding soil moisture values. It was done to understand the temporal variability in soil moisture within the collected sampling points. The optimal number of clusters were determined using the Pseudo F statistic. The optimal number of clusters at JPCREC (Figure 3.19) were seven and at SEGREC (Figure 3.20) were three. At SEGREC (Figure 3.21) there was no difference found between the soil health physical parameters and cluster id's whereas at JPCREC (Figure 3.22) differences were found between cluster ID's and bulk density ( $\text{g cm}^{-3}$ ) and wilting point (%).

### **3.4. Discussion**

The objective of the study was to evaluate short-term impact of LM density on soil physical properties in two hydrologically diverse soil types widely prevalent in Georgia and south-eastern

U.S. The soils at JPCREC, located above the fall line in Piedmont region range from a clay-to-clay loam soil textural classification and those at SEGREC, located below the fall line in the coastal plain region, are sand to loamy sand. For the same soil types, we also evaluated if the presence of a LM system appreciably alters soil moisture management zones in a short time by affecting the spatially distributed soil moisture dynamics of the field in a living mulch-cotton system. The differences in soils in the two regions were not represented by all measured soil physical properties. Field capacity and available water content varied significantly between the two regions while physical parameters like bulk density and wilting point could not distinguish between the variable soil types. While significant differences were observed in the bulk density in the SEGREC region, no other significant differences between the different treatments were observed after a period of two year. For the same region in 2022, Shome et al. 2023 showed significant differences in growth parameters until mid-growing season for JPCREC. Since no differences in soil physical health properties were observed in JPCREC in this study, it indicates that one-year post LM establishment does not affect soil physical health properties in clayey soils and any differences in plant growth can be attributed to other environmental factors. This observation is also partially supported for the sandy soils in SEGREC where a significant difference in bulk density was observed in 2022 but no differences in any yield or turnout parameters were found. The impact of LM on soil properties however does vary with LM density. Since the bulk density of soils changed with different VFS widths in sandy soils for a two year study, a change in soil porosity and consequently infiltration (Lipiec et al. 2006) and erodibility potential varied for these soils. Sanders et al., 2018 showed significant short-term effect of VFS width on agronomic parameters albeit in clayey soils that were also investigated in this work. Previously published research has typically evaluated the effect of LM systems in

different soil types over longer periods of time and observed differences over periods of three years or more. Hill et al., 2021 conducted their work on Cecil sandy loam (Hill et al. 2021) and observed lowered bulk density and increased porosity and infiltration after three years of LM establishment which would have resulted from changes to soil structure from LM roots.

However, we did not observe such differences in the same physical properties in similar soils over a period of one year in the clay soil type. Therefore, the effect of LM on hydrological ecosystem services like partitioning runoff and infiltration is soil-specific and varies with time.

Das et al. 2022 in another study studied the impact of LM based different conservation tillage practices on soil properties for a three-year period in erosion prone sandy soils. A cow pea LM with different combinations of no till, minimum till, and conventional till were assessed. After a three-year study, cowpea LM with minimum till was considered the best option as it increased the water holding capacity, maximized soil moisture content and lowered the bulk density when compared to other treatments. In this study, we found that the varying the density of LM could variable lower the bulk density in similar sandier soils as early as two years post establishment while the same cannot be said about water holding capacity and soil moisture content as observed by Das et al. 2022. Thus, it can be deduced that some ecosystem services from LM may be provided at the end of year two in lighter textured soils. But, when cluster ID was analyzed using with corresponding soil health physical parameters, it yielded different result. There was no difference found between cluster ID and soil health physical parameters at SEGREC whereas at JPCREC there were differences in bulk density and wilting points.

As the LM system started to impart the expected agroecosystem services at SEGREC (sandy soils), the study further investigated the impacts of SMNZ in a LM cotton system at both locations. The objective of this investigation was to determine if the varying LM systems

superimpose itself with the SMNZ. To understand this, a parsimonious RF model was created using apparent EC data. Studies have been done to delineate SMNZ using apparent EC as it describes the soil physical and chemical properties in high resolution less laborious manner (Carranza et al. 2021; Shao et al. 2021; Kargas et al. 2020). It also helps to make a parsimonious RF model with reduced number of classes and spatial continuity between them. This study reported that a RF model can predict soil moisture with apparent EC data with  $R^2$  of 0.70 and RMSE of 3.49. This is one of the first kind study done with varying LM densities in cotton thereby limiting the amount of research done in this sphere. Carranza et al. 2012 estimated root zone soil moisture with a parsimonious random forest model. However, it limited the results to specific sites which were data driven as extrapolation results were poor. In our study, while soil moisture data modeling provided good results, extrapolation of soil physical health parameters using ordinary kriging method yielded a very high error likely resulting from a small data size (Hughes and Lettenmaier 1981).

K medoids clustering was done to understand if the LM densities superimpose itself with the predicted SMNZ. K medoids clustering was chosen instead of K means as K medoids is more robust to noise and outliers as compared to k mean since it minimizes the sum of pairwise dissimilarities instead of a sum of squared Euclidean distances (Carranza et al. 2021). Analysis showed that LM did not appreciably alter the SMNZ. A comparison was further done within cluster ID for each location for the predicted soil moisture separated by normal, wet, and dry conditions at which apparent EC data were collected. At SEGREC, no difference was found within cluster IDs whereas at JPCREC there was difference within cluster IDs at predicted soil moisture at normal conditions. This difference can only be explained with further long-term

study rather than a short-term one-year study. K medoids clustering grouped the study area into optimized clusters and establish management zones respectively. The presence of LM didn't affect the clustering as opposed to the hypothesis of the study, thus indicating to the fact that a long-term study is required for the LM to actually affect the SMNZ. A pairwise test between clusters at both the locations establishes significant differences within predicted soil moisture except at JPCREC. There was no difference between cluster ID 2,3 and 1,3 and there is a difference between 1 and 2. This management zone doesn't superimpose with the different LM densities or VFS widths in the study area thus elucidating the fact that despite having a LM agroecosystem a soil moisture management zone is a necessity.

### **3.5. Conclusion**

In this study on evaluating the short-term impact of LM management on soil moisture dynamics and soil physical properties for variable soil types, we found that LM management does not impact soil moisture dynamics over the short term (one-year post establishment in clayey soils and two years post establishment in sandy soils). A few agroecosystem benefits can be experienced at the end of year two for sandy soils such as lower bulk densities. The hypothesis of this study did not hold true for either soil type over a short period of time as the soil moisture management zones did not overlap with the LM density in the study areas. Soil properties such as field capacity or available water content remained unchanged for either soil type irrespective of the length of the LM establishment. It is important to note that the study was limited to one year in clayey soils and two years in sandy soils and further long-term research is needed to understand the effects of LM on soil hydrology over a longer period of time. The results from

this study can inform growers on the immediate return on investment in terms of ecosystem services for different soils that they can expect from LM management in their fields.

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Tables

**Table 3.1: Plot number with associated VFS width for each location**

Plot no	Location	
	JPCREC	SEGREC
	VFS (cm)	
101	0	0
102	15	15
103	30	30
104	60	60
105	90	90
106	0	0
107	15	15
108	30	30
109	60	60
110	90	90
201	60	15

202	0	90
203	60	30
204	30	60
205	90	0
206	30	30
207	0	0
208	90	90
209	15	60
210	15	15
301	0	60
302	60	0
303	60	0
304	15	60
305	0	30
306	90	15
307	90	30
308	15	15
309	30	90
310	30	90
401	60	15
402	15	60
403	0	90
404	15	30

405	30	90
406	30	0
407	0	15
408	90	30
409	60	0
410	90	60

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Figure 3.4: A) EM 31 data collection via a fiber glass cart pulled by an UTV when there was no cotton stand present at the study locations. B) Hand-held EM 31 data collection when cotton stands were present in the study area.

Figure 3.5: The fall line in Georgia that separates the soil into Piedmont region having more clay content and Coastal Plains having more sand content in soils. Soil Texture Triangle with textural classification of JPCREC (red) and SEGREC (blue).

Figure 3.6: Soil physical health parameter A) significant difference between locations for Field Capacity at  $p=0.001$ ,  $t$  statistic=3.37 and  $df=59$  B) non-significant difference between locations for bulk density at  $p=0.536$   $t$  statistic= 0.622 and  $df=59$  C) non-significant difference between locations for wilting point at  $p=0.536$   $t$  statistic= 0.622 and  $df=59$  D) significant difference between locations for available water content at  $p=0.024$   $t$  statistic=2.32 and  $df=59$

Figure 3.7: The effects of different VFS width at SEGREC on A) Field capacity B) Bulk Density ( $p < 0.05$ ) C) Wilting point and D) Available water content

Figure 3.8: The effects of different VFS width at JPCREC on A) Field capacity B) Bulk Density C) Wilting point and D) Available water content.

Figure 3.9: Apparent electrical conductivity to assess within field variability A) significant between location for normal soil moisture conditions at  $p < 0.00001$  B) significant between location for wet soil moisture conditions at  $p < 0.0001$ .

Figure 3.10: Soil health physical parameters at JPCREC extrapolated using ordinary kriging A) FC B) BD C) WP D) AWC

Figure 3.11: Soil health physical parameters at SEGREC extrapolated using ordinary kriging A) FC B) BD C) WP D) AWC

Figure 3.12: The RF model rank to decide EM data to be used only for the model.

Figure 3.13: The Pseudo F-Statistic to determine the optimum number of clusters for Location SEGREC using K medoids method by setting random seed.

Figure 3.14: The Pseudo F-Statistic to determine the optimum number of clusters for Location JPCREC using K medoids method by setting random seed.

Figure 3.15: The Multivariate clustering at SEGREC with representative yield (larger circle more yield) and the corresponding VFSW.

Figure 3.16: The Multivariate clustering at JPCREC with representative yield (larger circle more yield) and the corresponding VFSW.

Figure 3.17: A pairwise comparison between the cluster ID with the predicted soil moisture separated by normal (A), wet (B) and dry conditions (C) for SEGREC at  $p < 0.0001$ .

Figure 3.18: A pairwise comparison between the cluster ID with the predicted soil moisture separated by normal (A), and wet conditions (B) for JPCREC at  $p < 0.0001$ .

Figure 3.19: The Pseudo F-Statistic to determine the optimum number of clusters for Location JPCREC using K medoids method by setting random seed

Figure 3.20: The Pseudo F- Statistic to determine the optimum number of clusters for Location JPCREC using K medoids method by setting random seed

Figure 3.21: The Multivariate clustering with soil health physical parameters at SEGREC

Figure 3.22: The Multivariate clustering with soil health physical parameters at JPCREC

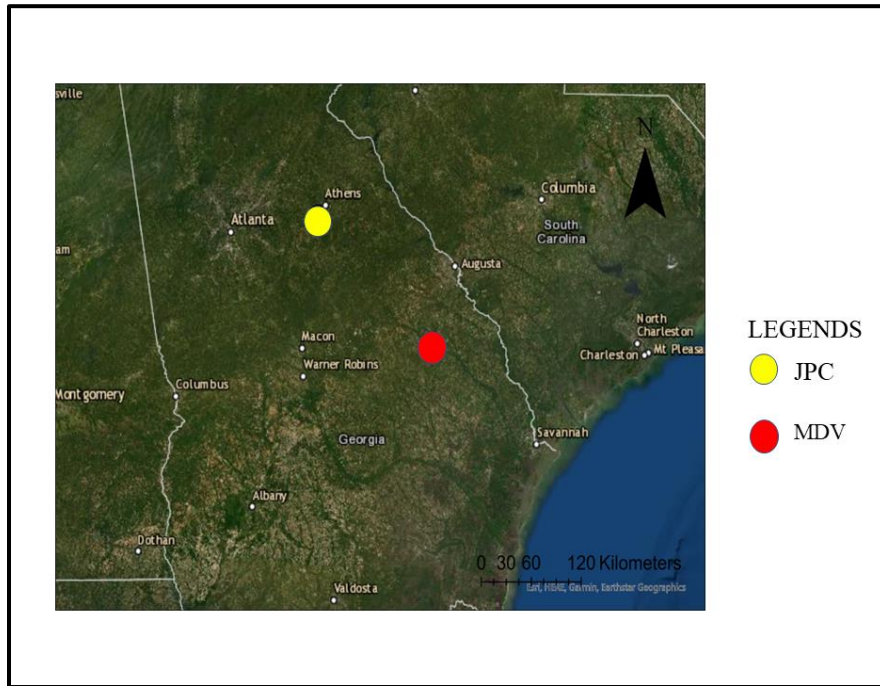


Figure 3.1



Figure 3.2



Figure 3.3

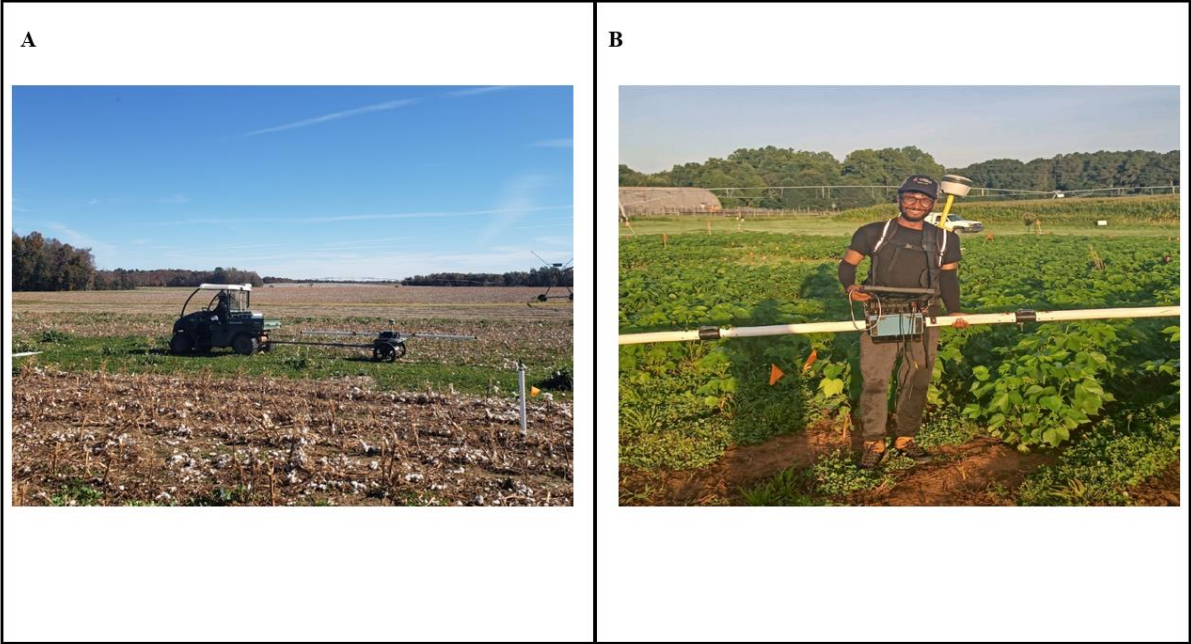


Figure 3.4

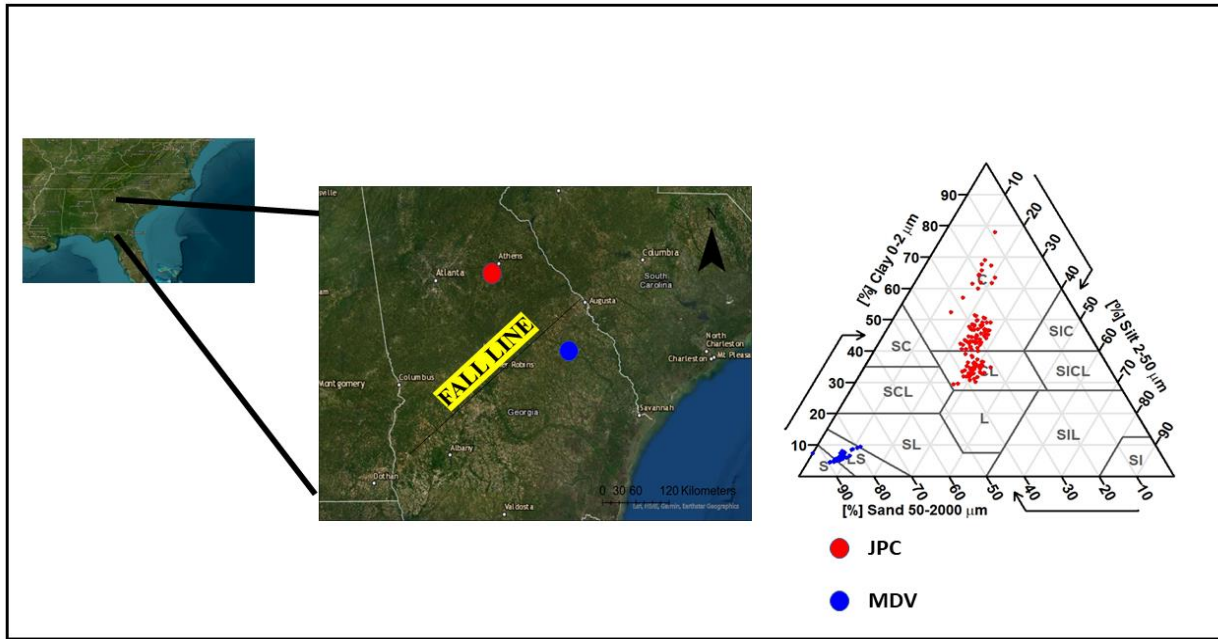


Figure 3.5

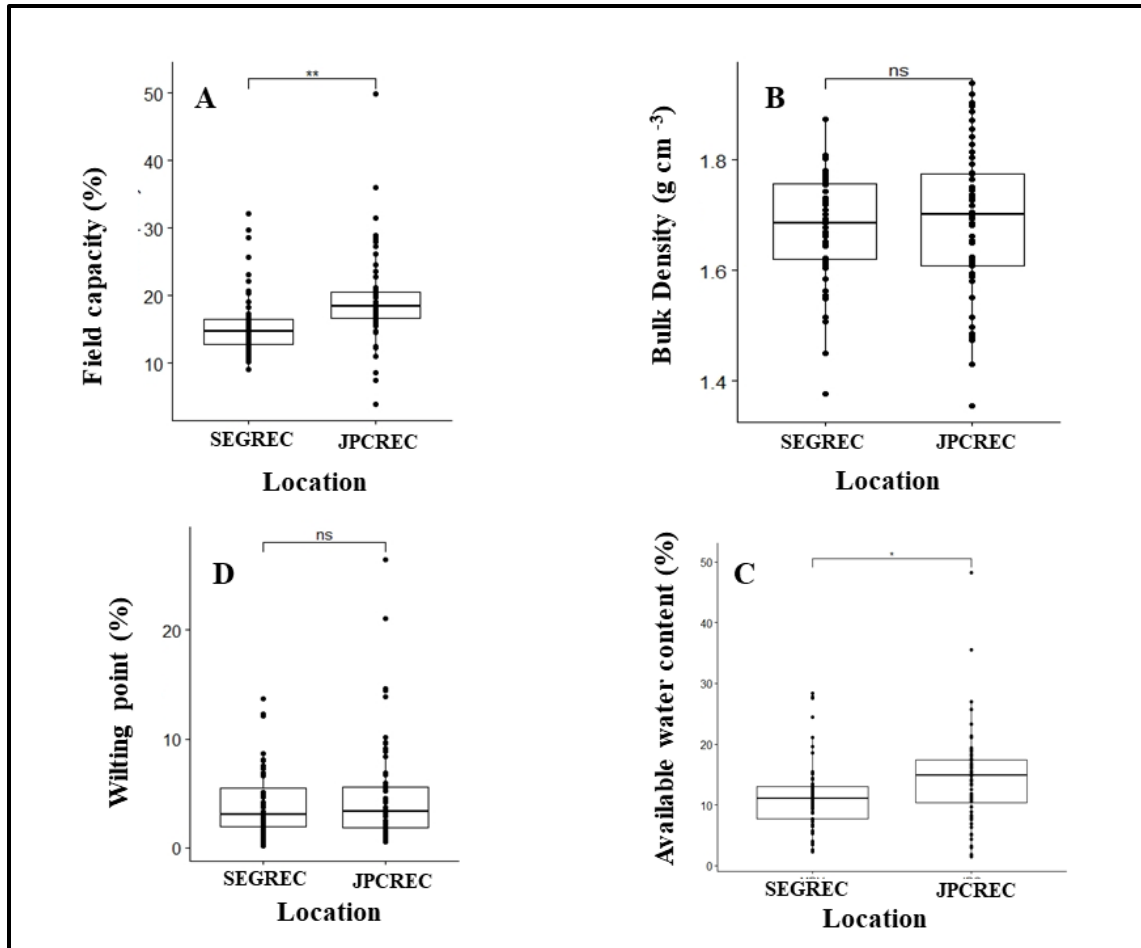


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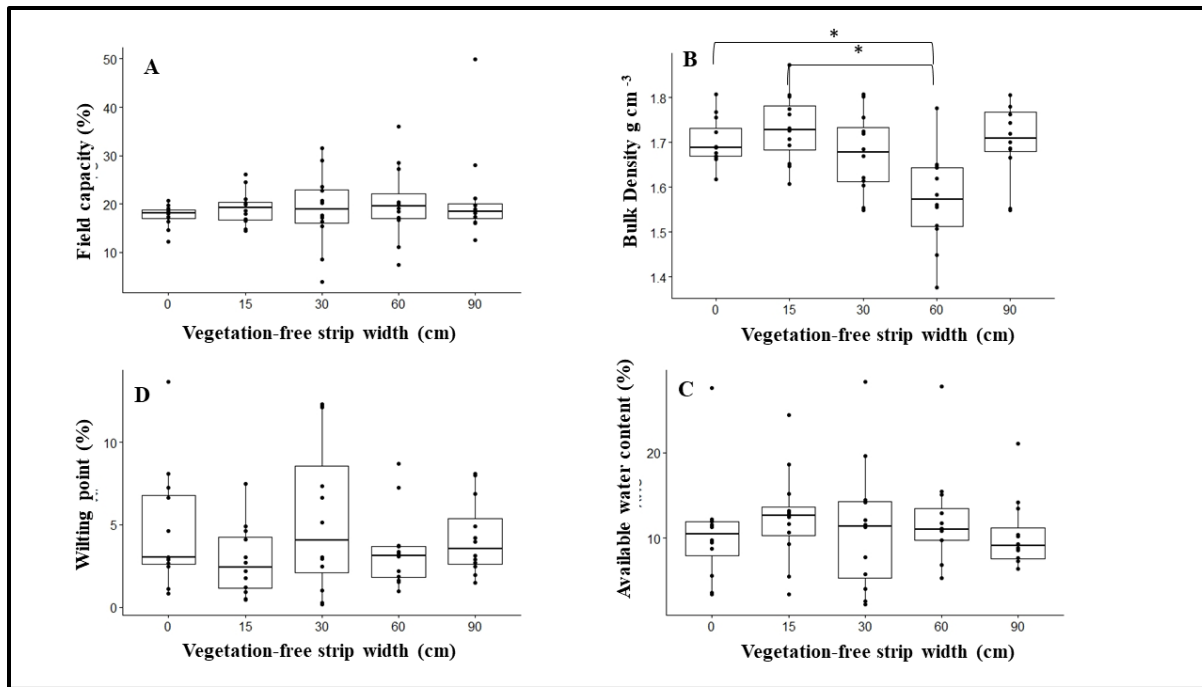


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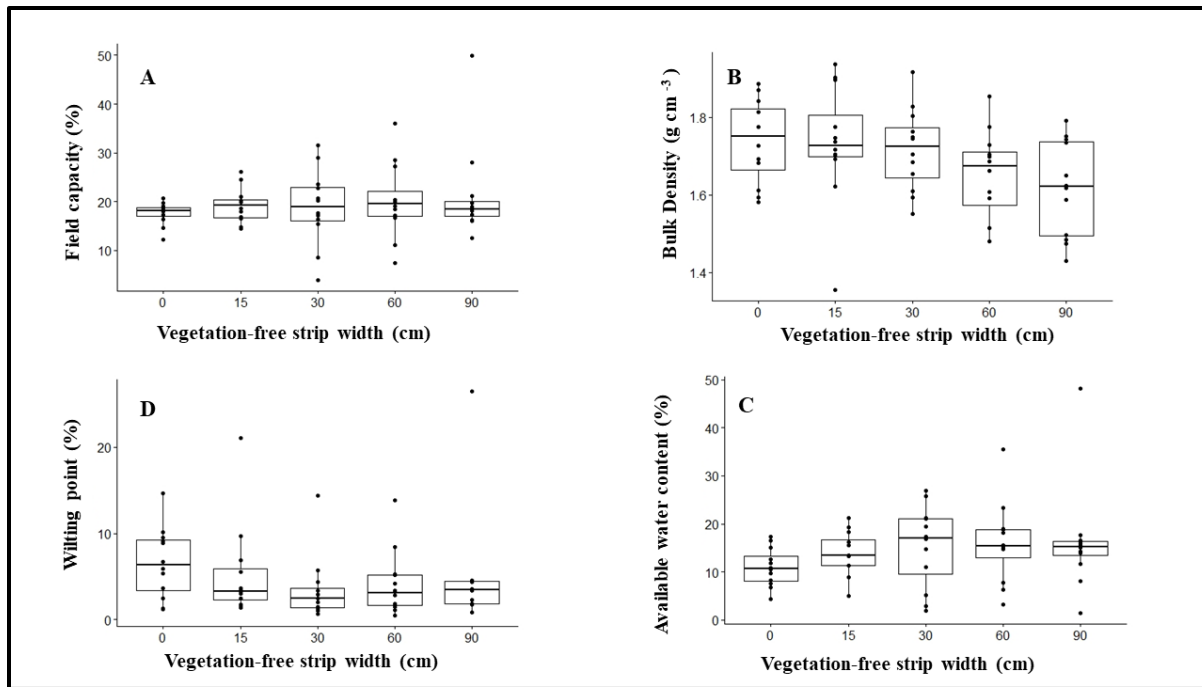


Figure 3.8

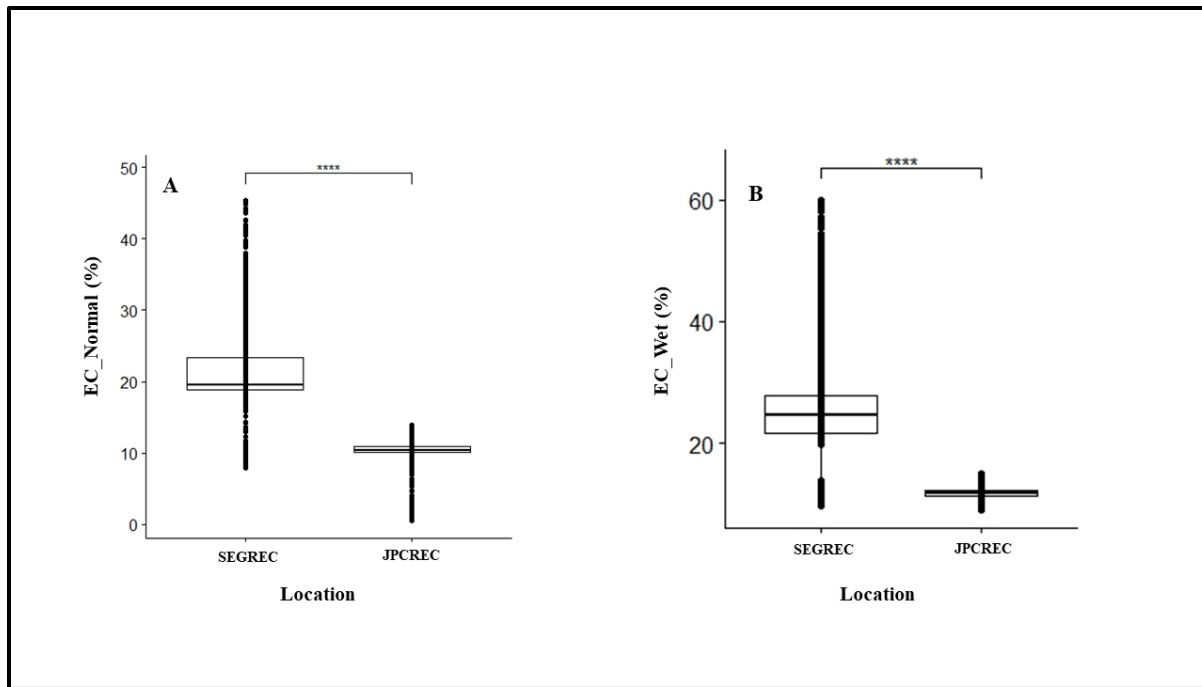


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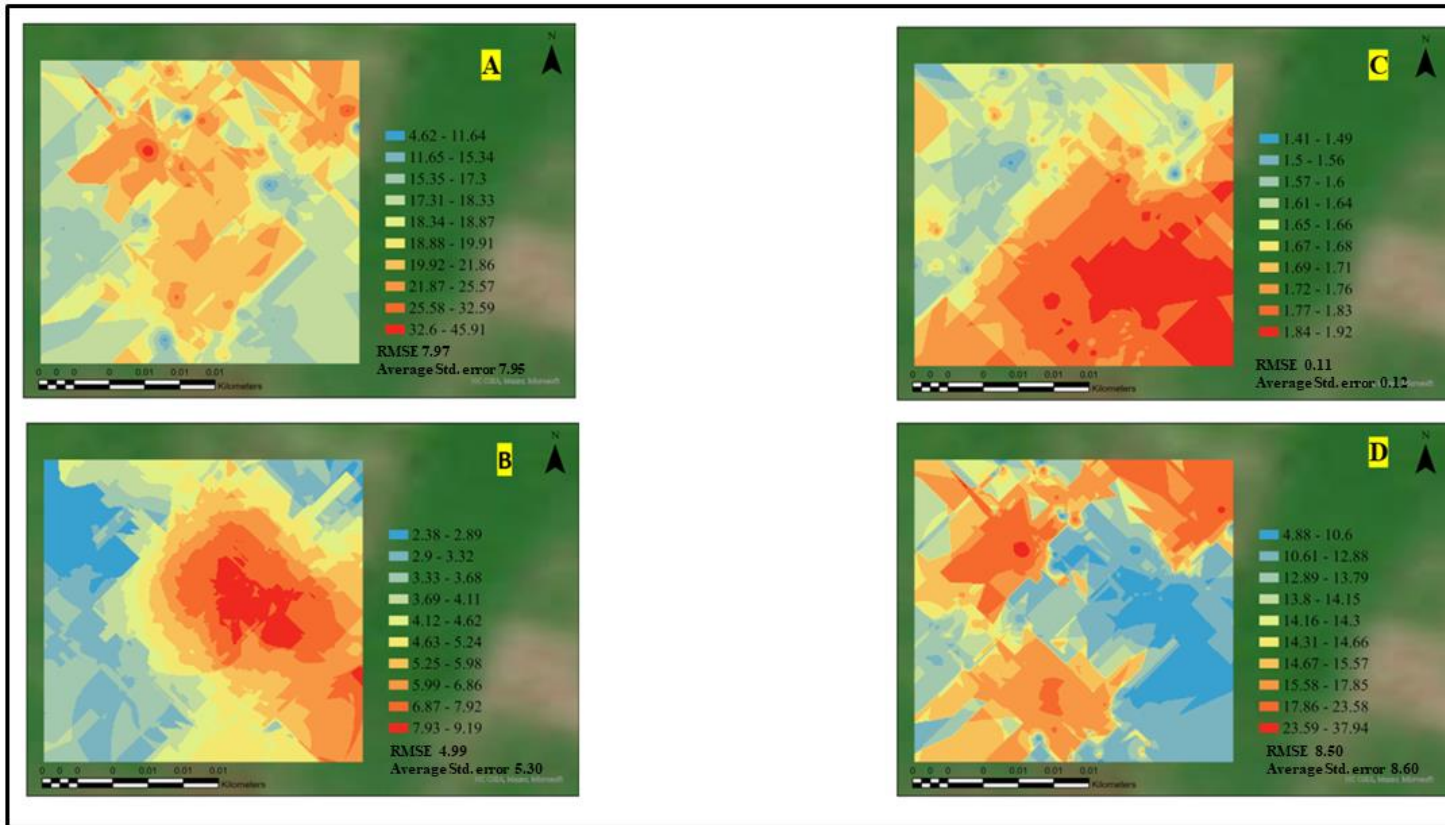


Figure 3.10

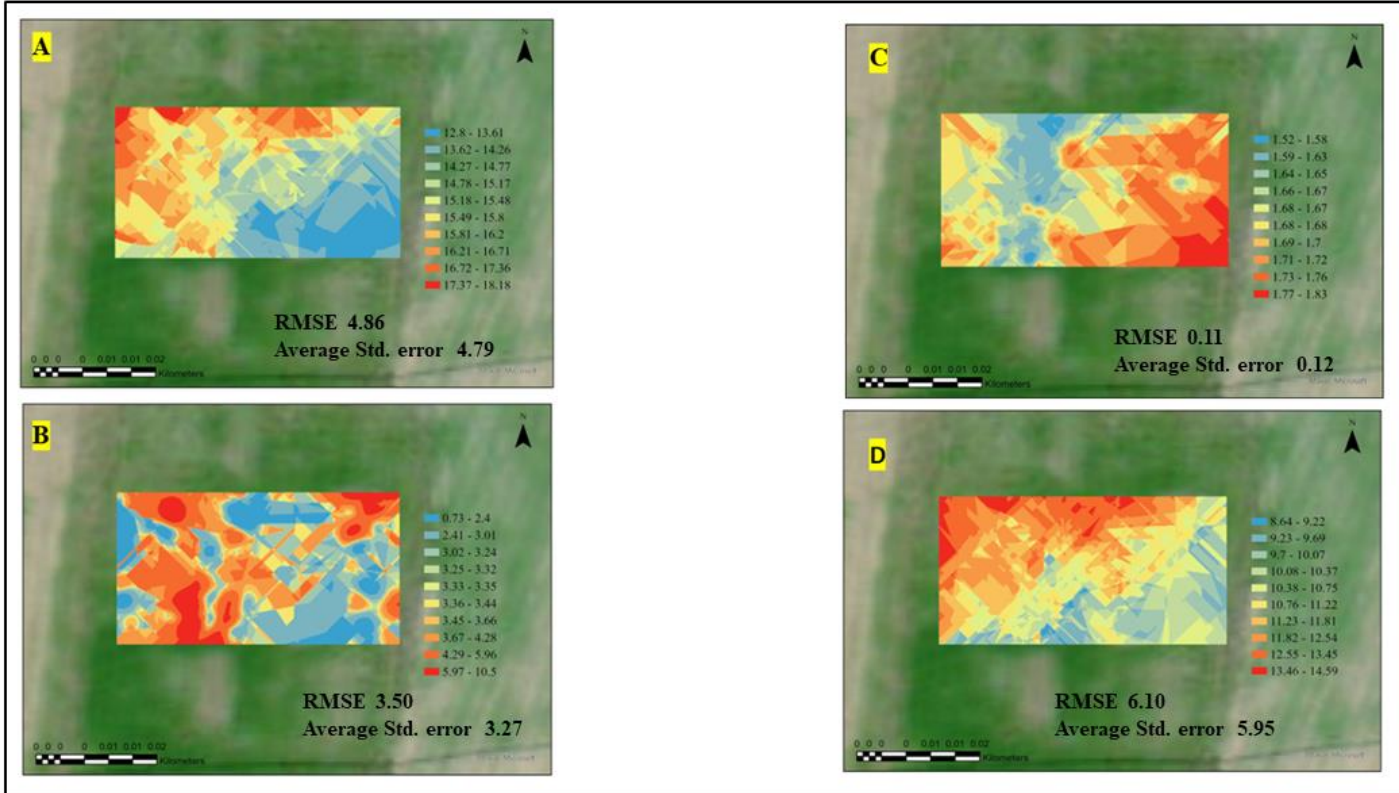


Figure 3.11

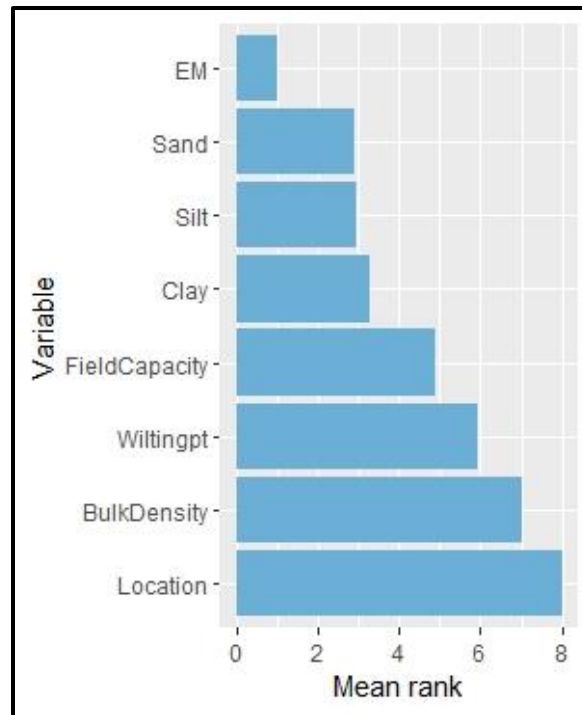


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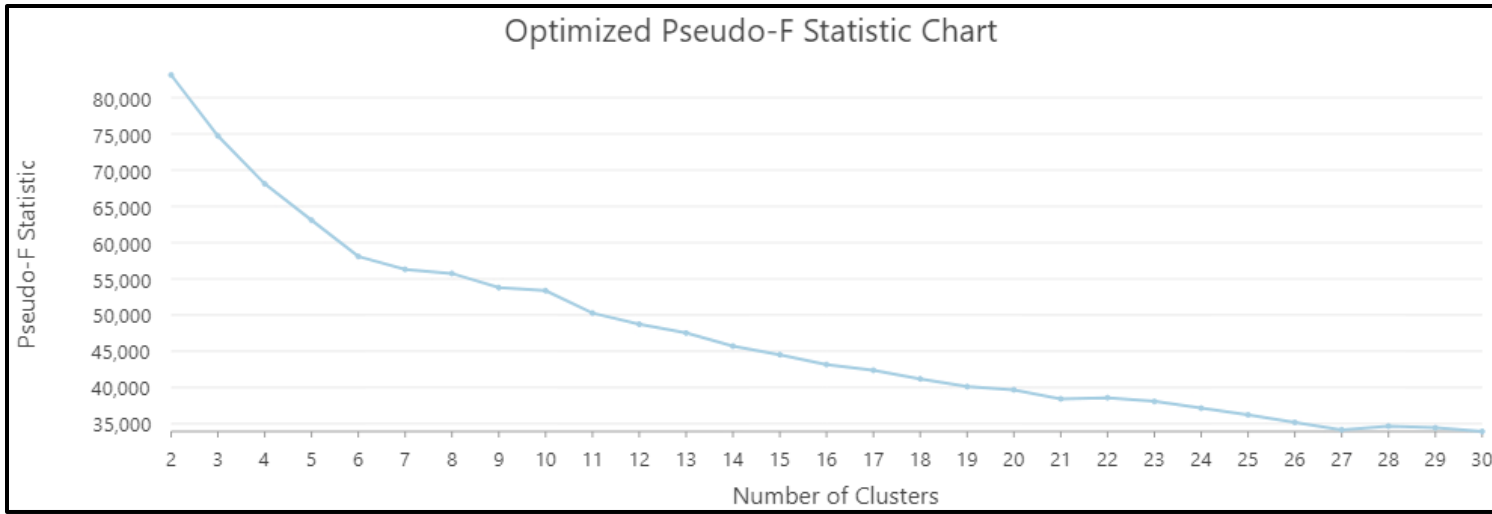


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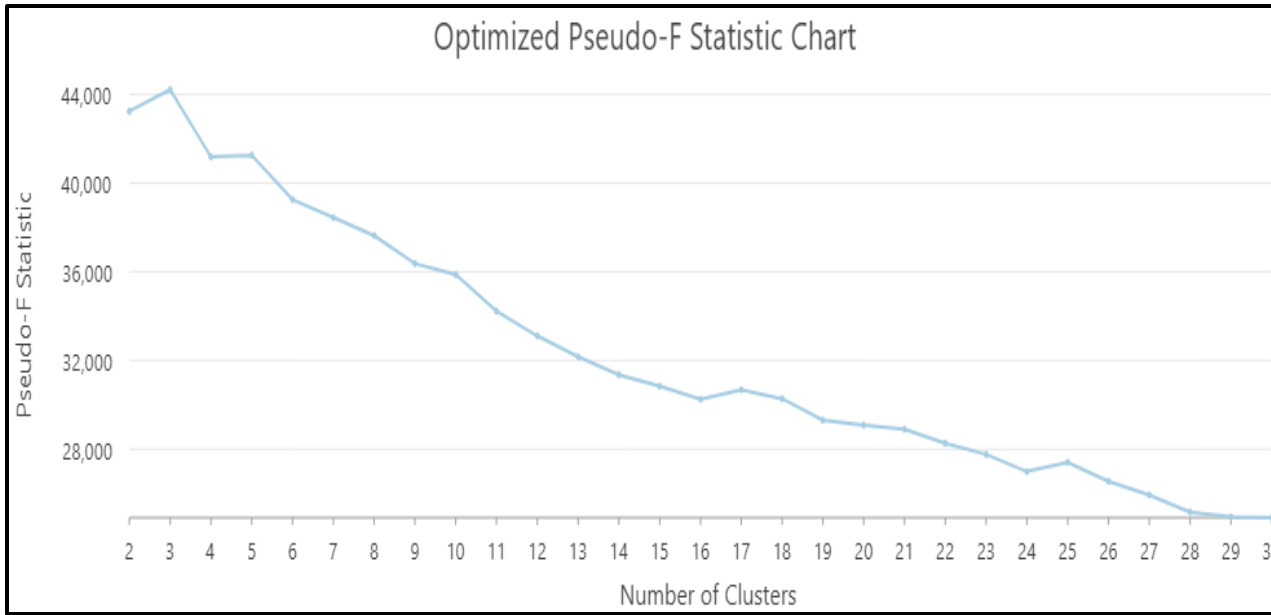


Figure 3.14

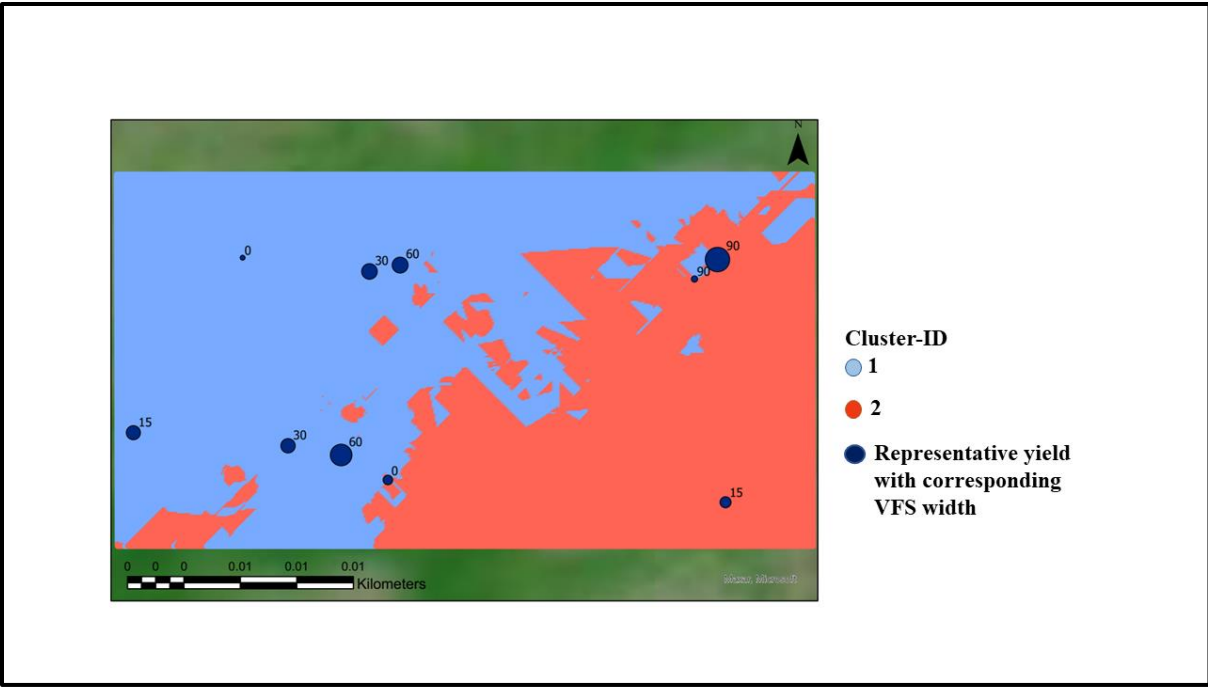


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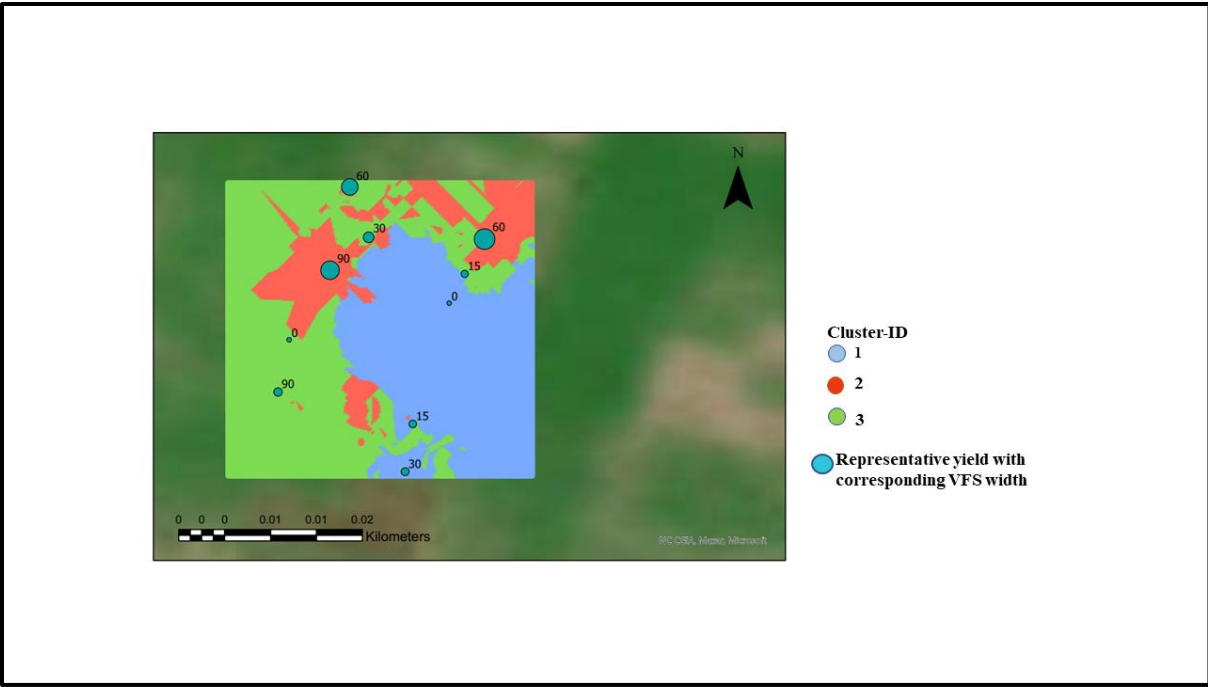


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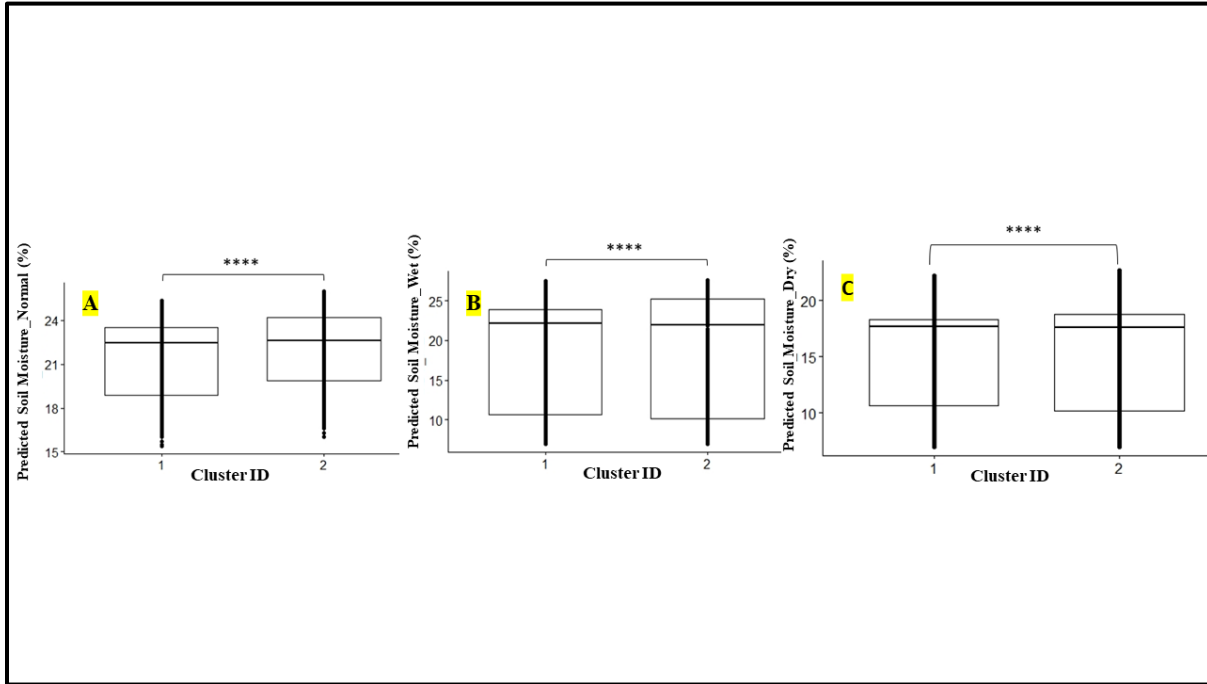


Figure 3.17

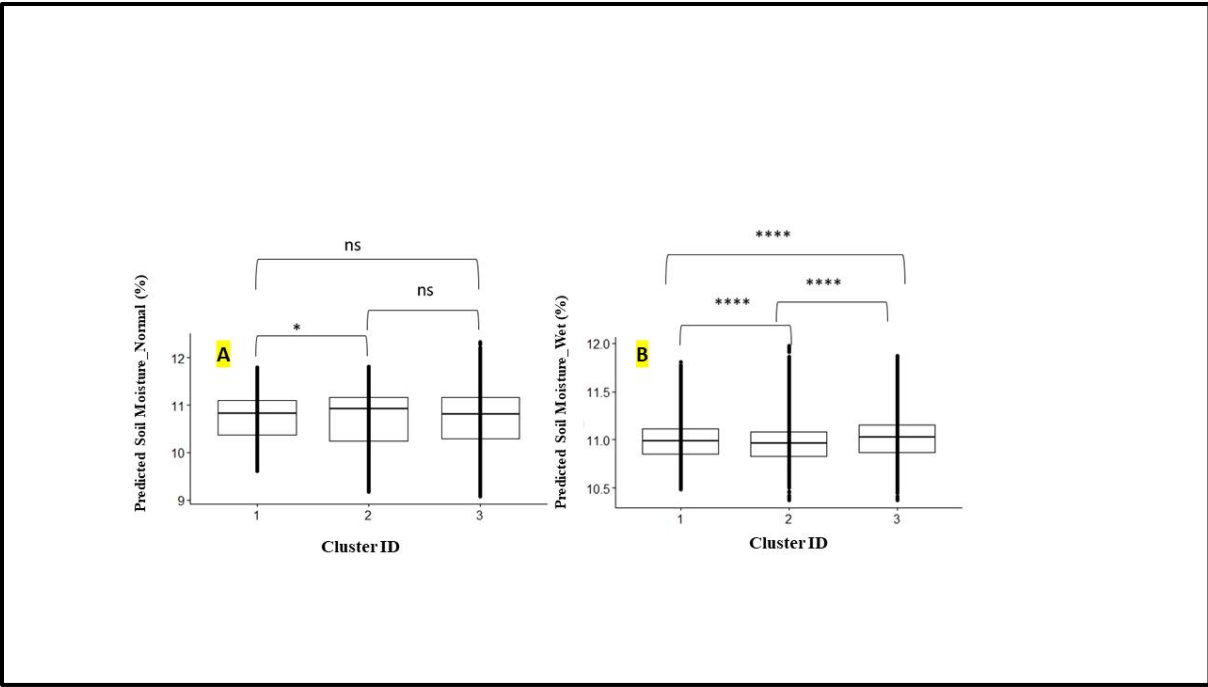


Figure 3.18

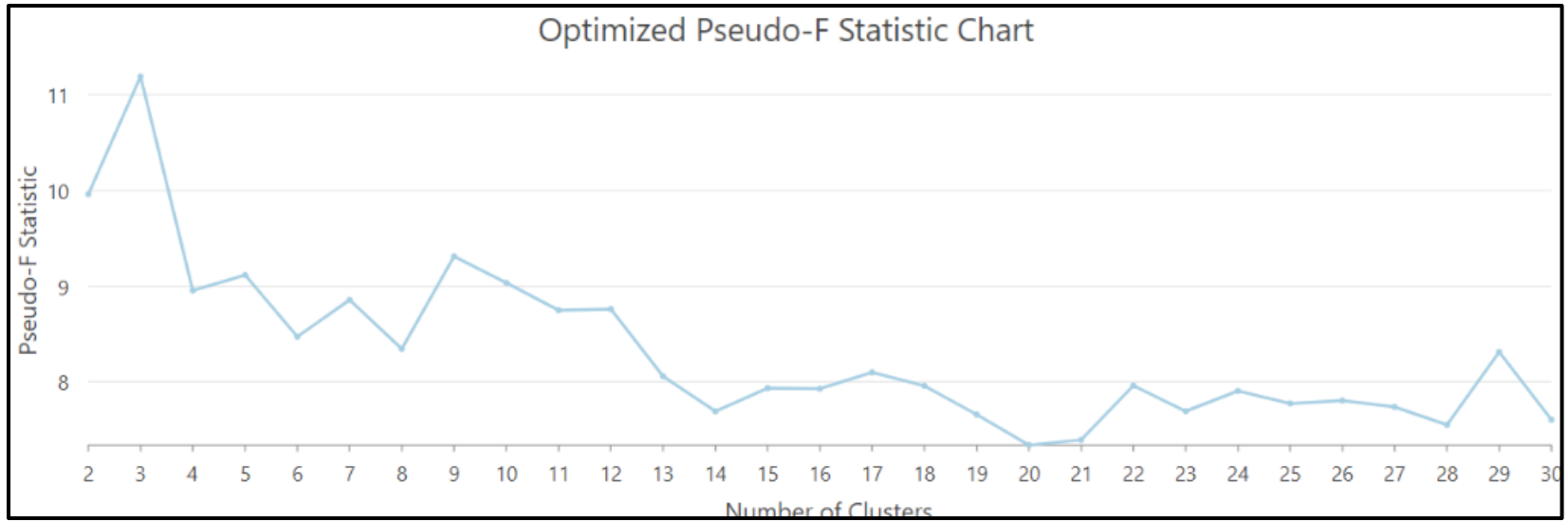


Figure 3.19

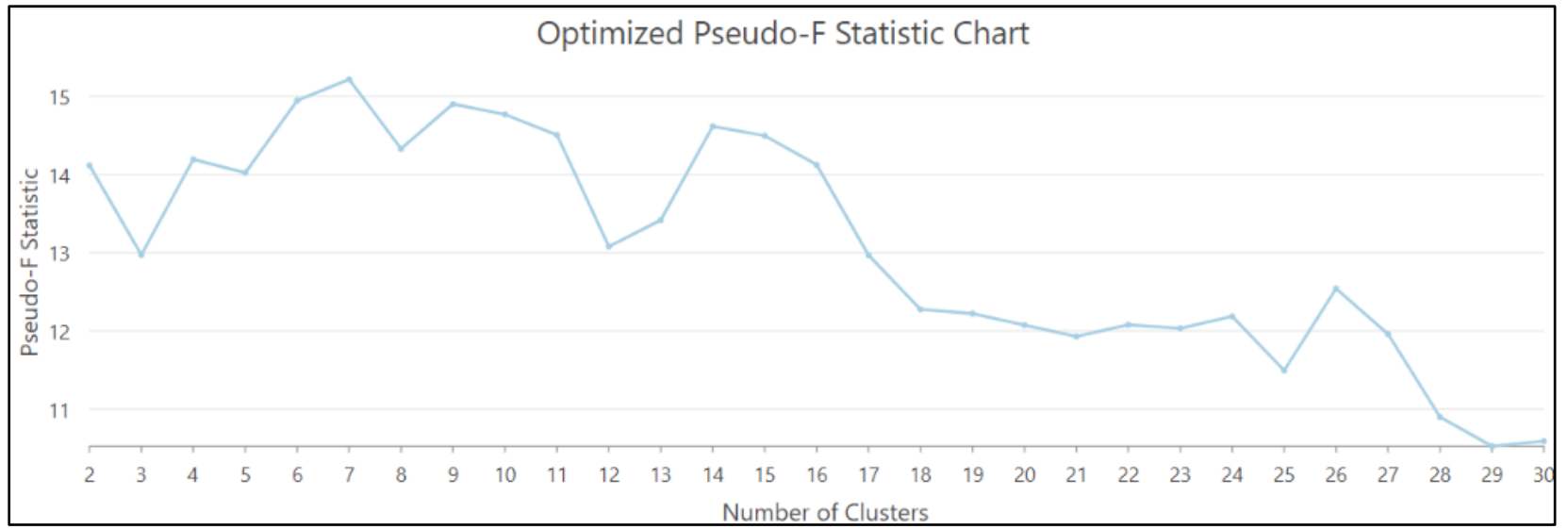


Figure 3.20

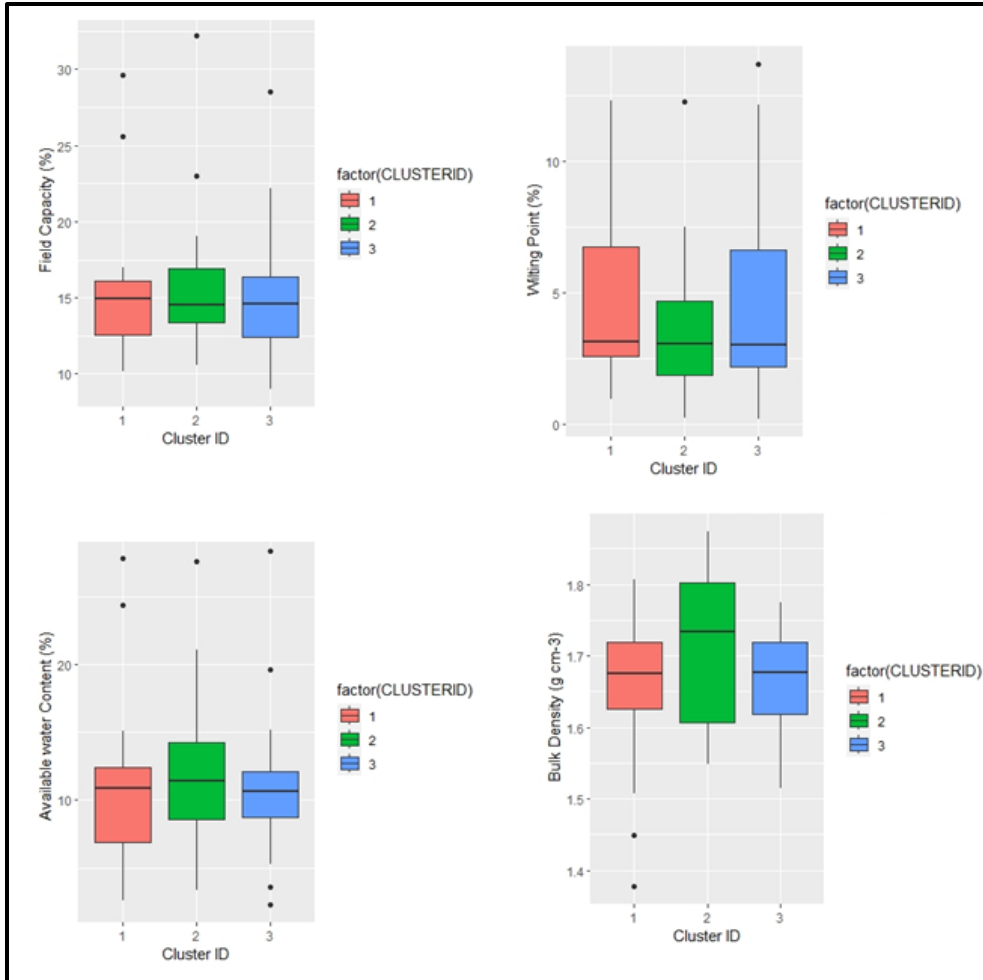


Figure 3.21

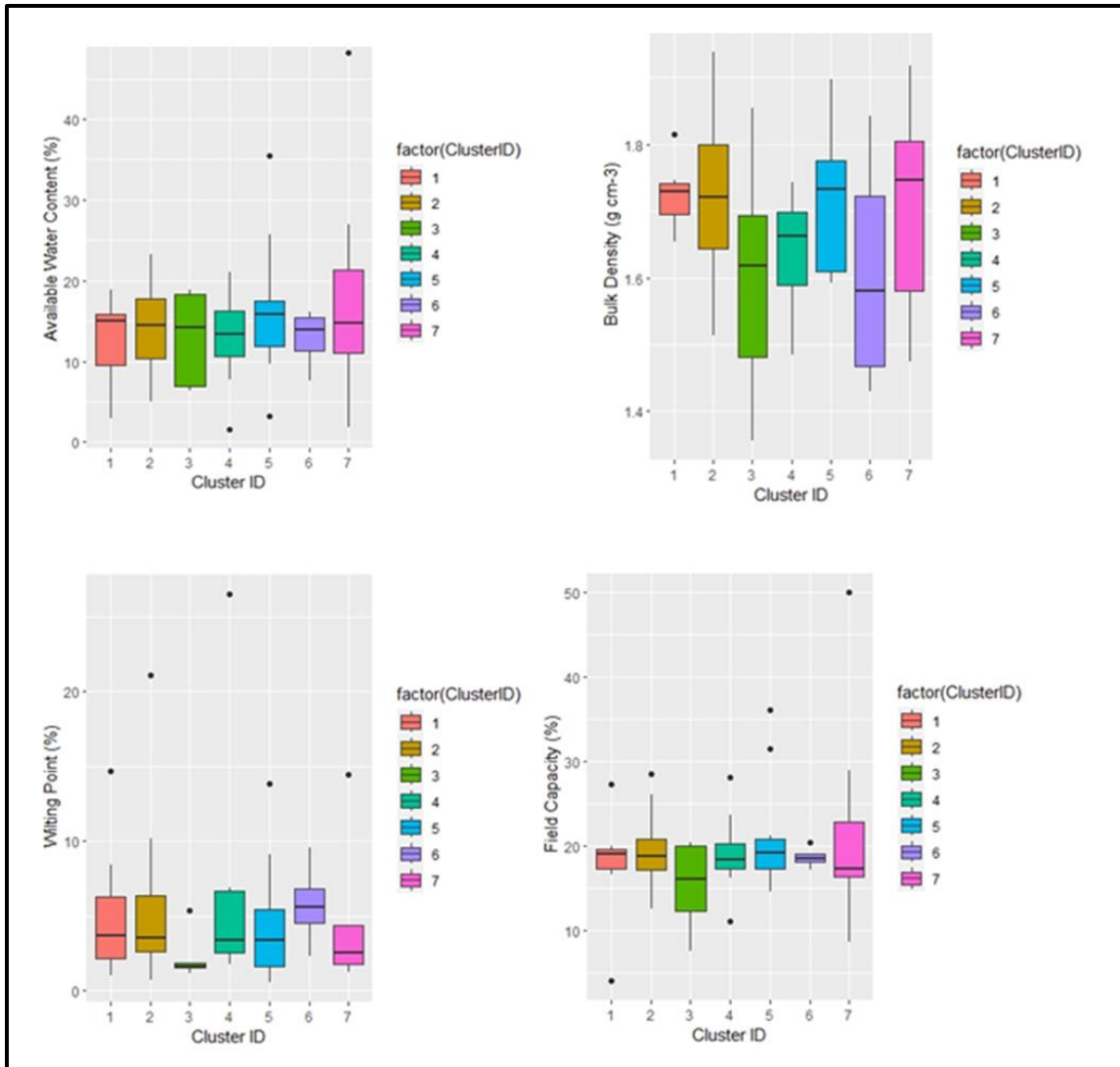
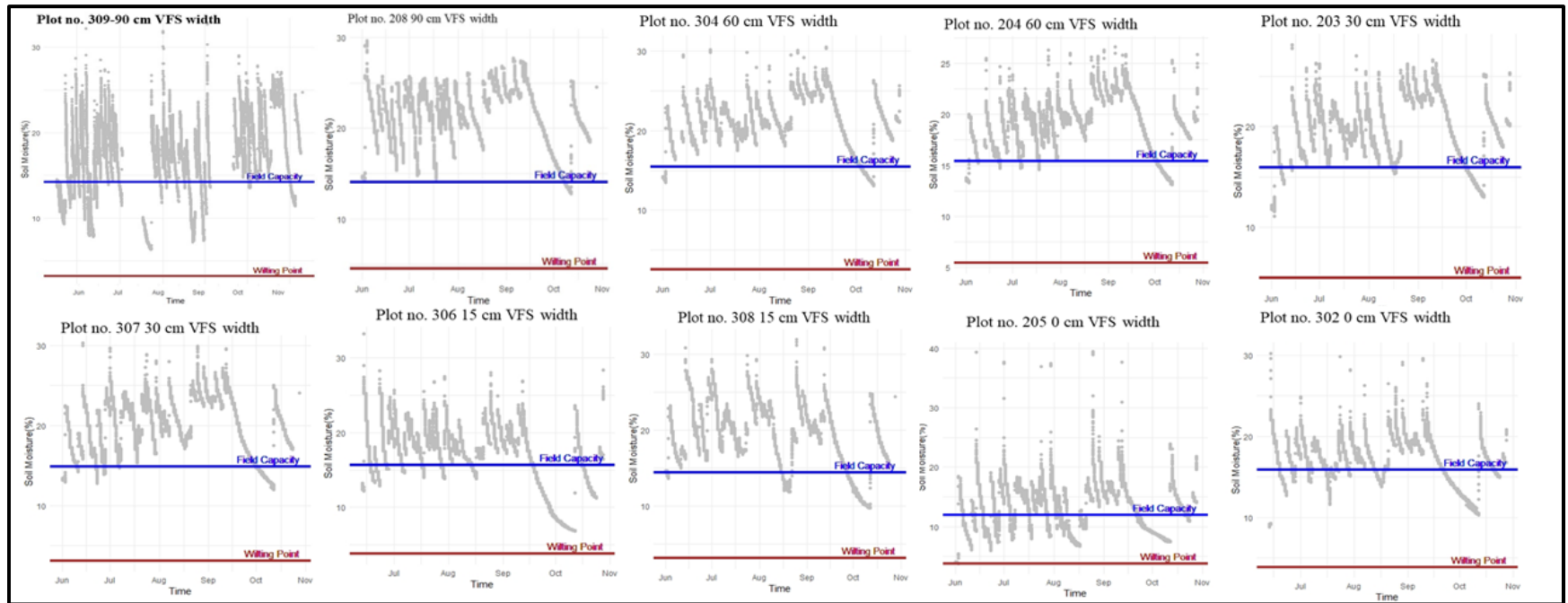


Figure 3.22

Appendix

The graph below shows the variable soil moisture collected with the help of TDR sensors installed at 10 cm depth in soil profile where the maximum root density of clover and cotton was found to exist. SEGREC had a consistent soil moisture above field capacity while at JPCREC the soil moisture was variable amongst plots with some being above field capacity and some below.

1)SEGREC



2) JPCREC

