Evaluation of Cover Crops, Conservation Tillage, and Nitrogen Management in Cotton Production in Southeastern Virginia

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Keywords: upland cotton, cover crops, conservation tillage, nitrogen management, fertilizer placement

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Academic Abstract

The response of upland cotton (*Gossypium hirsutum* L.) to legume and small grain cover crop establishment, in-season nitrogen (N) rate, and fertilizer N placement was investigated in two experiments located in coastal plain Virginia and North Carolina. The first experiment examined 1) soil compaction and cotton yield response to strip-tillage compared to no-tillage with a precision planted tillage radish and 2) the influence of legume mix, rye, and legume mix/rye combination cover crops with four in-season nitrogen (N) rates applied to cotton on cover crop biomass, cover crop nutrient uptake, soil compaction, soil N cycling, petiole nitrate-N (NO₃⁻-N) during the first week of bloom, cotton lint yield, and fiber quality parameters over two years. Legume mix cover crops resulted in greater N uptake, soil NO₃-N during the growing season, and lint yields compared to LMR, rye, and fallow treatments over both study years. Soil compaction and lint yields were not significantly different between strip-tilled and no-till with tillage radish treatments in either year. Relative lint yields after LM were maximized at 93% relative yield with 110 kg N ha⁻¹ applied in-season while relative lint yields for cotton following LM with 0 kg N ha⁻¹ applied reached 75%, measuring at least 9% higher than cotton following other cover crop treatments. The second experiment investigated the effect of five N rates (0, 45, 90, 135, and 180 kg N ha⁻¹) and three placement methods (broadcast, surface banded, and injected) on lint yield, petiole nitrate-N (NO₃⁻-N), lint percent turnout, and fiber quality parameters. Nitrogen rate and placement had a significant effect on lint yield but only N rate affected petiole NO₃⁻-N concentration. It was estimated that injecting fertilizer N requires an N rate of 133 kg N ha⁻¹ to achieve 95% relative yield while surface banded fertilizer N required a
rate of 128 kg N ha$^{-1}$ to produce 90% relative yield. A critical petiole NO$_3$-N concentration threshold of 5,600 mg NO$_3$-N kg$^{-1}$ was calculated to reach 92% relative yield. Other agronomic management practices such as cover crop termination timing, cover crop species blends, and number of fertilizer N applications are of interest in order to develop better recommendations and promote conservation agricultural practices in coastal plain Virginia and North Carolina.
Evaluation of Cover Crops, Conservation Tillage, and Nitrogen Management in Cotton Production in Southeastern Virginia

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General Audience Abstract

Upland cotton (Gossypium hirsutum L.) response to diverse species cover crop mixes, conservation tillage method, fertilizer N rate, and fertilizer N placement at side-dress was measured in two field studies conducted on the coastal plain soil in Virginia and North Carolina from 2016-2018. The objectives of the following research were to 1) examine the influence of two conservation tillage practices and four cover crop mixes on cover crop biomass production, soil compaction, cover crop nutrient uptake, soil N cycling, petiole nitrate (NO$_3$-N) and cotton lint yield and 2) measure cotton performance in response to five N rate and three placement application methods. Legume mix (LM) cover crops contained more N in biomass, resulting in higher soil NO$_3$-N during the growing season and higher lint yields at harvest compared to a legume mix and rye combination (LMR), rye, and fallow treatments. Soil compaction and lint yield were not significantly different between strip-tilled and no-till/tillage radish treatments in either year. Nitrogen rate and placement had a significant effect on lint yield but only N rate affected petiole NO$_3$-N concentration. Injection of fertilizer N required an N rate of 133 kg N ha$^{-1}$ to achieve 95% relative yield while surface banded fertilizer N required a rate of 128 kg N ha$^{-1}$ to produce 90% relative yield. A critical petiole NO$_3$-N concentration threshold of 5,600 mg NO$_3$-N kg$^{-1}$ was also calculated to reach 92% relative yield. Future application of these results can include investigation of optimal N source for Virginia cotton production, best N placement method for cotton grown in high residue systems, and an economic analysis to determine optimum agronomic management for Virginia coastal plain cotton production.
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1 Literature Review

Upland cotton (*Gossypium hirsutum* L.) is a perennial, tropical plant originating from Mexico, Guatemala, and northern South America. This textile fiber plant is now managed for annual crop production in temperate climates and comprises over 90% of international cotton production (Wendel and Cronn, 2003). In the United States, cotton is produced in seventeen states and accounts for more than $25 billion annually in services and products (USDA ERS, 2016). Virginia is the northernmost cotton-producing state on the East Coast and hectares in cotton production have increased from 2015 to 2018 (Virginia Department of Agricultural and Consumer Services, 2018). The wide geographic range of cotton production in the United States results in the need for a variety of management strategies that can be tailored depending on the region, soil type, and resources available. As more land in Virginia is utilized for cotton production, it is imperative to investigate and implement different tillage, cover cropping, and nutrient management systems that can maximize economic and environmental sustainability.

1.1 Conservation Tillage Systems

Choosing a tillage system is a cost-benefit decision based on cost, resources, and familiarity with a certain tillage system. The traditional method of soil cultivation for farming is referred to as conventional tillage. This process involves using machinery to disturb the soil surface prior to planting in order to create a uniform, fine seed bed (Siemens, 1980). Historically, conventional tillage has been the primary method used in Virginia to prepare land before cotton planting to disrupt the compacted, high strength layers that are present in Virginia’s coastal plain soils (Radford et al., 2001). These hardpan layers are ubiquitous in mid-Atlantic coastal plain soils and have the capacity to restrict cotton root growth (Radford et al., 2001). Though conventional and deep tillage practices may provide decreased soil compaction beneficial for cotton establishment, there are distinct disadvantages of using this tillage system. Conventional
tillage contributes to higher rates of soil erosion, soil moisture loss, and soil organic carbon loss (Busari et al., 2015). The last half century has seen a significant push from agronomists, government agencies, and conservationists to transition from conventional tillage to other forms of land preparation.

Alternative methods of tillage that aim to reduce soil disturbance are known as conservation tillage practices. The Conservation Technology Information Center (2002) defines conservation tillage as any type of soil cultivation that leaves at least 30% of the soil surface covered with crop residue after a previous harvest. Methods of conservation tilling such as no-till, strip-till, and mulch till have been recommended by government agencies and have been adopted by rate of 63% by cotton producers in Virginia (Reiter and Frame, 2016). Benefits of implementing a conservation tillage program include increasing soil organic matter, soil aggregation, and water holding capacity while decreasing nutrient runoff and soil erosion in comparison to conventional tillage (Holland 2004; Busari et al., 2015).

The transition to conservation tillage in cotton production can also provide economic benefits. Buman et al. (2005) also conducted a survey of field studies to determine how the adoption of no-till cotton production affected cotton yield. The authors found no decrease in yield and lower production costs associated with both no-till and strip till compared to conventional tillage (Buman et al., 2005). The authors also claim that lower production costs can offset the economic effect of lower yields in no-till systems that result from varied rainfall or climate conditions (Buman et al., 2005). Recent studies by Toliver et al. (2012) and Ogle et al. (2012) have supported these results and found little difference in yield between no-till and conventional tillage practices. More experimentation with different conservation tillage systems in cotton production are needed to evaluate their effects on yield among other parameters (Bauer and Busscher, 1996).
1.2 Cover Crops

Cover crops are frequently recommended to be used along with conservation tillage practices in order to promote greater sustainability and economic gain for cotton producers (Tyler et al., 2000). Cover crops are planted alongside or in rotation with cash crops to help reduce soil erosion, increase soil organic matter, and suppress weeds (Larson et al., 2001). Using cover crops in a conservation tillage system is especially recommended when planting cotton, a crop considered to be a greater erosion hazard than corn or soybean (Nyakatawa et al., 2001). The little surface residue left by cotton after harvesting is ineffective in controlling soil erosion by wind and water. Economic outcomes in cotton production are also tied to soil conservation efforts. McDaniel and Hajek (1984) found a 4% decline in cotton lint yield for every centimeter of topsoil loss, making the adoption of cover cropping and other soil conservation efforts an important part of maintaining revenue for years to come.

A wide variety of cover crop species can be implemented depending on the desired benefits, climate of the region, and cash crop that is being planted. The most common cover crops used in Virginia cotton production are winter wheat (Triticum aestivum) and rye (Secale cereale), two small grains crops with fibrous roots systems that are effective in controlling erosion and capturing nutrients in the soil profile (Schomberg et al., 2006). Wendt and Burwell (1985) conducted a study on no-till corn that showed the inclusion of a rye or wheat cover crop could decrease annual soil loss by 95% compared to a fallow system. Positive effects on yields have also been measured when using a rye cover crop. A study by Bauer and Busscher (1996) found that rye produced a positive effect on cotton yields in a conservation tillage system but not when planted with conventional tillage. Despite these benefits, the high biomass content and C:N ratio of these small grain covers also have an immobilizing effect on nitrogen (N) in the soil environment, making less N available to cotton and increasing fertilizer demands for maximum
cotton yields (Dabney et al., 2001, Reiter et al., 2008). Varco et al., (1999) described an increased rate of 120 kg N ha\(^{-1}\) required to produce maximum cotton lint yields following a rye cover crop and a rate of 96 kg N ha\(^{-1}\) required after winter fallow. This higher required N rate that is needed raises costs for cotton producers and needs to be considered when choosing a cover crop.

The higher fertilizer demands present with rye could be lowered if a legume is introduced into the mix. Leguminous cover crops such as crimson clover (\textit{Trifolium incarnatum}), hairy vetch (\textit{Vicia villosa} L.), and Austrian winter pea (\textit{Pisum sativum}) form a symbiotic relationship with \textit{Rhizobium} bacteria that can fix atmospheric N and contribute 32 - 45 kg N ha\(^{-1}\) to the soil N pool, which can reduce the need for fertilizer N in cotton production when cotton follows a pure legume cover crop stand (Brown et al., 1985; Tyler et al., 2000, Rochester et al., 2001; Sainju and Singh, 2008). A combination of both legume species and a small grain has the potential to offer the soil fertility benefits of both cover crops such as increased soil N, reduced N leaching, reduced soil erosion, and increased nutrient capture (Sainju et al., 2006). Bauer et al. (1993) showed that crimson clover and Austrian winter pea can supply adequate N and that incorporation of rye into the green manure system did not increase N fertilization requirements for a greater cotton yield or improved lint quality. Further research on the optimal cover crop mix that maximizes cotton N uptake, yield, and lint quality while minimizing additional fertilizer costs is still needed.

1.3 Nitrogen Management

An important aspect of cotton agronomy that affects yield, fiber quality, and environmental health is nutrient management. An essential element in nutrient management is N, a major component of chlorophyll, amino acids, and proteins in plants (Gerik et al., 1994). Nitrogen is also vital for the proper synthesis of the seed wall and subsequent lint development in
cotton, making N management a very economically important part of cotton growth (Arnall and Boman, 2012). Cotton is negatively affected by both under-fertilization and over-fertilization, as N deficiency limits leaf size, carbon dioxide assimilation, fruit development, yield, and water uptake, while N overabundance causes excessive vegetative growth, delayed maturity, and reduced lint yield and fiber quality (Hons et al., 2004).

Nitrogen application rates must be highly customized based on soil type, location, climate, tillage system, and potential yield in order to meet plant nutrient demands and limit negative impacts on environmental quality. While some have observed a linear relationship between N fertilization rates and lint yield, others have shown that yields can stagnate or even decrease after a certain rate (Fritschi et al., 2003; Boquet et al., 2005).

The current recommended N application rates for cotton producers in Virginia range from 67-135 kg N ha\(^{-1}\), where 23-27 kg N ha\(^{-1}\) is needed to produce a 218 kg bale of lint (Frame et al., 2016). The fertilization rate in Virginia depends on soil texture, with sandier soils requiring a Higher N rate (Frame et al., 2016). This may be due to the predisposition of sandy, well drained soils with shallow water tables, especially coastal plain soils, to leach N into groundwater and volatize N as gases in the atmosphere (Sallade and Sims, 1993). The balance of providing enough N for plant use and limiting N losses is a long-standing challenge facing agroecosystems.

The placement method of N can also influence rate and the efficiency of nutrient uptake in cotton (Mahler, 2001). The most common forms of N application are broadcast, surface banding, and injection (Gerik et al., 1998). Broadcasting is a method by which fertilizer is applied uniformly across an entire field, making it a fast and economical choice for producers but leaving the system vulnerable to denitrification, volatilization, and leaching, especially in no-till systems (Doran, 1980). The placement and dribbling of fluid N on the soil surface is called surface banding. This method requires less fertilizer and can improve nutrient use efficiency but
requires specific equipment for application (Mahler, 2001). Fertilizer injection can promote N uptake by roots through precise placement of fluid N below the soil surface and into the root zone (Thompson and Varco, 1996; Howard et al., 2001). Several studies on cotton response to N placement have shown the inefficiency of broadcasting N in cotton production (Thompson and Varco, 1996; Howard et al., 2001; Nkebiwe et al., 2016; Adviento-Borbe et al., 2018), but little research comparing multiple placement methods across fertilization rates has been done on the coastal plain soils of the southeastern United States.

1.4 Overall Objectives

The objectives of this research are to 1) examine the influence of two conservation tillage practices and four cover crop mixes on cover crop biomass production, soil compaction, cover crop nutrient uptake, soil N cycling, petiole nitrate (NO$_3$-N) and cotton lint yield and 2) measure cotton performance in response to five N rate and three placement methods of fertilizer N.

1.5 References


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2 Agronomic Efficiency of Cover Crop Mixes and Conservation Tillage in Cotton Production in Southeastern Virginia

2.1 Abstract

The impact of cover crop mixes and conservation tillage on nutrient cycling and uptake of cotton (Gossypium hirsutum L.) grown on the coastal plain soils of southeastern Virginia is not well described. The objective of this study was to examine 1) soil compaction and cotton yield response to strip-tillage compared to no-tillage with a precision planted tillage radish and 2) the influence of legume mix, rye, and legume mix/rye combination cover crops with four in-season nitrogen (N) rates applied to cotton on cover crop biomass, cover crop nutrient uptake, soil compaction, soil N cycling, petiole nitrate-N (NO$_3$-N) during the first week of bloom, cotton lint yield, and fiber quality parameters. Legume mix cover crops resulted in greater N uptake, soil NO$_3$-N during the growing season, and lint yields compared to LMR, rye, and fallow treatments over both study years. Soil compaction and lint yields were not significantly different between strip-tilled and tillage radish treatments in either year. Cover crop mix and N rate had a significant (P<0.0001) impact on lint yield, where relative lint yields after LM were maximized at 93% relative yield with 110 kg N ha$^{-1}$ applied in-season. Relative lint yields for cotton following LM with 0 kg N ha$^{-1}$ applied reached 75%, measuring at least 9% higher than cotton following other cover crop treatments. Legume cover crops preceding cotton may allow for the reduction in fertilizer N recommendations for cotton production in southeastern Virginia.

**Keywords** - upland cotton, cover crops, conservation tillage, tillage radish, nitrogen cycling

2.2 Introduction

Conservation agriculture addresses the need for high production output and decreased environmental footprint through implementing practices such as conservation tillage, cover cropping, crop rotation, and nutrient management (Franzluebbers, 2008). A recent
comprehensive report published by the United States Department of Agriculture (USDA) Economic Research Service (ERS) identified the southern seaboard region of the country to have the highest rates of both conservation tillage and cover crop adoption (Wade et al., 2015). The authors also noted that cotton producers in this region planted a lower share of cotton in no-till and strip-till systems than the average share for crops in conservation tillage systems (Wade et al., 2015). To encourage higher rates of adoption by cotton producers in the southeastern U.S. coastal plain and Virginia, a greater depth of research is needed to see the interactions between different conservation management strategies and cotton production.

The transition from conventional agricultural practices to conservation agriculture provides producers with a variety of tillage choices. Baker et al. (2002) describes the varied and sometimes interchangeable terms that refer to conservation tillage practices. No-tillage (no-till), reduced tillage, strip-tillage (strip-till), chemical fallow, and ridge tilling are all frequently labeled as conservation tillage techniques (Baker et al., 2002). No-till leaves the soil mostly undisturbed except for planting while strip-tillage involves planting the seed into a narrow strip that is 25-40 cm wide and maintains residue cover (Busari et al., 2015; Wade et al., 2015). Tillage method is a major concern in Virginia because of the hardpan layers commonly present in the E horizon of coarse-textured, coastal plain soils (Gorucu et al., 2006).

Mixed results have been derived when testing how cotton responds to no-till and strip-till systems. Sainju et al. (2006) found that lint yields and nitrogen (N) uptake were equal to or better in a cover cropped no-till system than with cover cropped strip-till. Daniel et al. (1999) and Mitchell et al. (2012) found no difference in lint yield or soil quality between the two conservation tillage systems. Buman et al. (2005) also reported little difference between lint yield when comparing no-till and strip-till in cotton production but noted lower production and labor costs in no-till systems compared to strip-till. Other research has determined strip-till to be
the premier conservation tillage system for cotton production on the basis of yield and profitability (Khalilan et al., 2004; Schomberg et al., 2006; Raper et al., 2007). Comparison of these two conservation tillage methods is warranted to provide updated management recommendations for coastal plain soils in Virginia.

Biological tillage in no-till systems through the use of cover crops may also help alleviate soil compaction in coastal plain cotton production systems. Biological tillage refers to the creation of pores by cover crop roots which can then be used as low resistance pathways by roots of the succeeding crop (Chen and Weil, 2010). Research conducted on the efficacy of biological tilling in cotton has mostly focused on rye (*Secale cereale*) and tillage radish (*Raphanus sativus*). The taproot morphology present in tillage radish makes it much more efficient in breaking up compacted soil than rye, but results relating to its effect on cotton yields are mixed. Norsworthy et al. (2011) determined that rye did not affect cotton yields but plots with tillage radish had lower yields. Marshall et al. (2016) found significant reductions in soil compaction due to the forage radish cover crop and a higher cotton lint yield in their no-till study on a coastal plain soil in South Carolina. Though the potential for lessening soil compaction in coastal plain soils may make forage radish an attractive option for cotton producers, more research needs to be conducted relating cover crop types and tillage method in this region before it can be established as a viable option for cotton production.

Cover crops can have a marked impact on N dynamics in cotton production systems and can require adjusted rates of N fertilization in-season. Higher rates of N fertilization to produce optimum lint yields with rye, a common cover crop for cotton, have been reported by a series of agronomic studies (Varco et al., 1999; Howard et al., 2001; Reiter et al., 2008; Ducamp et al., 2012). This is due to the high C:N ratio in small grain biomass that immobilizes available soil N for microbial consumption, thus decreasing the amount of N available to cotton (Reiter et al.,
Legume cover crops are able to introduce N into the soil through N fixation and lower the C:N ratio, thus lessening the amount of N needed to maximize yields for cotton production systems (Larson et al., 2001; Rochester et al., 2001; Boquet et al., 2004). Other studies have found that legumes can introduce over 150 kg N ha\(^{-1}\) into the soil environment; however, this N may not be mineralized from residues in phase with highest cotton N demands, which occurs at mid-bloom for boll development (Zablotowicz et al., 2011). A combination of both rye and legumes in cotton production systems may provide the benefits of a small grain cover crop, such as nutrient capture, while also adding N to the soil, possibly easing the higher N rate requirement that is needed after a pure rye cover (McCracken et al., 1994; Kuo and Sainju, 1998). Sainju and Singh (2008) determined that a vetch-rye mix produced similar cotton N uptake, soil N storage, and N fertilization requirements compared to a pure vetch cover on a sandy loam soil in Georgia. Further investigation of cotton response to diverse cover crop mixes across N application rates is warranted to understand the impacts of conservation agriculture on the coastal plain of Virginia.

The objectives of this research were to 1) compare the use of precision-planted tillage radishes as a replacement for strip-tillage to alleviate in-row soil compaction on coastal plain soils in Virginia and 2) evaluate the impact of legume and small grain cover crops on N cycling and cotton performance in southeastern.

2.3 Materials and Methods

2.3.1 Experimental Site and Design

This study was conducted at the Tidewater Agricultural Research and Extension Center in Suffolk, VA from 2016-2018. Soil characteristics for the two field sites can be found in Table 2.1 while total precipitation can be found in Fig 2.1. Strip-tilled corn (\textit{Zea mays} L.) was grown and harvested before this experiment at both sites. Corn was chosen to precede cotton in this experiment in order to plant cover crops early in the fall to promote maximum biomass
establishment. The experiment was a randomized complete block design, with a split-plot treatment design where cover crop mix was the whole plot treatment. Four cover crop treatments of fallow, rye (Secale cereale), legume mix (crimson clover: Trifolium incarnatum, hairy vetch: Vicia villosa R., woolypod vetch: Vicia villosa R. ssp. dasycarpa), and a legume mix/rye combination was also planted in the fall after corn harvest. The legume mix (LM) and legume mix/rye combination (LMR) consisted of only crimson clover and woolypod vetch in 2018 due to seed availability issues for hairy vetch in that year. Wheeler© rye (Moore Seed, Elsie, MI) was used in 2017 while Dura rye (variety not stated) was used the following year. The seeding rates for the cover crops were 84 kg ha⁻¹ of rye, 34 kg ha⁻¹ of the LM split evenly amongst included species, and 56 kg ha⁻¹ of rye with 28 kg ha⁻¹ of legumes species for LMR. The subplots consisted of a 2 x 4 factorial treatment design corresponding to the tillage and N rate treatments. Four replications were implemented in each year. Each whole plot was eight rows wide (0.91 m rows) x 49.1 m long. Sub-plots consisted of four rows having precision tillage radish (Raphanus sativus) planted in the fall and cotton planted no-till in the spring while the other four rows was strip-tilled in the spring. Tillage radish seed was drilled at a rate of 6.7 kg ha⁻¹. All cover crops were planted with a no-till drill. Cover crops received 34 kg N ha⁻¹, 45 kg P ha⁻¹, and 56 kg K ha⁻¹ of starter fertilizer in the fall in order to promote maximum biomass establishment.

Four total N application rates were applied at 0 kg N ha⁻¹, 45 kg N ha⁻¹, 90 kg N ha⁻¹, and 135 kg N ha⁻¹ at side-dress. Starter N fertilizer was applied at cotton planting at a rate of 28 kg N ha⁻¹ 5 cm from the row and 5 cm below the seed using a two-row Monosem planter outfitted with a coulter and fertilizer knife (Monosem Inc, Edwardsville, KS). The remaining N was applied at the matchhead square growth stage, the point where the cotton flower bud is 2-3 mm in diameter and one-third developed (Ritchie et al., 2007). This consisted knifing in a fluid UAN (32-0-0) + ammonium thiosulfate (21-0-0-26S) blend (UTS) at the experimental rate. Phosphorous (P) and
potassium (K) was applied prior to planting according to Virginia's soil test recommendations for upland cotton. Cover crop termination occurred during the late April or early May in each year with 2.3 liters ha⁻¹ of Roundup® (Monsanto, St. Louis, MO) and Liberty® (BASF, Ludwigshafen, Germany) (Table 2.2). This was followed by planting Phytogen® 340 W3FE cotton (Corteva Agroscience, Indianapolis, IN) into the rows term (Table 2.2). An agronomic management schedule can be found in Table 2.2.

2.3.2 Soil Measurements

Prior to cotton planting, soil compaction was measured to the depth at which soil resistance is equal to 2,068 kPa using an analog Dickey-john dial penetrometer. Three measurements were taken from each tillage subplot at 0 cm, 15 cm, 30 cm, and 45 cm from the row. Soil ammonium (NH₄-N) and nitrate (NO₃-N) concentration were also measured from each subplot receiving 0 kg N ha⁻¹ at 0-15, 15-30, 30-60, and 60-90 cm depths from May through September. Soil samples were air dried and extracted with 2M KCl. Ammonium-N and NO₃-N concentrations for each sampling depth were determined using the cadmium reduction colorimetric method (QuickChem Method 12-107-04-1-B) on a Lachat 8500 flow injection colorimeter (Lachat Instruments, Loveland, CO).

2.3.3 Plant Measurements

Prior to cover crop termination in the spring of each year, biomass was sampled from 0.25 m² of cover crop whole plots and dried at 60° C. Samples were ground to a pass through a 1 mm sieve and a nutrient analysis for N, phosphorus (P), potassium (K), and sulfur (S) was conducted by Water Agricultural Laboratories (Camilla, Georgia). Twenty-four cotton petiole samples were randomly sampled from the fourth true main stem leaf in the first and fourth row of each subplot during the 1st week of bloom. Petiole samples were then dried at 60° C, ground through a 1 mm sieve, and extracted with 2% acetic acid. Extracts were analyzed for NO₃-N
using the cadmium reduction colorimetric method (QuickChem Method 3-107-04-1-A) on a
Lachat 8500 flow injection colorimeter (Lachat Instruments, Loveland, CO).

2.3.4 Harvesting

Twenty-five bolls were collected prior to mechanical harvest from random plants in the
center two rows of each plot for lint quality analysis. A commercial two-row cotton picker
modified for small plots research was used to harvest the center two rows of each plot. A 10 saw
micro-gin was used to determine lint proportion from seed cotton using the 25 boll samples. Lint
was sent to the USDA Cotton Program Classing Office in Florence, South Carolina where it was
analyzed for quality via high volume analysis instrument (HVI) to measure fiber length, strength,
micronaire, and uniformity. Relative yield was determined to limit site-year differences and was
calculated as individual plot lint yield divided by the highest average treatment yield per year.

2.3.5 Statistical Analysis

The study was analyzed as a randomized complete block design with split-plot treatment
design having a 2 x 4 factorial treatment design in the sub-plots with four replications. An
ANOVA was performed to determine differences among treatments in cover crop biomass, cover
crop nutrient uptake, soil compaction, soil NO$_3$-N, soil NH$_4$-N, petiole NO$_3$-N, lint yield, and lint
quality parameters using PROC GLIMMIX in SAS 9.3 (SAS, Cary, North Carolina). A
comparison of fit analysis using the extra sum-of-squares F test was performed in GraphPad
Prism version 8.0.0 for Mac (GraphPad Software, San Diego, CA) to determine the best
regression model fit per relative lint yield following each cover crop treatment versus applied N
rate in-season. Relative yield data was combined over the two growing seasons for this
comparison of fit analysis. Cover crop treatments and corresponding relative lint yields that were
best fit by a quadratic, second order polynomial regression curve were subjected to the quadratic
plateau model using PROC NLIN in SAS 9.3 to determine the optimal N rate applied in-season
for cotton grown after that cover crop treatment. All comparison analyses were measured at an $\alpha = 0.10$ level of significance.

### 2.4 Results

#### 2.4.1 Cover Crop Biomass

Planting a cover crop had significant effects on total biomass production in both 2017 ($P<0.0001$) and 2018 ($P<0.0001$) (Tables 2.3; Table 2.4). In 2017, total biomass amounts from rye, LM, and LMR was significantly higher than plots that were fallow (Table 2.3). In 2018, plots with LM and LMR produced more biomass than plots with rye and fallow treatments, respectively (Table 2.4). Nitrogen uptake in biomass was also greater in mixes with a legume species compared to rye and fallow plots in 2017 (Table 2.3). Legume mix had the highest N uptake in 2018, followed by LMR, rye, and fallow plots (Table 2.4). Rye, LM, and LMR biomass took up significantly higher P compared to fallow biomass in both years ($P<0.0001$) (Table 2.3; Table 2.4). LM and LMR biomass took up significantly higher K and S compared to fallow and rye biomass in both years ($P<0.0001$) (Table 2.3; Table 2.4).

#### 2.4.2 Soil Compaction

In 2017, the depth to the root restrictive layer was not significantly different between strip-tilled plots and plots with no-till and precision-planted tillage radish ($P=0.55$). The only significant effects determining the depth to the root restrictive layer was distance from the corn row ($P<0.0001$) and cover crop treatment ($P=0.01$). The further away from the corn row, the shallower the depth to the root restrictive layer (Fig. 2.2). Inclusion of a cover crop resulted in a statistically greater depth to the root restrictive layer compared to fallow plots (Fig. 2.2). In 2018, the only significant factor ($P<0.0001$) determining vertical distance to root restrictive layer was the distance from the corn row (Fig. 2.3). Tillage type ($P=0.86$) and cover crop ($P=0.433$) treatment did not have a significant effect on soil compaction in 2018 (Fig. 2.3)
2.4.3 Soil NO\textsubscript{3}-N and NH\textsubscript{4}-N

In 2017, soil NO\textsubscript{3}-N concentrations generally decreased over the growing season (Fig. 2.4). Cover crop treatment (P<0.0001) and sampling depth (P<0.0001) had significant effects on soil NO\textsubscript{3}-N in each month of the growing season. Mean NO\textsubscript{3}-N concentration in plots after LM was significantly higher than other cover crop treatments in all months. Sampling depth (P<0.0001) was significant throughout the growing season and the interaction between cover crop treatment and sampling depth was significant in all months except July (Supplementary Table 1, Appendix A). In 2017, soil NO\textsubscript{3}-N concentrations fluctuated over sampling depths during the growing season. Soil NO\textsubscript{3}-N concentrations were greater in the 0-30 cm sampling depths in plots with LM during the beginning of the growing season, but from July to September soil NO\textsubscript{3}-N concentrations were greater after LM from 30-90 cm in the soil profile compared to the 0-30 cm sampling depths (Fig. 2.4). The only significant factor in determining soil NH\textsubscript{4}-N in 2017 was sampling depth (P<0.0001) while cover (P=0.19) and the interaction of cover and depth (P=0.97) were not significant (Table 2.5). The 0-15 cm soil depth contained the highest concentration of soil NH\textsubscript{4}-N and soil NH\textsubscript{4}-N concentrations decreased with sampling depth (Table 2.5).

In 2018, mean NO\textsubscript{3}-N concentrations were lower compared to the 2017 growing season (Fig. 2.4; Fig 2.5). Cover crop treatment (P<0.0001) had a significant effect on soil NO\textsubscript{3}-N in all months except for September (P=0.12). Mean NO\textsubscript{3}-N concentration in plots after LM were significantly higher than other cover crop treatments in all months except September. Sampling depth (P<0.0001) was significant across the growing season, whereby soil NO\textsubscript{3}-N was highest in the top 0-15 cm sampling layer in each month (Fig. 2.5). The interaction of cover and sampling depth was only significant in the month of May (Supplementary Table 2, Appendix A). Like in 2017, the top 0-15 cm of soil after LM contained the highest amount of soil NO\textsubscript{3}-N amongst
treatments in May (Fig. 2.4; Fig. 2.5). Cover crop treatment did not have an effect on soil NH₄-N concentrations in 2018, but sampling depth (P<0.0001) was significant in May and September of that year (Table 2.6). The 60-90 cm depth had the highest soil NH₄-N compared to the other sampling depths in 2018 (Table 2.6). The interaction between cover and sampling depth was insignificant with respect to soil NH₄-N in each month (P=0.65).

2.4.4 Petiole NO₃-N Concentrations

In 2017, cover crop treatment (P=0.09) and N rate (P<0.0001) affected petiole NO₃-N concentrations during the first week of bloom. Petiole NO₃-N concentrations increased as applied N rate increased to 90 kg N ha⁻¹ (Fig. 2.6). The mean petiole NO₃-N concentration for cotton grown after LM (7,050 mg NO₃-N kg⁻¹) was statistically higher than petiole concentrations after fallow (5,336 mg NO₃-N kg⁻¹). Petiole concentrations after rye (6,244 mg NO₃-N kg⁻¹) and LMR (5,754 mg NO₃-N kg⁻¹) were statistically similar to petiole NO₃-N concentrations in cotton following LM and fallow treatments. An interaction between cover crop and N rate was not detected (P=0.20) in 2017. In 2018, petiole NO₃-N concentrations were lower than petiole NO₃-N concentrations during 2017 (Fig. 2.6; Fig. 2.7). Only N rate had significant effects (P<0.0001) on petiole NO₃-N concentrations during the first week of bloom in 2018 (Fig. 2.6). Petiole NO₃-N concentrations increased as applied N rate increased to 135 kg N ha⁻¹ (Fig. 2.7). Cover crop treatment (P=0.35) and the interaction of cover crop and N rate (P=0.60) were not significant in determining petiole NO₃-N in 2018.

2.4.5 Lint Yields

Lint yields varied from 1,014 to 2,687 kg ha⁻¹ in 2017 (data not shown). Cover crop treatment (P<0.0001), N rate (P<0.0001), and their interaction (P=0.004) all had significant effects on lint yield in 2017. Tillage treatment did not have a significant effect on lint yield (P=0.84). Lint yield generally increased with increasing applied N rate (Fig. 2.8). Each cover crop treatment
resulted in statistically different lint yields. The mean lint yield across all N rates was greatest following LM (1,696 kg ha\(^{-1}\)), LMR (1,603 kg ha\(^{-1}\)), fallow (1,473 kg ha\(^{-1}\)), and rye (1,358 kg ha\(^{-1}\)) cover crop treatments. Figure 2.8 shows the lint yield response to cover crop treatment and N rate in 2017.

Lint yields varied from 513 to 1,834 kg ha\(^{-1}\) in 2018 (data not shown). Cover crop treatment (P=0.015) and N rate (P<0.0001) had significant effects on lint yield in 2018. Tillage treatment did not have a significant effect (P=0.94) on lint yield. Mean lint yield (1,303 kg ha\(^{-1}\)) following LM was significantly higher compared to the other cover crop treatments (Fig. 2.9). Lint yield generally increased with increasing N rate (Fig. 2.9). No interaction of cover crop treatment and N rate (P=0.26) was detected in 2018. Figure 2.9 shows the lint yield response to cover crop treatment and N rate in 2018.

Comparison of fits analysis determined that H\(_0\) should not be rejected for response of relative lint yield to applied N following fallow (P=0.65), rye (P=0.96), and LMR (P=0.93) cover crop treatments, indicating that the best fit regression model is a first order polynomial (Fig. 2.10). An optimal N rate using the quadratic plateau model was thus not calculated for relative lint yield following fallow, rye, and LMR cover crop treatments across applied N rates (Fig 2.10). The null hypothesis (H\(_0\)) was rejected for relative lint yield response to applied N after LM (P=0.05), indicating that relative lint yield response to applied in-season N rate after LM was best fit by a second order polynomial, or a quadratic polynomial (Fig. 2.10). The quadratic plateau model determined that cotton relative lint yield is maximized at 110 kg N ha\(^{-1}\) applied in-season to achieve a relative lint yield of 93% at harvest (Fig. 2.10).

2.4.6 Lint & Fiber Quality

In 2017, N rate had a significant effect (P=0.019) on lint percent turnout but cover crop treatment (P=0.23) and the interaction of cover crop treatment and N rate (P=0.16) was not
determined to have a significant effect (Table 2.7). Lint harvested after applied N rates of 0, 45, and 90 kg N ha\(^{-1}\) had statistically higher (45%) mean lint percent turnout compared to lint sampled after 135 kg N ha\(^{-1}\) applied in-season (44%; Table 2.7). In 2018, neither cover crop treatment, N rate, or their interaction had an impact on lint percent turnout (Table 2.8). Nitrogen rate was the only treatment that affected fiber quality in this study. In 2017, N rate (P=0.016) had a significant effect on fiber micronaire, where fiber micronaire decreased with increasing N rate (Table 2.7). Cover crop treatment (P=0.53) and the interaction between N rate and cover crop treatment (P=0.57) did not have a significant effect on fiber micronaire. Nitrogen rate also had a significant effect (P=0.06) on fiber strength in 2017, where cotton applied with 90-135 kg N ha\(^{-1}\) produced fibers with lower strength compared to cotton applied with 0-90 kg N ha\(^{-1}\) (Table 2.7). Fiber strength and uniformity was not affected by treatments in 2017 (Table 2.8). In 2018, fiber strength (P=0.005), fiber length (P=0.002), and fiber uniformity (P=0.004) increased with increasing applied N rate (Table 2.8). Fiber micronaire was not affected by any treatments in 2018 (Table 2.8).

2.5 Discussion

The aim of the present study was to determine how legume and small grain cover crops in a conservation tillage system influence nutrient cycling and cotton performance in southeastern Virginia. Our results concerning biomass production and nutrient uptake are in agreement with other research (Boquet et al., 2004; Zabloutowicz et al., 2011). Predictably, winter cover crops produced a higher amount of biomass compared to fallow plots with winter weeds in both years. The smaller biomass yield observed from rye plots in 2018 may be attributed to variety differences between the two study years. The rye variety used in the second year of the study was Dura rye, a variety that is adapted to colder climates and does not establish as much biomass compared to the Wheeler\(^{®}\) rye planted during the previous trial year. This resulted in lower
biomass production in cover crop treatments including rye in 2018 compared to the previous year. The current study also observed the greatest N uptake in mixes with legume species, most likely due to the N fixation occurring during legume growth. Kuo and Sainju (1998) reported a similar uptake pattern in their study comparing ratios of hairy vetch and cereal rye in cover crop mixes. While N has been the major macronutrient of study in cover crop research, the present study observed a significant amount of K and S uptake with treatments containing legume species compared to rye and fallow treatments. Legume species have been shown to be a sink for cations like K, while bicultural mixes with legumes and Brassica species can be catch crops for mineral S (Groffman et al., 1987; Anugroho et al., 2010; Couedel et al., 2018). The capability of legume species cover crops to absorb K and S may result in increased availability in the soil during the growing season. Coastal plain cotton often has K and S deficiencies, so future studies should investigate contributions of cover crops to coastal plain soil fertility.

Higher N uptake from N fixation in LM biomass translated to higher soil NO$_3$-N over the growing season after LM. This finding reflects several other studies on legume species contributions to soil mineral N concentration through N fixation (Schomberg and Endale, 2004; Zablotowicz et al., 2011). Lower amounts of soil NO$_3$-N following the mixes that included cereal rye may be the result of N immobilization, the conversion of plant-available N into organic matter and making it unavailable for plant uptake (Sullivan and Andrews, 2012). Soil NH$_4$-N concentrations responded inconsistently according to cover crop treatment and sampling depth across years. Ammonium-N is often the preferred form of N for assimilation by microbes and most NH$_4$-N is nitrified to NO$_3$-N in the aerated, sandy soils present in this study (Geisseler et al., 2010). This often leaves very little NH$_4$-N left compared to NO$_3$-N for plant uptake (Robertson, 1997). Lack of a NH$_4$-N response to cover crop treatment has been reported elsewhere (Isse et al., 1999; Dean and Weil, 2009). Soil NH$_4$-N concentrations have also been
determined to vary greatly within regions, possibly explaining the variability across depths and sites observed here (Nieder et al., 2011).

Planting a tillage radish in a no-till system was determined to be an acceptable substitute for strip-tilling in this experiment with regard to the depth to the root restrictive layer in soil. Licht and Al-Kaisi (2005) also observed similar soil penetration resistance for both strip-tilled and no-till soil. The capabilities of Brassica species to penetrate compacted soil and lessen soil strength for the following crop has been well established (Williams and Weil, 2004; Chen and Weil, 2010; Marshall et al., 2016). The 2017 growing season did see a difference in soil compaction from cover crop treatments, where all cover crop treatments had a deeper mean depth to the root restrictive layer compared to the fallow treatment (Fig. 2.2). Decreased soil strength after cover crops has been reported in several studies (Raper et al., 2000, Rosolem et al., 2002, Chen and Weil, 2010). This effect was only observed at the site used in 2017, however (Fig. 2.2, Fig. 2.3).

Mineral N contributions from the LM treatment were apparent when measuring cotton response. Petiole analysis showed increased mean petiole NO$_3$-N after LM in 2017 compared to other cover crop treatments. This finding is in agreement with Foote et al. (2014), who also saw elevated petiole NO$_3$-N concentrations after planting a legume species. Only N rate had an effect on petiole NO$_3$-N in 2018, which may have been due to the overall lower soil NO$_3$-N measured in 2018 compared to 2017 (Fig. 2.4, Fig. 2.5). Elevated NO$_3$-N concentrations in LM biomass, soil, and petioles translated to elevated lint yields after LM in both years. Lint yields were lower in 2018 compared to 2017, corresponding to the lesser soil NO$_3$-N and petiole NO$_3$-N observed in 2017 (Fig. 2.8, Fig. 2.9). Several studies have reported cotton yield increases after legume species cover crops, mostly attributed to greater concentrations of mineral N present in the soil after legume species establishment and subsequent N fixation (Torbert et al., 1996; Zablowtowicz et al., 2011). While some studies have questioned whether soil N after legume
species can be mineralized in time for peak cotton demand, yield response after legumes were significantly higher compared to the other cover crop treatments in the present study (Dabney et al., 2001; Zablowtowicz et al., 2011). Lower yields after inclusion of rye in LMR compared to yields after LM is due to the tendency of small grains to immobilize soil NO₃-N mineralized by legumes in the LMR treatment, thus making it unavailable to the proceeding cotton for boll development (Schomberg and Endale, 2004; Reiter et al., 2008).

Though lint yields were greater in 2017 than in 2018, lint yield response to N rate based on preceding cover crop was similar in both years. Predictably, yield generally increased as the applied in-season N rate on cotton increased. Relative yields following LM were optimized at 93% relative yield plateau with 110 kg N ha⁻¹ applied in-season while cotton following LMR, rye, and fallow treatments did not reach a relative yield plateau between 0 and 135 kg N ha⁻¹ applied in-season. Even with 0 kg N ha⁻¹ applied in-season, relative yields after LM reached 76%, which was 20%, 22%, and 9% higher than relative yields after fallow, rye, and LMR with 0 kg N ha⁻¹ applied at side-dress, respectively (Fig. 2.10) A lower optimal N rate for cotton following legume species has been reported before (Rochester et al., 2001; Sainju et al., 2006; Sainju and Singh, 2008; Foote et al., 2014), as has the increased N requirement for cotton grown after rye (Reiter et al., 2008). Implementation of LM resulted in clear yield advantages for cotton grown on the coastal plain soils of southeastern Virginia. This finding differs from another experiment on sandy loam soils by Sainju et al. (2006), who claims that a preceding hairy vetch/rye mix with 65 kg N ha⁻¹ applied in-season produced greater N efficiency and cotton yields in their study. Their measured lint yields were 200-300 kg ha⁻¹ less than the present study on average, however (Sainju et al., 2006). Sainju et al. (2006) also found increased N leaching at a 30 cm soil depth after a pure hairy vetch stand compared to a hairy vetch/rye mix. We did
measure elevated NO$_3$-N leaching in the 30-90 cm sampling depths after LM in our study, but only at the site used in 2017.

Conservation tillage method was also tested to investigate how strip-tilled cotton yields compare to cotton grown in a no-till system with a precision-planted tillage radish. Lint yields did not significantly differ between strip-tilled and no-till treatments in both. Comparable lint yields between two conservation tillage method have been reported elsewhere while others have shown that strip-tillage can produce higher yields compared to no-till (Wiatrak et al., 2005; Sainju et al., 2006, Marshall et al., 2016). Nutrient acquisition and release by tillage radishes may have also contributed to competitive yields compared to strip-tillage. Dean and Weil (2009) observed a significant uptake of soil NO$_3$-N in fall-planted tillage radishes that was subsequently released from the radish after decomposition during the spring months. Additional nutrient contributions from tillage radishes may have thus increased lint yields in the present study. Inclusion of a no-till treatment with no cover crops in a future study can help determine lint yield response to planting a tillage radish on coastal plain soils.

Lint percent turnout and fiber quality response varied during both study years. High N rates resulted in a lower percent lint turnout in 2017 but no treatment effects were observed for lint percent turnout measured in 2018. Lint percent turnout has been reported to vary under a variety of experimental and environmental conditions, thus making our varied results unsurprising (Verhalen et al., 2003; Reiter et al., 2008; Boman, 2012). Satisfactory lint percent proportions from seed cotton can range from 38-42%, implying that the lint percent turnout yielded under the present experimental conditions (44-45%) are acceptable for producers (Boman, 2012). Fiber quality parameters were only affected by applied N rate, but results were not consistent across the study years. In 2017, fiber micronaire and strength decreased with increasing N fertilization while length and uniformity were unaffected. However, in 2018, fiber
uniformity, length, and strength increased with applied N rate while fiber micronaire was not affected by N rate. Other research regarding fiber quality response to N management has produced mixed results as well (Bauer and Roof, 2004; Read et al., 2006; Saleem et al., 2010; Gormus and El Sabagh, 2016). Fiber quality has also been reported to rely on cotton variety more than agronomic management decisions (Saleem et al. 2010). While it is unknown why the single variety used here (PHY 340 W3FE) responded differently to treatments across years, environmental factors such as moisture, temperature, pest exposure, and sunlight can also impact fiber quality response within a growing season (Davidonis et al., 2004). Nevertheless, the fiber quality measurements derived from the cotton grown in this study is satisfactory for purchase based on the standards reported by Cotton Incorporated (Cotton Incorporated, 2019).

2.6 Conclusions

This study aimed to understand nutrient cycling and cotton response on coastal plain soils by implementing four cover crop mixes, two conservation tillage methods, and four in-season N rates. Overall, LM produced greater N uptake in cover crop residue, soil NO$_3$-N during the growing season, and lint yields compared to LMR, rye, and fallow treatments. Cotton grown after LM with 0 kg N ha$^{-1}$ applied in season produced relative lint yields similar to cotton grown after LMR, rye, and fallow treatments at 90-135 kg N ha$^{-1}$ applied in season. An optimal N rate of 110 kg N ha$^{-1}$ applied to cotton after LM was calculated to reach 93% relative lint yield plateau across years. Finally, the use of a precision planted tillage radish in a no-till system was determined to be a comparable substitute to strip-tillage based on both pre-plant soil compaction measurements and lint yields. Future extensions of this research could involve investigations of K and S release from legume residue to the soil or tillage radish contributions to nutrient cycling on coastal plain soils.
2.7 References


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https://doi.org/10.1007/s00374-004-0795-6

https://www.uvm.edu/vtvegandberry/NMP/PANFromCoverCrops.pdf


2.8 Figures & Tables

Figure 2.1 Monthly precipitation at the Tidewater Agricultural Research and Extension Center in Suffolk, VA during the 2017 and 2018 growing seasons.
Figure 2.2 Pre-plant soil penetrometer measurements to the root restrictive layer (2,068 kPa) in strip-tilled and no-till plots with tillage radish plots before the 2017 growing season. Non-significant differences between the depth to the root restrictive layer between tillage systems is marked at each sampling distance with “ns.”
Figure 2.3 Pre-plant soil penetrometer measurements to the root restrictive layer (2,068 kPa) in strip-tilled and no-till plots with tillage radish before the 2018 growing season. Significant differences in the depth to the root restrictive layer is indicated by ‘**’. Non-significant differences between the depth to the root restrictive layer between tillage systems is marked at each sampling distance with “ns.”
Figure 2.4 Soil NO$_3$-N concentrations after fallow, rye, legume mix, and legume mix/rye combination cover crop termination at 0-15, 15-30, 30-60, and 60-90 cm sampling depths from May-September in 2017.
Figure 2.5 Soil NO₃-N concentrations after fallow, rye, legume mix, and legume mix/rye combination cover crop termination at 0-15, 15-30, 30-60, and 60-90 cm sampling depths from May-September in 2018.
Figure 2.6 Petiole NO$_3$-N concentrations in 2017 during the first week of bloom in cotton grown after four different cover crop mixes across four in-season N rates at side-dress.

Figure 2.7 Petiole NO$_3$-N concentrations in 2018 during the first week of bloom in cotton grown after four different cover crop mixes across four in-season N rates at side-dress.
Figure 2.8 Lint yield response from cotton grown after four cover crop mixes and four in-season N rates applied at side-dress in 2017.

Figure 2.9 Lint yield response from cotton grown after four cover crop mixes and four in-season N rates applied at side-dress in 2018.
Figure 2.10 Cotton relative lint yield (%) response to in-season N rate after four cover crop mixes where “y” refers to relative lint yield and “N” refers to applied in-season N rate in the corresponding regression equations. Relative lint yield values were averaged across two growing seasons. The intersection of red, dotted lines indicates an optimal N rate of 110 kg N ha\(^{-1}\) and maximum relative yield of 93% calculated by the quadratic plateau model for cotton grown after the legume mix (LM) over both study years. Optimal N rates were not calculated for cotton following fallow, rye, and LMR treatments due to the linear fit of the response curves.
Table 2.1 Soil characteristics during the 2017 and 2018 growing season for the two field sites at the Tidewater Agricultural Research and Extension Center in Suffolk, VA.

<table>
<thead>
<tr>
<th>Year</th>
<th>Location</th>
<th>Series</th>
<th>Texture</th>
<th>Family</th>
</tr>
</thead>
<tbody>
<tr>
<td>2017</td>
<td>Suffolk, VA</td>
<td>Lynchburg</td>
<td>Fine sandy loam</td>
<td>Fine-loamy, siliceous, semi active, thermic Aeric Paleaquults</td>
</tr>
<tr>
<td>2018</td>
<td>Suffolk, VA</td>
<td>Rains</td>
<td>Fine sandy loam</td>
<td>Fine-loamy, siliceous, semi active, thermic Typic Paleaquults</td>
</tr>
</tbody>
</table>

Table 2.2 Agronomic management timeline for cover crop and cotton production 2017-2018.

<table>
<thead>
<tr>
<th>Study Year</th>
<th>Cover Crops Planted</th>
<th>Cover Crop Termination</th>
<th>Cotton Planted</th>
<th>Side-dress N</th>
<th>Petiole Sampling</th>
<th>Defoliation</th>
<th>Cotton Harvest</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>May 19, 2017†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

† Two applications of herbicide were required in 2017 for complete cover crop termination
Table 2.3 Total biomass and nutrient uptake from four different cover crop treatments from 2016-2017.

<table>
<thead>
<tr>
<th>Cover Crop</th>
<th>Total Biomass</th>
<th>N</th>
<th>P</th>
<th>K</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fallow</td>
<td>1,052 b†</td>
<td>19 c</td>
<td>3 b</td>
<td>36 c</td>
<td>2 b</td>
</tr>
<tr>
<td>Rye</td>
<td>5,871 a</td>
<td>66 b</td>
<td>12 a</td>
<td>100 b</td>
<td>5 b</td>
</tr>
<tr>
<td>LM†</td>
<td>4,656 a</td>
<td>172 a</td>
<td>15 a</td>
<td>150 a</td>
<td>9 a</td>
</tr>
<tr>
<td>LMR</td>
<td>5,896 a</td>
<td>149 a</td>
<td>16 a</td>
<td>169 a</td>
<td>10 a</td>
</tr>
</tbody>
</table>

*† Values with the same letter are not significantly different for means separation at α = 0.10 within columns
‡ LM, Legume Mix; LMR, Legume Mix and Rye combination*

Table 2.4 Total biomass and nutrient uptake from four different cover crop treatments from 2017-2018.

<table>
<thead>
<tr>
<th>Cover Crop</th>
<th>Total Biomass</th>
<th>N</th>
<th>P</th>
<th>K</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fallow</td>
<td>1,084 c†</td>
<td>14 d</td>
<td>3 b</td>
<td>22 c</td>
<td>2 b</td>
</tr>
<tr>
<td>Rye</td>
<td>3,794 b</td>
<td>44 c</td>
<td>10 a</td>
<td>80 b</td>
<td>4 b</td>
</tr>
<tr>
<td>LM†</td>
<td>5,658 a</td>
<td>163 a</td>
<td>16 a</td>
<td>147 a</td>
<td>8 a</td>
</tr>
<tr>
<td>LMR</td>
<td>5,394 a</td>
<td>114 b</td>
<td>14 a</td>
<td>180 a</td>
<td>10 a</td>
</tr>
</tbody>
</table>

*† Values with the same letter are not significantly different for means separation at α = 0.10 within columns
‡ LM, Legume Mix; LMR, Legume Mix and Rye combination*
Table 2.5 Main effect of sampling depth on soil NH$_4$-N concentrations during the 2017 growing season.

<table>
<thead>
<tr>
<th>Depth Cm</th>
<th>May</th>
<th>June</th>
<th>July</th>
<th>August</th>
<th>September</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-15</td>
<td>5 a</td>
<td>9 a</td>
<td>4 ab</td>
<td>9 ns‡</td>
<td>7 a</td>
</tr>
<tr>
<td>15-30</td>
<td>3 b</td>
<td>8 b</td>
<td>5 a</td>
<td>7</td>
<td>5 b</td>
</tr>
<tr>
<td>30-60</td>
<td>4 ab</td>
<td>6 c</td>
<td>3 bc</td>
<td>8</td>
<td>5 b</td>
</tr>
<tr>
<td>60-90</td>
<td>3 b</td>
<td>6 c</td>
<td>2 c</td>
<td>8</td>
<td>5 b</td>
</tr>
</tbody>
</table>

ANOVA $P > F$  <0.0001  <0.0001  0.0991  0.1753  <0.0001

† Values with the same letter are not significantly different for means separation at $\alpha = 0.10$ within columns
‡ NS, not significant at $\alpha = 0.10$ within columns

Table 2.6 Main effect of sampling depth on soil NH$_4$-N concentrations during the 2018 growing season.

<table>
<thead>
<tr>
<th>Depth cm</th>
<th>May</th>
<th>June</th>
<th>July</th>
<th>August</th>
<th>September</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-15</td>
<td>2 c †</td>
<td>4 ns‡</td>
<td>3 ns</td>
<td>5 ns</td>
<td>4 b</td>
</tr>
<tr>
<td>15-30</td>
<td>2 c</td>
<td>4</td>
<td>2</td>
<td>5</td>
<td>4 b</td>
</tr>
<tr>
<td>30-60</td>
<td>3 b</td>
<td>4</td>
<td>3</td>
<td>5</td>
<td>4 b</td>
</tr>
<tr>
<td>60-90</td>
<td>4 a</td>
<td>4</td>
<td>3</td>
<td>5</td>
<td>5 a</td>
</tr>
</tbody>
</table>

ANOVA $P > F$  <0.0001  0.7923  0.3634  0.1750  0.0012

† Values with the same letter are not significantly different for means separation at $\alpha = 0.10$ within columns
‡ NS, not significant at $\alpha = 0.10$ within columns
Table 2.7 Main effect of nitrogen (N) rate on lint proportion from seed cotton (sd. ctn) and fiber quality characteristics from cotton grown in 2017.

<table>
<thead>
<tr>
<th>N Rate (kg N ha(^{-1}))</th>
<th>Lint Proportion (g lint / g sd. ctn)</th>
<th>Micronaire</th>
<th>Strength (g tex(^{\dagger}))</th>
<th>Length (cm)</th>
<th>Uniformity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.45 a(^\dagger)</td>
<td>4.75 a</td>
<td>31.0 a</td>
<td>2.9 ns(^\dagger)</td>
<td>84.5 ns</td>
</tr>
<tr>
<td>45</td>
<td>0.45 a</td>
<td>4.72 ab</td>
<td>31.0 a</td>
<td>2.9</td>
<td>84.4</td>
</tr>
<tr>
<td>90</td>
<td>0.45 a</td>
<td>4.65 bc</td>
<td>30.5 b</td>
<td>2.9</td>
<td>84.3</td>
</tr>
<tr>
<td>135</td>
<td>0.44 b</td>
<td>4.61 c</td>
<td>30.5 b</td>
<td>2.9</td>
<td>84.3</td>
</tr>
</tbody>
</table>

ANOVA \(P > F\) 0.0191 0.0162 0.0634 0.2454 0.6643

\(^\dagger\) Values with the same letter are not significantly different for means separation at \(\alpha = 0.10\) within columns

\(^\dagger\) NS, nonsignificant at \(\alpha = 0.10\) within columns

Table 2.8 Main effect of nitrogen (N) rate on lint proportion from seed cotton (sd. ctn) and fiber quality characteristics from cotton grown in 2018.

<table>
<thead>
<tr>
<th>N Rate (kg N ha(^{-1}))</th>
<th>Lint Proportion (g lint / g sd. ctn.)</th>
<th>Micronaire</th>
<th>Strength (g tex(^{\dagger}))</th>
<th>Length (cm)</th>
<th>Uniformity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.44 ns(^\dagger)</td>
<td>5.16 ns</td>
<td>32.2 b(^\dagger)</td>
<td>2.8 b</td>
<td>83.2 c</td>
</tr>
<tr>
<td>45</td>
<td>0.44</td>
<td>5.15</td>
<td>32.2 b</td>
<td>2.8 b</td>
<td>83.6 b</td>
</tr>
<tr>
<td>90</td>
<td>0.44</td>
<td>5.17</td>
<td>32.8 a</td>
<td>2.8 b</td>
<td>83.8 ab</td>
</tr>
<tr>
<td>135</td>
<td>0.44</td>
<td>5.16</td>
<td>33.1 a</td>
<td>2.9 a</td>
<td>84.1 a</td>
</tr>
</tbody>
</table>

ANOVA \(P > F\) 0.5824 0.9613 0.0051 0.0002 0.0004

\(^\dagger\) NS, nonsignificant at \(\alpha = 0.10\) within columns

\(^\dagger\) Values with the same letter are not significantly different for means separation at \(\alpha = 0.10\) within columns
3  Cotton Response to Nitrogen Rate and Placement in North Carolina

3.1 Abstract

Proper nitrogen (N) management in upland cotton (Gossypium hirsutum L.) production is imperative to achieve yield goals. There is a need to detail the proper application rate and placement method of N fertilizer on the coarse-textured soils of coastal plain Virginia and North Carolina. The objective of this study was to measure cotton performance in response to N rate and placement application method. Five N rates (0, 45, 90, 135, and 180 kg N ha\(^{-1}\)) and three placement methods (broadcast, surface banded, and injected) were evaluated from 2016-2018 at two locations in Virginia and one location in North Carolina, USA. Lint yield, petiole nitrate-N (NO\(_3\)-N), lint percent turnout, and fiber quality parameters were measured to determine the impact of N rate and placement. Nitrogen rate and placement had a significant effect on lint yield but only N rate affected petiole NO\(_3\)-N concentration. Quadratic plateau analysis on relative yield estimated that injecting fertilizer N requires an N rate of 133 kg N ha\(^{-1}\) to achieve 95% relative yield while surface banded fertilizer N required a rate of 128 kg N ha\(^{-1}\) to produce 90% relative yield. A critical petiole NO\(_3\)-N concentration threshold of 5,600 mg NO\(_3\)-N kg\(^{-1}\) was calculated to reach 92% relative yield. Neither rate nor placement had a significant effect on fiber quality parameters. Other considerations such as fertilizer N source, timing, and number of applications should be tested with rate and placement to provide updated management recommendations to cotton producers in coastal plain Virginia and North Carolina.

*Keywords* - upland cotton, nitrogen management, fertilizer placement, petiole analysis

3.2 Introduction

Nitrogen (N) is an essential nutrient for upland cotton (Gossypium hirsutum L.) development. Cotton needs to accumulate between 250 and 300 kg N ha\(^{-1}\) to achieve proper boll development and maximum yield potential (Ali, 2015). Application of N fertilizer is an integral
part of any successful cotton production system. Nitrogen fertilization practices can have negative consequences when under applied or applied in excess, thus requiring a precise application rate to meet yield goals (Hons et al., 2004). Concern about the environmental fate of applied N through leaching, runoff, and greenhouse gas emissions are also motivations for improving N management and efficiency in the agroecosystem (Shah et al., 2017). Several variables can affect nitrogen use efficiency and yield in cotton production. Nitrogen rate, placement method, source, timing, soil texture, and climate are all variables of interest when evaluating N management practices for economic and environmental sustainability.

Rainfed cotton response to N fertilization rate has not been well documented on the coastal plain of Virginia and North Carolina. General recommendations for Virginia cotton production are N rates of 67-135 kg N ha\(^{-1}\) while the state of North Carolina recommends 33-90 kg N ha\(^{-1}\) (Frame et al., 2016; Crozier and Hardy 2019). Specific, optimal N rates determined by other studies have varied considerably. A study conducted in South Carolina by Bauer et al. (1993) reported an optimal N rate of 57 kg N ha\(^{-1}\) on a Norfolk sandy loam to reach yields near 1,200 kg ha\(^{-1}\), whereas Torbert and Reeves (1994) found that the use of strip-tillage to alleviate soil compaction was more important than elevated N rates in determining lint yield and N efficiency on coastal plain soils. Main et al. (2013) reported that 150 kg N ha\(^{-1}\) of total N, from both residual soil and fertilizer N, was required to maximize yields across several soil types used in their study. Application of an optimal N rate to cotton on coastal plain soils may prevent N leaching while maximizing cotton yields. Movement of nitrate-N (NO\(_3\)-N) to groundwater via leaching is a major environmental concern on the southeastern coastal plain due to the high rainfall, warm temperatures, and sandy textured soils characteristic of this region (Pisani et al., 2017). Determination of an optimal N rate for this region can prevent unnecessary N inputs, thus benefitting both environmental quality and cotton producers.
Another facet of N management that affects optimal rate is fertilizer placement method, which influences N availability and use efficiency by crops (Johnston and Fowler, 1991). Mixed results have been reported on the efficiency and yield outcomes of different N placement methods for cotton (Howard et al., 2001; Shah et al., 2002; Reiter et al., 2008). Roberts et al. (1999) reported optimal N rates between 92 and 112 kg N ha\(^{-1}\) but concluded no significant differences in yield between broadcast and injected N. Greater efficiency of injected N due to proximity of fertilizer placement to roots has also been reported (Thompson and Varco, 1996). The placement of a fluid band on the soil surface has also been compared to broadcasted N as a placement method (Guthrie, 1991; Shah et al., 2002). Few studies in field crops research have compared multiple placement methods for side-dressed N in a single experiment.

The mobility of N in coarse-textured coastal plain soils makes it difficult to assess in-season N status and possible yield outcomes. Petiole NO\(_3\)-N concentrations measured during the first week of bloom is a common indicator of in-season N status for cotton due to the simplicity and accuracy of petiole testing (McConnell et al., 1993). While petiole NO\(_3\)-N concentrations frequently correlate with applied N rate, correlation with lint yield has been weak in past studies (Wood et al., 1992; Bronson et al., 2001). Mitchell and Baker (2000) claimed that the sufficiency range for proper cotton nutrition in Georgia during the first week of bloom was 4,500 – 12,500 mg NO\(_3\)-N kg\(^{-1}\). Critical thresholds of petiole NO\(_3\)-N to meet satisfactory yields have also been investigated in several older studies, but introduction of modern, high yielding cotton varieties warrants reassessment of these critical concentrations in Virginia and North Carolina (Lutrick et al., 1986). Constable et al. (1991) also notes that critical petiole NO\(_3\)-N thresholds vary with soil type and location. Determination of a precise critical petiole NO\(_3\)-N concentration to predict yields would be a useful reference for N management practices in Virginia and North Carolina.
The objectives of this study were to (1) determine how various N application rates and placement methods influence cotton yield and fiber quality on coastal plain soils in Virginia and North Carolina; and (2) assess the critical petiole nitrate (NO$_3$-N) concentration at early bloom for strip-tilled cotton on coastal plain soils.

3.3 Materials and Methods

3.3.1 Experimental Design

A field experiment at two sites per year was replicated over three cotton growing seasons (2016-2018) for a total of six site-years. The experiment was conducted at the Tidewater Agricultural Research and Extension Center in Suffolk, Virginia (2016-2018), the Peanut Belt Research Station in Lewiston-Woodville, North Carolina (2016-2017), and a site in Yale, Virginia (2018). The soils had sandy loam and loamy fine sand textures across sites (Table 2.1). The cotton variety planted at all locations was Phytogen® 333 WRF (Corteva AgroSciences, Indianapolis, IN) and was planted in 3.65 (four rows) x 10.66 m plots with four replications at each site. Starter N fertilizer was applied to all plots at a rate of 34 kg N ha$^{-1}$ in a band 5 cm on the side of the row and 5 cm below the seed at planting except for the 0 kg N ha$^{-1}$ treatment. Nitrogen rates and placement strategies were arranged in a 4x3 randomized design with four total N application rates (45 kg N ha$^{-1}$, 90 kg N ha$^{-1}$, 135 kg N ha$^{-1}$, and 180 kg N ha$^{-1}$) and three placement methods at side-dress: broadcasting, surface banding, and injection. In-season (side-dress) fertilizer N was applied at matchhead square, the crop stage when cotton flower buds are 2-3 mm in diameter and one-third developed (Ritchie et al., 2007). Nitrogen sources for each placement method included a granular urea (46-0-0) + ammonium sulfate (AMS, 21-0-0-24S) blend for broadcast treatments and fluid urea ammonium nitrate (UAN, 32-0-0) and ammonium thiosulfate (ATS, 12-0-0-26S) solution for surface banded and injected treatments. Broadcast treatments were hand-applied evenly across plots. Injected N was applied 15 cm from the row.
and 10 cm deep with a Yetter Generation III fertilizer coulter (Yetter Farm Equipment, Colchester, IL). Surface banded N was placed 46 cm on the side of the row by lifting the side-dress toolbar and dribbling fluid out of the injection coulter. Phosphorus (P) and potassium (K) were applied prior to planting according to Virginia soil test recommendations for cotton (Maguire and Heckendorn, 2017). Sulfur was balanced using ATS and AMS across N application rates at 22 kg S ha\(^{-1}\).

3.3.2 Soil Measurements

Pre-plant soil samples were taken at 0-15, 15-30, 30-60, and 60-90 cm at each site. Soil samples were air dried and extracted with 2M KCl. Ammonium-N (NH\(_4\)-N) and NO\(_3\)-N concentrations for each sampling depth were determined using the cadmium reduction colorimetric method (QuickChem Method 12-107-04-1-B) on a Lachat 8500 flow injection colorimeter (Lachat Instruments, Loveland, CO). Total pre-plant soil NH\(_4\)-N and NO\(_3\)-N concentrations can be found in Table 2.1.

3.3.3 In-season Petiole Sampling

Twenty-four cotton petiole samples from the fourth main stem true leaf were collected from random plants in the first and fourth row of each plot. Petiole samples were collected after the first week of bloom. Petiole samples were then dried at 60 °C, ground through a 1-mm sieve, and extracted with 2% acetic acid. Extracts were analyzed for NO\(_3\)-N using the cadmium reduction colorimetric method (QuickChem Method 3-107-04-1-A) on a Lachat 8500 flow injection colorimeter (Lachat Instruments, Loveland, CO).

3.3.4 Lint Yield and Quality

Twenty-five bolls were collected from the center two rows of all test plots. A commercial two-row cotton picker modified for small plots research was used to harvest the remaining cotton to determine lint yield. Boll samples were ginned with a 10-saw micro-gin was to calculate lint
weight from seed cotton weights. Relative lint yield was determined for each N rate and placement method to standardize absolute yields across years and locations. Relative yield was calculated by taking individual plot lint yield and dividing it by the highest average lint yield within each site-year. Lint samples were sent to the USDA Cotton Program Classing Office in Florence, SC, where they were analyzed for micronaire, length, strength, and uniformity measurements via high volume instrument (HVI) analysis.

3.3.5 Statistical Analysis

The PROC GLIMMIX procedure in SAS 9.3 was used to determine treatment effects on lint yield, petiole NO$_3$-N, lint percent turnout, fiber uniformity, fiber micronaire, fiber strength, and fiber length among the four N rates and three placement methods (SAS Institute, Cary, NC). Tukey's HSD was then used to identify any specific treatment differences.

A quadratic plateau model using the PROC NLIN procedure in SAS 9.3 (SAS Institute, 2012) was used to determine optimum N application rates and maximum relative lint yield for each placement method in a combined analysis of all site-years. Absolute lint yield to determine the yield response to N rate for each placement method per site-year was analyzed using regression analyses in Sigma Plot 12.5 (SigmaPlot, San Jose CA). The quadratic plateau model in PROC NLIN was also used to determine the maximum petiole NO$_3$-N concentration reached in response to relative yield. A significance level of $P = 0.10$ was used for all statistical comparisons.

3.4 Results

3.4.1 Lint Yield

Cotton was responsive to applied N at all sites, except Lewiston in 2016 where Hurricane Matthew caused yield loss (Table 3.2). The site-year was thus excluded from the combined statistical analysis. Lint yields ranged from 834 to 2,175 kg ha$^{-1}$ across five site-years (data not
shown). In the combined analysis, both N rate (P<0.0001) and placement method (P=0.03), and their interaction (P=0.04) had an effect on lint yield. Lint yield generally increased as applied N rate increased (Fig. 3.1). The main effect of placement resulted in lint yield measured from plots after injected N having significantly higher yields (1,528 kg ha\(^{-1}\)) compared to yields after surface banding (1,469 kg ha\(^{-1}\)) and broadcasting (1,433 kg ha\(^{-1}\)).

Table 2 shows the quadratic equation, optimal N rate, and maximum lint yield derived from regression analysis for each placement method during each site-year. The quadratic plateau model applied for each placement treatment across site-years determined optimal N rates of 128 kg N ha\(^{-1}\) for surface banded N and 133 kg N ha\(^{-1}\) for injected N to reach maximum relative yield (Fig. 3.1). In contrast, relative yields after broadcast N did not reach an optimum N rate even at 180 kg N ha\(^{-1}\) applied, the highest N rate established in this experiment (Fig. 3.1). Relative yields at optimum N rates were 94%, 90%, and 95% for broadcast, surface banded, and injected treatments, respectively.

3.4.2. In-season Petiole Sampling

Petiole NO\(_3\)-N concentrations were affected by N rate (P<0.0001) but not placement (P=0.49). The interaction between N rate and placement was not significant (P=0.91). The concentration of petiole NO\(_3\)-N increased as applied N rate increased (Fig. 3.2). Quadratic plateau analysis calculated a critical petiole NO\(_3\)-N threshold of 5,600 mg NO\(_3\)-N kg\(^{-1}\) to achieve 92% of relative yield (Fig. 3.3). Fertilization N rates between 90 and 135 kg N ha\(^{-1}\) is required to meet the concentration threshold of 5,600 mg NO\(_3\)-N kg\(^{-1}\) (Fig. 3.2).

3.4.3 Cotton Lint Quality

Nitrogen rate (P=0.49), placement (P=0.34), and their interaction (P=0.81) were not significant in determining lint percent turnout (Table 3.3). Fiber quality parameters including
micronaire, length, strength, and uniformity were also unaffected by N rate, placement method, or their interaction (Table 3.3).

3.5 Discussion

In the present study, we evaluated cotton in-season N nutritional status, lint yield, and fiber quality response to N rate and placement methods in the coastal plain of Virginia and North Carolina. Greater lint yields occurred with lower optimal N rates after surface banded or injected N compared to broadcasting. Localized application of N near the root zone through injection or surface banding places N where it can be immediately taken up by the root system, thus preventing N losses through volatilization or leaching (Shapiro et al., 2016). Broadcasting N across a wider spatial area results in a lower amount of N immediately available to the crop (Lemon, 2009). More broadcast N is then required to meet cotton nutritional demands and yield goals, explaining why relative yields after broadcast N did not reach a quadratic plateau in this study (Fig. 3.1). The inefficiency of broadcasting compared to more precise N placement methods has been reported in several studies concerning cotton and other field crops (Howard and Tyler, 1989; Hultgreen and Leduc, 2003; Nkebiwe et al., 2016).

Unlike broadcasting, optimal N rates and corresponding relative lint yields could be calculated for cotton grown after surface banded and injected N. Both placement methods reduced the surface area where N could interact with the soil compared to broadcasting, thus reducing the potential for transformation by microorganisms (Nkebiwe et al., 2016). Nitrogen placed near cotton roots at matchhead square through injection and surface banding may be expected to be more available to cotton and thus result in an increased number of nodes, fruiting positions, and bolls. Placement of N under the soil surface through injection resulted in a 5% higher relative yield with a 5 kg N ha⁻¹ higher optimal N rate compared to surface banding (Fig. 3.1). Injecting N was thus only slightly more efficient than surface banding in terms of the
relative yield of rainfed cotton lint produced per unit of applied N. These results most closely resemble findings from Fox and Piekielek (1993), who determined that surface banded and injected fluid UAN/ATS at side-dress produced comparable corn yields across application rates in the mid-Atlantic US. The present cotton study demonstrates that surface banded and injected N at rates of 128 kg N ha\(^{-1}\) and 133 kg N ha\(^{-1}\) are satisfactory methods to produce lint yields higher than 1,500 kg ha\(^{-1}\) on coastal plain soils in Virginia and North Carolina.

Nitrogen source effects can be confounded with placement method when measuring yield response due to N loss to the atmosphere (Venterea et al., 2005). Broadcast ammonium nitrate (AN) has been reported to produce higher lint yields compared to surface banded or injected fluid UAN, presumably because urea-containing fertilizers like UAN are more susceptible to NH\(_3\) volatilization via urease enzyme activity (Howard et al., 2001; Reiter et al., 2008). Ammonium nitrate is not affected by the enzyme urease, so broadcast applications of AN may result in an optimal N rate lower than 180 kg N ha\(^{-1}\) (Howard et al., 2001; Reiter et al., 2008). Urea is more commonly used as a granular N source in modern cotton production due to the safety and security issues surrounding AN, but volatilization losses are generally higher after granular urea application compared to fluid UAN, thus possibly explaining the inefficiency of broadcasted applications reported here (Fox et al., 1996). Application timing has also been reported to interact with placement method in determining cotton yields but was not a variable of interest in the present study (Reiter et al., 2008). Further understanding of N response curves in cotton production would benefit from testing different fertilizer placement method under a variety of N sources, application timings, and other management variables to specify yield capabilities in coastal plain Virginia and North Carolina.

Our study also determined that petiole sampling in the first week of bloom can also be a useful predictor of yield outcomes in coastal plain Virginia and North Carolina. The critical
petiole NO$_3$-N concentration of 5,600 mg NO$_3$-N kg$^{-1}$ to reach 92% relative yield established in this study is reflective of older research investigating cotton tissue nutrition on sandy loam soils (Lutrick et al., 1986, Mitchell and Baker, 2000). Lint yields at and beyond this critical threshold exceeded yields from older studies by 300 kg ha$^{-1}$, most likely due to the use of modern, high yielding varieties. Yin and Main (2015) reported that N fertilization rates of 90-146 kg N ha$^{-1}$ were required to meet critical leaf N concentrations and maximum yields in their Tennessee study. Their findings therefore correspond closely with the results presented here, where an optimal N rate between 90 and 135 kg N ha$^{-1}$ met the critical petiole NO$_3$-N threshold for cotton grown on coastal plain Virginia and North Carolina (Fig. 3.3). The optimal N rates of 128 and 133 kg N ha$^{-1}$ applied via surface banding and fertilizer injection can thus reach the critical petiole NO$_3$-N concentration established here.

Lint and fiber quality characteristics were unaffected by rate and placement method in this study. This finding contradicts past studies that have reported that N rate typically influences lint percent turnout more than placement. Elbehar (1991) noted lower lint percentages as higher N rates were applied and concluded that increased N availability to cotton plants can result in more N allocation to the seed, thus decreasing lint percentage. Fiber quality parameters such as fiber micronaire, length, and strength did not show any response to N rate or placement in this study (Table 3). Many have reported general improvement of fiber quality parameters with increasing N rate (Bauer and Roof, 2004; Read et al., 2006; Gormus and El Sabagh, 2016). Saleem et al. (2010) however, concluded that fiber quality was more influenced by variety choice than N fertilization rate in their study. Considering that only one variety was used in this study, the fiber quality of other common varieties used in Virginia and North Carolina may respond to N fertilization differently. Testing multiple cotton varieties under rate and placement experiments may elucidate fiber quality response more clearly.
3.6 Conclusions

The present study provides updated information on cotton response to N rate and placement strategy in coastal plain Virginia and North Carolina. Quadratic plateau analysis indicates that the optimal N rate for surface banded application (128 kg N ha\(^{-1}\)) was slightly lower than fertilizer injection (133 kg N ha\(^{-1}\)), but injection produced 5% higher relative yield (95%) compared to surface banding (94%) at the higher optimal N rate. Both placement methods yielded over 1,500 kg ha\(^{-1}\) or 90% of the relative yield. These N rates are higher than recommended rates for this region but also correspond to lint yields that are 300-400 kg ha\(^{-1}\) higher (1,500-1,600 kg ha\(^{-1}\)) than the state averages in Virginia and North Carolina. This study also affirms a critical petiole NO\(_3\)-N threshold of 5,600 mg NO\(_3\)-N kg\(^{-1}\) at first bloom to achieve over 92% relative yield at harvest. Lint parameters and fiber quality were unaffected by N rate and placement methods as implemented in the current study. These results suggest that surface banded and injected N at application rates of 128 and 133 kg N ha\(^{-1}\), respectively, are efficient rate and placement methods for cotton production on the coarse-textured soils of coastal plain Virginia and North Carolina.

3.7 References


https://doi:10.2134/agronj1986.00021962007800060021x


https://doi:10.2134/agronj1993.00021962008500060011x


https://doi: 10.2489/jswc.72.5.519


3.8 Figures & Tables

**Figure 3.1** Cotton relative yield response to broadcast, surface banded, and injection fertilizer N placement across N application rates for the five responsive site-years. Intersecting dotted, red lines indicate the join point calculated from the PROC NLIN quadratic plateau model. Surface banded N placement reached 90% of relative yield at an optimal N rate of 128 kg N ha\(^{-1}\) while injected N placement reached 95% of relative yield at an optimal N rate of 133 kg N ha\(^{-1}\). The quadratic plateau calculated for broadcasted N fertilizer was outside the experimental range and is not denoted in this figure.
Figure 3.2 Cotton petiole NO$_3$-N concentrations after first bloom in response to applied N rate. Different letters indicate significant differences according to Tukey's HSD (P<0.10). The dotted line indicates the critical petiole NO$_3$-N threshold at first bloom as determined by the PROC NLIN quadratic plateau model to achieve 92% relative yield plateau (see Fig. 3.3).
Figure 3.3. Relationship between petiole NO$_3$-N and relative yield across five sites-years. The dotted red line represents the critical petiole NO$_3$-N concentration and corresponding relative yield (5,600 mg kg$^{-1}$ at 92% relative yield) calculated from the PROC NLIN quadratic plateau model.
Table 3.1 Soil series, soil N concentrations from 0-90 cm, and agronomic management schedule at experimental sites from 2016-20

<table>
<thead>
<tr>
<th>Year</th>
<th>Location</th>
<th>Soil Series</th>
<th>Pre-plant soil NO$_3$-N</th>
<th>Pre-plant soil NH$_4$-N</th>
<th>Planting date</th>
<th>Side-dress N application date</th>
<th>Harvest date</th>
</tr>
</thead>
<tbody>
<tr>
<td>2016</td>
<td>Suffolk, VA</td>
<td>Eunola loamy fine sand</td>
<td>7</td>
<td>9</td>
<td>Apr. 26</td>
<td>June 30</td>
<td>Oct. 19</td>
</tr>
<tr>
<td></td>
<td>Lewiston, NC</td>
<td>Goldsboro sandy loam</td>
<td>7</td>
<td>4</td>
<td>May 19</td>
<td>July 7</td>
<td>Oct. 20</td>
</tr>
<tr>
<td>2017</td>
<td>Suffolk, VA</td>
<td>Eunola loamy fine sand</td>
<td>8</td>
<td>4</td>
<td>June 2</td>
<td>July 11</td>
<td>Dec. 1</td>
</tr>
<tr>
<td></td>
<td>Lewiston, NC</td>
<td>Lynchburg sandy loam</td>
<td>15</td>
<td>12</td>
<td>May 30</td>
<td>July 13</td>
<td>Nov. 15</td>
</tr>
<tr>
<td>2018</td>
<td>Suffolk, VA</td>
<td>Kenansville loamy sand</td>
<td>10</td>
<td>4</td>
<td>May 4</td>
<td>June 19</td>
<td>Oct. 9</td>
</tr>
<tr>
<td></td>
<td>Yale, VA</td>
<td>Slagle fine sandy loam</td>
<td>12</td>
<td>3</td>
<td>May 8</td>
<td>June 28</td>
<td>Dec. 3</td>
</tr>
</tbody>
</table>
Table 3.2 Goodness of fit, regression equations, optimal N rates, and maximum lint yields determined by the quadratic plateau model using the PROC NLIN command for three different placement types across three years and three locations.

<table>
<thead>
<tr>
<th>Year</th>
<th>Location</th>
<th>Placement</th>
<th>Equation</th>
<th>$R^2$</th>
<th>$P &gt; F$</th>
<th>Optimal† N rate</th>
<th>Max. Lint yield‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>2016</td>
<td>Suffolk, VA</td>
<td>Broadcast</td>
<td>$y = 976 + 6.56N - 0.019N^2$</td>
<td>0.70</td>
<td>&lt;0.0001</td>
<td>175</td>
<td>1,374</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Surface Band</td>
<td>$y = 1002 + 8.95N - 0.035N^2$</td>
<td>0.65</td>
<td>&lt;0.0001</td>
<td>114</td>
<td>1,350</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Injection</td>
<td>$y = 1037 + 8.02N - 0.025N^2$</td>
<td>0.78</td>
<td>&lt;0.0001</td>
<td>155</td>
<td>1,492</td>
</tr>
<tr>
<td>2016</td>
<td>Lewiston, NC</td>
<td>Broadcast</td>
<td>$y = 918 - 1.90N - 0.005N^2$</td>
<td>0.05</td>
<td>ns</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Surface Band</td>
<td>$y = 1003 - 1.10N - 0.000N^2$</td>
<td>0.51</td>
<td>0.0205</td>
<td>BR ††</td>
<td>876</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Injection</td>
<td>$y = 800 + 2.70N - 0.01N^2$</td>
<td>0.38</td>
<td>0.0561</td>
<td>BR</td>
<td>856</td>
</tr>
<tr>
<td>2017</td>
<td>Suffolk, VA</td>
<td>Broadcast</td>
<td>$y = 1123 + 4.31N - 0.006N^2$</td>
<td>0.61</td>
<td>0.0003</td>
<td>OR ††</td>
<td>1,683</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Surface Band</td>
<td>$y = 1133 + 8.02N - 0.028N^2$</td>
<td>0.52</td>
<td>0.0011</td>
<td>108</td>
<td>1,478</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Injection</td>
<td>$y = 1190 + 6.88N - 0.018N^2$</td>
<td>0.53</td>
<td>0.0015</td>
<td>OR</td>
<td>1,916</td>
</tr>
<tr>
<td>2017</td>
<td>Lewiston, NC</td>
<td>Broadcast</td>
<td>$y = 1412 + 3.27N - 0.005N^2$</td>
<td>0.32</td>
<td>0.0369</td>
<td>OR</td>
<td>1,702</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Surface Band</td>
<td>$y = 1430 + 5.71N - 0.019N^2$</td>
<td>0.38</td>
<td>0.0161</td>
<td>133</td>
<td>1,633</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Injection</td>
<td>$y = 1466 + 5.98N - 0.021N^2$</td>
<td>0.31</td>
<td>0.0364</td>
<td>84</td>
<td>1,641</td>
</tr>
<tr>
<td>2018</td>
<td>Suffolk, VA</td>
<td>Broadcast</td>
<td>$y = 940 + 13.99N - 0.039N^2$</td>
<td>0.91</td>
<td>&lt;0.0001</td>
<td>179</td>
<td>1,954</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Surface Band</td>
<td>$y = 1000 + 12.03N - 0.031N^2$</td>
<td>0.80</td>
<td>&lt;0.0001</td>
<td>OR</td>
<td>1,912</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Injection</td>
<td>$y = 1023 + 17.21N - 0.067N^2$</td>
<td>0.88</td>
<td>&lt;0.0001</td>
<td>124</td>
<td>1,791</td>
</tr>
<tr>
<td>2018</td>
<td>Yale, VA</td>
<td>Broadcast</td>
<td>$y = 1100 + 4.66N - 0.007N^2$</td>
<td>0.74</td>
<td>0.006</td>
<td>OR</td>
<td>1,859</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Surface Band</td>
<td>$y = 1067 + 4.79N - 0.004N^2$</td>
<td>0.65</td>
<td>0.0087</td>
<td>OR</td>
<td>1,800</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Injection</td>
<td>$y = 1109 + 6.18N - 0.012N^2$</td>
<td>0.66</td>
<td>0.0377</td>
<td>OR</td>
<td>1,824</td>
</tr>
</tbody>
</table>

† Optimal N rate determined by the quadratic plateau model to reach maximum yield.
‡ The average yield at 180 kg N ha⁻¹ is shown when the optimal N rate was calculated to be above 180 kg N ha⁻¹
§ N rate, "N", replaces "x" in the quadratic equation while "y" refers to lint yield.
¶ ns, Means within columns are not significantly different at α = 0.1
# NC, Not calculated due to insignificant P value at α = 0.10 significance level
†† BR, The optimal N rate determined by the quadratic plateau model in the PROC NLIN command is below 45 kg N ha⁻¹, the lowest N rate tested per placement in the present study.
‡‡ OR, The optimal N rate determined by the quadratic plateau model in the PROC NLIN command is beyond 180 kg N ha⁻¹, the highest N rate tested in the present study.
Table 3.3 Lint proportion from seed cotton (sd. ct.) and fiber quality parameter response to four N rates and three placement methods averaged over three years at two locations in Virginia and one location in North Carolina. The site at Lewiston, NC in 2016 was excluded due to a lack of lint yield response to N rate at this location.

<table>
<thead>
<tr>
<th>Placement</th>
<th>N Rate</th>
<th>Lint Proportion</th>
<th>Micronaire</th>
<th>Strength</th>
<th>Length</th>
<th>Uniformity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>kg N ha⁻¹</td>
<td>g lint / g sd. ct.</td>
<td>g tex⁻¹</td>
<td>cm</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Broadcast</td>
<td>45</td>
<td>0.45 ns⁺</td>
<td>4.85 ns</td>
<td>31.5 ns</td>
<td>2.9 ns</td>
<td>83.8 ns</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>0.45</td>
<td>4.74</td>
<td>31.0</td>
<td>2.9</td>
<td>85.0</td>
</tr>
<tr>
<td></td>
<td>135</td>
<td>0.44</td>
<td>4.73</td>
<td>31.6</td>
<td>3.0</td>
<td>84.5</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>0.44</td>
<td>4.70</td>
<td>31.4</td>
<td>3.0</td>
<td>84.0</td>
</tr>
<tr>
<td>Surface Banded</td>
<td>45</td>
<td>0.44</td>
<td>4.77</td>
<td>31.5</td>
<td>3.0</td>
<td>84.4</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>0.45</td>
<td>4.76</td>
<td>31.4</td>
<td>2.9</td>
<td>84.3</td>
</tr>
<tr>
<td></td>
<td>135</td>
<td>0.44</td>
<td>4.66</td>
<td>31.7</td>
<td>3.0</td>
<td>84.1</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>0.44</td>
<td>4.64</td>
<td>31.6</td>
<td>2.9</td>
<td>83.7</td>
</tr>
<tr>
<td>Injection</td>
<td>45</td>
<td>0.45</td>
<td>4.67</td>
<td>31.3</td>
<td>3.0</td>
<td>83.9</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>0.45</td>
<td>4.75</td>
<td>31.4</td>
<td>2.9</td>
<td>83.6</td>
</tr>
<tr>
<td></td>
<td>135</td>
<td>0.44</td>
<td>4.72</td>
<td>31.2</td>
<td>3.0</td>
<td>83.7</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>0.44</td>
<td>4.65</td>
<td>31.8</td>
<td>2.9</td>
<td>83.9</td>
</tr>
</tbody>
</table>

⁺ ns, Means within columns are not significantly different at α = 0.1
4 Conclusion

In 2018, cotton hectares rose 14% and 7% in the coastal plain region of Virginia and North Carolina, respectively. Recent promotion and education programs concerning conservation agricultural practices such as reduced tillage, cover crop establishment, and other soil health improvement efforts have warranted investigation into conservation cultural practices for cotton production in this region. Two main soil fertility challenges of focus in coastal plain cotton production is soil compaction and N management. The main objectives of this research were to properly assess legume and small grain cover crop contributions to cotton fertility on coastal plain soils, compare no-till with deep-rooted radish cover crops to strip-till as a method of conservation tillage, and investigate the effect of N rate and placement method on cotton performance and petiole nutritional status in Virginia and North Carolina.

No-till cotton grown after precision-planted tillage radishes performed similarly to strip-tilled cotton and the depth to the root restrictive layer was not significantly different between the conservation tillage systems. Legume mix cover crops (LM) containing a blend of crimson clover and hairy vetch/woolypod vetch absorbed more N from soil in biomass, produced higher soil NO$_3$-N concentrations after termination, and ultimately resulted in higher lint yields across N application rates at side-dress compared to fallow, cereal rye, and legume mix/rye (LMR) combination cover crop treatments. Relative lint yield after LM reached a quadratic plateau at 93% with 110 kg N ha$^{-1}$ applied in-season while relative lint yield after fallow, rye, and LMR treatments did not reach a plateau when applied with 0-135 kg N ha$^{-1}$ in season. This optimal fertilizer N rate for cotton after LM cover crop residue is lower than the optimal N rates determined for broadcast, surface banded, and injected placement methods (180, 128, and 133 kg N ha$^{-1}$) in a fallow system, implying that establishing LM cover crops in Virginia allows for fertilizer N reductions of 23-70 kg N ha$^{-1}$ to reach similar relative yields than with no cover crop.
Two components of N fertilizer management - rate and placement - were also investigated in this research. Three N placement methods at side-dress (broadcast, surface banded/dribbled, and injection) were tested at five N rates (0, 45, 90, 135, 180 kg N ha\(^{-1}\)) to determine how placement method affects the optimal N rate to apply to cotton on coastal plain soils. Broadcasting of a granular UAS was determined to be an inefficient form of N application due to the non-specific placement across the soil and subsequent increased potential for volatilization associated with the N source used. Optimal N rates of 128 kg N ha\(^{-1}\) and 133 kg N ha\(^{-1}\) were calculated for surface banding and injecting a fluid UAN/ATS blend. Both placement methods breached 1,500 kg ha\(^{-1}\) of lint at these N rates and were similarly efficient based on relative yield achieved per unit of applied N. Their greater N efficiency is due to the concentrated placement of fertilizer N near the cotton root zone and a lower area of soil applied with N compared to broadcast N. A critical petiole NO\(_3\)-N threshold of 5,600 mg NO\(_3\)-N kg\(^{-1}\) during the first week of bloom was also calculated to predict 92% relative yield at harvest for strip-tilled cotton in Virginia and North Carolina. This concentration offers a target for producers so cotton in-season N status can be evaluated and corrected before the window for cotton N uptake closes after the third week of bloom.

This research demonstrates that legume species blends can provide enough N for 75% relative yield for cotton grown on the coarse-textured soils of coastal Virginia and North Carolina. Green manure cover crops have been a long-standing option to synthetic fertilizer N but remain an unpopular N source in southeastern Virginia due to the higher cost and time investment associated with cover crop management relative to fertilizer N application. This research also provides evidence supporting the current effort for Virginia cotton producers to transition to conservation tillage methods such as strip-till or no-till. The optimal N rates calculated for cotton applied with surface banded and injected N at side-dress fall on the higher
end of the recommended N rate range (67-135 kg N ha⁻¹) for Virginia cotton producers (Frame et al., 2016). This finding affirms past speculation that increasing the application rate is needed to exceed average yields (1,100 kg of lint per ha⁻¹) on sandy loam and fine sandy loam soils in this region (Frame et al., 2016). Other aspects of fertilizer management such as timing, number of applications, and N source are of interest in determining the best N agronomic management practices for cotton producers on coastal plain Virginia and North Carolina. The research described here provides evidence for the soil N fertility and yield advantages from cover crop establishment on coastal plain soils as well as updated information regarding N placement at side-dress and petiole N nutrition for strip-tilled cotton in Virginia and North Carolina.
Appendix A

**Supplementary Table 1.** Effect of cover crop treatment and sampling depth interaction on soil NO$_3$-N concentrations over the 2017 growing season.

<table>
<thead>
<tr>
<th>Cover Crop</th>
<th>Depth</th>
<th>Soil Nitrate-N (mg NO$_3$-N kg$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cm</td>
<td>May</td>
</tr>
<tr>
<td>Fallow</td>
<td>0-15</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>15-30</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>30-60</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>60-90</td>
<td>3</td>
</tr>
<tr>
<td>Rye</td>
<td>0-15</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>15-30</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>30-60</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>60-90</td>
<td>1</td>
</tr>
<tr>
<td>LM§</td>
<td>0-15</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>15-30</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>30-60</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>60-90</td>
<td>5</td>
</tr>
<tr>
<td>LMR</td>
<td>0-15</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>15-30</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>30-60</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>60-90</td>
<td>1</td>
</tr>
</tbody>
</table>

ANOVA $P > F$<br> 0.0001   0.0015   0.4567   0.0003   <0.0001

† Values with the same letter are not significantly different for means separation at $\alpha = 0.10$ within columns
‡ NS, the interaction between cover crop treatment and sampling depth was not significant at $\alpha = 0.10$ within columns
§ LM, Legume Mix; LMR, Legume Mix and Rye combination
**Supplementary Table 2.** Effect of cover crop treatment and sampling depth interaction on soil NO$_3$-N concentrations over the 2018 growing season.

<table>
<thead>
<tr>
<th>Cover Crop</th>
<th>Depth cm</th>
<th>May</th>
<th>June</th>
<th>July</th>
<th>August</th>
<th>September</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fallow</td>
<td>0-15</td>
<td>5 b†</td>
<td>11 ns‡</td>
<td>6 ns</td>
<td>2 ns</td>
<td>2 ns</td>
</tr>
<tr>
<td></td>
<td>15-30</td>
<td>2 cd</td>
<td>4 ns‡</td>
<td>4 ns</td>
<td>1 ns</td>
<td>2 ns</td>
</tr>
<tr>
<td></td>
<td>30-60</td>
<td>2 cd</td>
<td>7 ns‡</td>
<td>4 ns</td>
<td>1 ns</td>
<td>1 ns</td>
</tr>
<tr>
<td></td>
<td>60-90</td>
<td>2 cd</td>
<td>4 ns‡</td>
<td>2 ns</td>
<td>1 ns</td>
<td>2 ns</td>
</tr>
<tr>
<td>Rye</td>
<td>0-15</td>
<td>2 cd</td>
<td>6 ns‡</td>
<td>5 ns</td>
<td>2 ns</td>
<td>3 ns</td>
</tr>
<tr>
<td></td>
<td>15-30</td>
<td>1 cd</td>
<td>2 ns‡</td>
<td>3 ns</td>
<td>1 ns</td>
<td>1 ns</td>
</tr>
<tr>
<td></td>
<td>30-60</td>
<td>1 cd</td>
<td>3 ns‡</td>
<td>2 ns</td>
<td>1 ns</td>
<td>2 ns</td>
</tr>
<tr>
<td></td>
<td>60-90</td>
<td>1 cd</td>
<td>2 ns‡</td>
<td>2 ns</td>
<td>1 ns</td>
<td>1 ns</td>
</tr>
<tr>
<td>LM§</td>
<td>0-15</td>
<td>2 a</td>
<td>10 ns‡</td>
<td>12 ns</td>
<td>2 ns</td>
<td>3 ns</td>
</tr>
<tr>
<td></td>
<td>15-30</td>
<td>2 cd</td>
<td>4 ns‡</td>
<td>6 ns</td>
<td>1 ns</td>
<td>2 ns</td>
</tr>
<tr>
<td></td>
<td>30-60</td>
<td>1 cd</td>
<td>4 ns‡</td>
<td>5 ns</td>
<td>2 ns</td>
<td>1 ns</td>
</tr>
<tr>
<td></td>
<td>60-90</td>
<td>1 cd</td>
<td>2 ns‡</td>
<td>2 ns</td>
<td>1 ns</td>
<td>2 ns</td>
</tr>
<tr>
<td>LMR</td>
<td>0-15</td>
<td>3 c</td>
<td>7 ns‡</td>
<td>6 ns</td>
<td>3 ns</td>
<td>2 ns</td>
</tr>
<tr>
<td></td>
<td>15-30</td>
<td>1 cd</td>
<td>3 ns‡</td>
<td>4 ns</td>
<td>1 ns</td>
<td>2 ns</td>
</tr>
<tr>
<td></td>
<td>30-60</td>
<td>1 cd</td>
<td>2 ns‡</td>
<td>2 ns</td>
<td>1 ns</td>
<td>1 ns</td>
</tr>
<tr>
<td></td>
<td>60-90</td>
<td>2 cd</td>
<td>2 ns‡</td>
<td>1 ns</td>
<td>1 ns</td>
<td>1 ns</td>
</tr>
</tbody>
</table>

ANOVA $P > F$  

0.0071 0.6510 0.3258 0.4290 0.3820

† Values with the same letter are not significantly different for means separation at $\alpha = 0.10$ within columns
‡ NS, the interaction between cover crop treatment and sampling depth was not significant at $\alpha = 0.10$ within columns
§ LM, Legume Mix; LMR, Legume Mix and Rye combination