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I am submitting herewith a dissertation written by Marcia Peireira Quinby entitled "CORN (Zea mays L.) PRODUCTION IN LIVING MULCH SYSTEMS, GRAZING POTENTIAL, AND ECONOMIC VIABILITY." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Plant Sciences.

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GRAZING POTENTIAL AND ECONOMIC VIABILITY OF CORN (Zea mays L.) PRODUCTION IN LIVING MULCH SYSTEMS

A Dissertation Presented for the

Doctor of Philosophy

Degree

The University of Tennessee, Knoxville

Márcia Pereira Quinby

August 2022

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ABSTRACT

Living mulch (LM) is a practice in which forages are grown simultaneously with the main crop, serving as a living cover throughout the growing season. The LM systems were developed to alleviate concerns of soil depletion and reduce negative effects of tillage on soil productivity. In addition, legumes used, it can decrease the reliance on N fertilizer. The use of corn in LM has been previously studied due to the crop being a large commodity in the U.S. In addition, the ability to graze the LM after corn production can increase land use efficiency. To determine the benefits of LM in the Southeastern U.S., two studies were developed. The first study had WC (Trifolium repens L. [WC]) LM and a mixture of crimson clover (Trifolium incarnatum L.) and cereal rye (Secale cereale L. [CCCR]), in Spring Hill, TN from 2018 to 2021. Cull cows were used for the grazing period of four weeks before planting and after harvest of corn. The study evaluated the botanical composition (BC), LM mass (LMM), nutritive value (NV), corn silage and grain production, and cows average daily gain (ADG). The second experiment contained WC LM seeded with corn silage and grain in different N levels to determine the best level of fertilization when utilizing WC. The production of corn at harvest, botanical composition, and LM mass throughout the corn growing season were assessed. Lastly, economic analysis was performed in both projects to determine the viability of the system.

iv

TABLE OF CONTENTS

INTRODUCTION
Living mulch species (e. g. white clover, crimson clover, and cereal rye) 4
Living Mulch Grazing7
Living mulch economics
Objectives
References11
CHAPTER I: Corn Production in Living Mulch Systems in the Southeastern U.S.
Abstract
Introduction
Materials & Methods
Measurements and Management
Living mulch botanical composition, mass, and nutritive value
Corn production and nutritive value
Grazing
Economic analysis
Statistical analysis
Results and Discussion
Weather
Botanical composition (BC)
Living mulch mass (LMM)

	Crude Protein value of LM	35
	Corn production and nutritive value (NV)	36
	Grazing	39
	Economic analysis	44
	Conclusions	44
	Appendix	46
	References	66
CHAPTI	ER II Nitrogen requirements for corn production in white clover living mulc	ch
systems		
	Abstract	74
	Introduction	75
	Materials & Methods	76
	Measurements and Management	77
	LM botanical composition, mass, and nutritive value	78
	Corn production and nutritive value	79
	Statistical analysis	80
	Results & Discussion	80
	Weather	80
	Experiment I	81
	Experiment II	86
	Conclusion	90

Apper	ndix		
Refer	ences	105	
CHAPTER III:	Assessment of yield and partial net returns of corn proc	luction in LM	
systems in different N LEVELS			
Abstra	act	111	
Introd	luction		
Mater	ial & Methods	113	
Eco	onomic analysis		
Sta	tistical analysis		
Resul	ts & Discussion	115	
We	ather	115	
Cor	rn production	115	
Par	tial net returns and implications of the LM system		
Concl	usions		
Apper	ndix		
Refer	ences		
CONCLUSIONS & FUTURE DIRECTIONS			
VITA 130)		

LIST OF TABLES

Table 1-1: Grazing period, forage sampling dates and number of animals used on
paddocks of corn growing with living mulch in 2020 and 2021 in Spring Hill, TN 46
Table 1-2: Precipitation (mm) and temperature (°C) from 2018 to 2021 field preparation
and growing season, and 30-year average in Spring Hill, TN 47
Table 1-3: Botanical composition (g kg ⁻¹) of living mulch (LM) (crimson clover/cereal
rye, CCCR; and white clover, WC) and its differences between beginning and end of
the corn growing season of two consecutive years in Spring Hill, TN
Table 1-4: Living mulch mass (LMM) (kg ha ⁻¹) of white clover (WC) and a crimson
clover-cereal rye mixture (CCCR) during the corn growing season of two
consecutive years (2020 and 2021) in Spring Hill, TN
Table 1-5: Crude protein levels (g kg ⁻¹) of white clover (WC) and a crimson clover-cereal
rye mixture (CCCR) as a living mulch during the corn growing season of two
consecutive years (2020 and 2021) in Spring Hill, TN
Table 1-6: Corn grain and silage production (DM t ha ⁻¹) grown in living mulch systems
(crimson clover and cereal mixture, CCCR; white clover, WC) of two consecutive
growing seasons (2020 and 2021) in Spring Hill, TN
Table 1-7: Nutritive value of unfermented and fermented silage grown in living mulch
systems during two consecutive growing seasons (2020 and 2021) in Spring Hill,
TN

- Table 1-9: Botanical composition (g kg⁻¹) of living mulch (LM) (crimson clover/cereal rye, CCCR; and white clover, WC) and its differences between beginning and end of each 28-d grazing period during two consecutive years (2020 and 2021) in Spring Hill, TN.
 57

- Table 1-13: Average Daily Gain (ADG, kg) of cows in living mulch (LM) (crimson clover/cereal rye, CCCR; and white clover, WC) paddocks at the beginning and end of each 28-d grazing period during two consecutive years in Spring Hill, TN. 63
- Table 1-14: Cost of production in 65% moisture (\$ ha⁻¹), corn yield (t ha⁻¹), revenue (\$ ha⁻¹), and profit (\$ ha⁻¹) of corn production of silage and grain in no-LM (living

mulch) systems compared to CCCR (crimson clover-cereal rye mixtures) and white clover (WC) LM in Spring Hill, TN during 2020 and 2021 growing seasons. 64 Table 1-15: Difference in cost of production (\$ ha⁻¹) between no-LM (living mulch) and LM systems (CCCR, crimson clover-cereal rye; white clover, WC), \$ per head, and \$ per head per day in Spring and Fall grazing in 2020 and 2021 growing seasons in Table 2-1: Living mulch (LM) botanical composition (DM g kg⁻¹) grown with silage corn during two consecutive growing seasons (2020 and 2021) in Spring Hill, TN...... 92 Table 2-2: Total living mulch (LM) mass (LM and weeds) (DM kg ha⁻¹) grown with silage corn during two consecutive growing seasons (2020 and 2021) in Spring Hill, Table 2-3: Living Mulch (LM) crude protein (CP), grown with silage corn during two Table 2-4: Corn silage production (DM ton ha⁻¹) during two consecutive growing seasons Table 2-5: Nutritive value (g kg⁻¹) of fermented and unfermented silage during two Table 2-6: Living mulch (LM) botanical composition (DM g kg⁻¹) grown with grain corn during two consecutive growing seasons (2020 and 2021) in Spring Hill, TN...... 99

Table 2-7: Total living mulch (LM) mass (LM and weeds) (DM kg ha-1) grown with
grain corn during two consecutive growing seasons (2020 and 2021) in Spring Hill,
TN
Table 2-8: Living Mulch (LM) crude protein (CP), grown with grain corn during two
consecutive growing seasons (2020 and 2021) in Spring Hill, TN 102
Table 2-9: Corn grain production (Ton ha ⁻¹) during two consecutive growing seasons
(2020 and 2021) in Spring Hill, TN
Table 2-10: Nutritive value (g kg ⁻¹) of corn grain during two consecutive growing
seasons (2020 and 2021) in Spring Hill, TN 104
Table 3-1: Variety, plating date, and seeding rates of LM grown in 2020 and 2021 in
Spring Hill, TN
Table 3-2: Variety, planting date, and seeding rates of LM grown in 2020 and 2021 in
Spring Hill, TN
Table 3-3: Annual budget of corn silage production within each assigned treatment with
and without living mulch (LM) at different N fertilizer rates in Spring Hill, TN 122
Table 3-4: Annual budget for corn grain production within each assigned treatment with
and without living mulch (LM) at different N fertilizer rates in Spring Hill, TN 123
Table 3-5: Yield of corn silage and grain in 2020 and 2021 growing seasons, and partial
net returns within treatments in Spring Hill, TN124

LIST OF FIGURES

Figure 1-1: Precipitation (mm) of June 2021 growing season and 30-year average in
Spring Hill, TN
Figure 1-2: Botanical composition (BC) of living mulch (LM) (crimson clover/cereal rye,
CCCR; and white clover, WC) paddocks during the 2020 and 2021 corn growing
season in Spring Hill, TN 50
Figure 1-3: Living mulch mass (LMM) (kg ha ⁻¹) of CCCR and WC during the growing
season of 2020 and 2021 in Spring Hill, TN
Figure 1-4: Botanical composition (BC) of living mulch (LM) (crimson clover/cereal rye,
CCCR; and white clover, WC) paddocks during each 28-d grazing period on spring
and fall of 2020 and 2021 in Spring Hill, TN59
Figure 2-1: Precipitation (mm) and temperature (°C) from 2018 to 2021 field preparation
and growing season, and 30-year average in Spring Hill, TN
Figure 3-1: Precipitation (in) and temperature (°F) from 2018 to 2021 field preparation
and growing season, and 30-year average in Spring Hill, TN

INTRODUCTION

Corn (*Zea mays* L.) is the major cereal crop of the U.S. (Boyer & Hannah, 2000). The U.S. alone is responsible for 32% of the world corn production (FAS, 2022); and its holistic use, from human to animal consumption (Revilla et al., 2021; Hallauer, 2004), make this crop an important commodity around the world. Corn is a warm-season annual plant from the grass family, with fibrous root system, a single leaf at each node. Depending on the hybrid, it can reach 3.5-m in height (Tollenar & Dwyer, 1999).

Corn is fed as silage or grain for animal production (Klopfenstein et al., 2013), especially in dairy production systems (Putnam & Delcurto, 2020). Total corn production in the Southeastern U.S. has increased 77% from 2007 to 2017, and silage is one of the most important forages for dairy producers (NASS Quick Stats^{1,} 2020). Silage is a method of forage preservation through fermentation in an anaerobic environment, allowing the feed to be ready for cattle consumption (Muck et al., 2020). However, even though total corn production increased, silage production has simultaneously decreased 15% (NASS Quick Stats^{1,} 2020) due to the sharp decline in dairy production in this region (Rahelizatovo & Gillespie, 1999).

Although corn grain production keeps increasing, only 8% of the crop grain area is harvested in the Southeastern states (NASS, 2022). For this reason, strategies to increase the land use efficiency are necessary. In addition, corn production in the Southeastern U.S. is usually affected by poor soil health, and issues such as erosion can result in low yields. Strategies such as no-till (Cassel et al., 1995) and living mulch (LM) systems are helpful to overcome these issues.

Living mulch is a system in which the forage species are established before or simultaneously with the row crop and maintained as a living cover throughout the growing

season (Hartwig & Ammon, 2002). One of the first known studies utilizing LM systems was conducted by Adrien Pieters in 1927, where the author demonstrates the different types of green manure that can be adopted by farmers to maintain soil health. In this report, LM is referred as a "companion crop", where clovers were seeded with corn and oats; and, after harvesting the grain, the clovers were harvested for hay production (Pieters, 1927). In general, LM systems were developed to alleviate concerns of soil depletion and find reduce negative effects of tillage on soil productivity, since farmers would rely solely on tillage for weed control before the introduction of herbicides (Paine & Harrison, 1993).

While conventional production can negatively affect the soil health (Kibblewhite, et al., 2008), LM systems could have the opposite effect. The LM system can improve soil structure by reducing soil erosion (Siller et al., 2016) and restricting weed growth in between corn rows (Sanders et al., 2018). In addition, the U.S. and Europe are trying to encourage producers to reduce the use of synthetic N by recommending the use of green manures, legumes, and organic fertilizers (Prasad, 1998). When utilizing legumes as a LM, the association with *Rhizobium* spp. in legume roots leads to an influx of N, increasing the N pool for plant uptake (Peoples & Craswell, 1992).

Nitrogen is an essential macronutrient for plant growth because it is responsible for amino acid and protein synthesis, and it is a component of the chlorophyll molecule (Barker & Culman, 2020; Tripathi et al., 2014) for photosynthesis. To meet the N demand of crops, synthetic N fertilizers are often used in most cash crop production systems. However, these synthetic N fertilizers can have harmful side effects, such as acid rain and contamination of ground waters due to nitrate concentration, and ammonia volatilization (Prasad, 1998). For this

reason, the use of legumes to ease the amount of synthetic fertilizer can ultimately provide N to the corn (Andrews et al., 2018) through N transfer.

The N transfer occurs through forage decomposition (Sanders et al., 2018), N from the root exudates (Paynel et al., 2001; Ta et al., 1986), or arbuscular mychorrizal fungi (AMF) association (Thilakarathna et al., 2016). According to Soumare et al. (2020), AMF aids BNF (Biological Nitrogen Fixation) by the direct or indirect interaction with microorganisms that have N-fixing functions. In addition, lower N fertilization should be applied to assure that the N uptake comes from the BNF rather than chemical fertilization (Ledgard et al., 2001). Enriquez-Hidalgo et al. (2006), it found that synthetic N fertilization reduces the amount of fixed N with increased N fertilizer. The BNF is an energetically costly system, thus, when N is available in soils, the roots association do not occur effectively. According to Kunelius (1974), N fertilization decreases the nodulation and weight of the nodules in legume, which leads to less BNF. Therefore, intensive management and adequate environmental conditions are necessary for the success of the LM system.

A study conducted in Nova Scotia showed that the LM system is limited in climates with lower temperatures, because the LM leads to lower soil temperature, delaying corn emergence, development, and increasing its sensitivity to frost (Martin et al., 1999). In the other hand, a study conducted in Georgia concluded that LM systems can be successful in areas with warmer soils (Andrews et al., 2018), since higher soil temperatures can increase soil mineralization (Guntiñas et al., 2012) by releasing important nutrients for plant growth.

According to Siller et al., (2016), the LM systems can decrease soil loss through erosion by 77% and P and N loss by 80%. Deguchi et al. (2017) observed that P fertilization might not be necessary in silage corn grown under LM systems, due to a potential increased mycorrhizal

association with the corn. Püschel et al. (2017) found that mychorrizal associations not only increase the P uptake, but also increase the total plant N through BNF. According to Israel (1987), P increases the number of nodules and fresh weight in soybeans; These. Therefore, these associations have a great impact in increasing crop production (Messa & Savioli, 2021) due to the increase in root contact surface by the fungal mycelia that develop in the roots (Smith & Smith, 2012) and for the increase in P acquisition that increases the efficiency of BNF by the increase of nodule mass (Divito & Sadras, 2014)

To maximize cash crop production, suppression of the LM is necessary to avoid competition between corn and LM for nutrients and water (Sanders et al., 2017; Affeldt et al., 2004; Hoffman et al., 1993). Zemenchik et al. (2000) studied kura clover as LM and found that, if the LM was not suppressed, it delayed corn emergence;. Ginakes et al. (2020) found that suppression of LM through tillage methods provided greater soil N, thus increasing grain yields. Similar responses were found using partial rototilling to suppress LM two weeks after corn emergence (Grubinger et al., 1990).

Even though LM systems are, for the most part, beneficial, they can also produce some challenges. Several studies observed that corn yield tended to be lower under living mulch systems than corn grown conventionally (Hill, et al., 2021; Andrews et al., 2018; Sanders et al., 2018; Ochsner et al., 2010). However, the benefits in local biodiversity must be considered, given the current emphasis in agricultural sustainable practices (Jordan et al., 2007).

Living mulch species (e. g. white clover, crimson clover, and cereal rye)

White clover is a cool-season perennial legume used for hay production, grazing, and cover crops; and it can be used both in mixtures or as a monoculture (Ball et al., 2007; Gibson &

Hollowell, 1966). White clover has the potential to biologically fix more than 200 kg of N per ha⁻¹ (Wedin & Russelle, 2020). Given current sustainable efforts to reduce the use of synthetic N fertilization, the reliability on legumes can help the production of a neighboring species (Caradus et al., 1995).

Van Eekeren et al. (2009) observed that introducing white clover to a grassland helps to maintain soil structure and increase supply of nutrients through the soil; Carlsen et al. (2012) found that white clover has the ability to release chemical compounds that regulate the soil microbial community to inhibit weed growth; and Chapman et al. (2016) concluded that the contribution of white clover to grass mixtures increased the forage accumulation in pastures, decreasing the need for synthetic N fertilization. Legume plants also have species-specific advantages when incorporated into a system. Brtnicky et al. (2021) observed that aboveground dry matter (DM) biomass was greater in crimson clover (*Trifolium incarnatum* L.) than white clover, but the root fresh biomass was greater in white clover than crimson clover. Yet, both species can be successfully utilized in forage systems.

Crimson clover was first introduced in the U.S. in the early 1800's (Westgate, 1913). It is a winter annual forage legume easily spotted by its red vibrant inflorescence. It is widely used in pasture, hay, or green manure (Ball et al., 2007). Crimson clover thrives in well-drained soil with moderate fertility (Duggar et al., 1925). Since it is a legume, it does not require N fertilization as it can fix up to 190 kg N ha⁻¹ (Wedin & Russelle, 2020). In a study by Knight, 1967, crimson clover overseeded in grass swards increased the overall forage mass. Dyck & Liebman (1994) studied the use of crimson clover as green manure to suppress lambsquarter (*Chenopodium album*) and aid sweet corn growth, and observed that crimson clover suppressed the emergence and growth of weeds while not negatively affecting the germination and growth of sweet corn.

However, the seeding of crimson clover should be done before planting the main crop to avoid crimson clover establishment issues due to shading (Brooker et al., 2020). Youngerman et al. (2018) found that, although cover crops aid in the suppression of weeds, the negative relationship between corn density and cover crop biomass suggests that lower corn density would be beneficial to these systems to allow greater light transmission to the cover crop. Also, although crimson clover has slow N release (Dyck and Liebman, 1994), when used as a cover crop for corn production, it can release N before corn demand (Ranells and Wagger, 1996). Therefore). Therefore, it is recommended that corn should be planted soon after incorporation of crimson clover cover crop (Yang et al., 2020).

Crimson clover is often grown with cereal rye (*Secale cereale* L.) as a cover crop in row crops production for weed suppression and soil moisture conservation (Geddes & Gulden, 2021; Hodgskiss et al., 2021; Vann et al., 2018; Wiggins, et al., 2016). Cereal rye is considered an N scavenger (Andrews et al., 2020; Ranells and Wagger, 1997) and for this reason, must be suppressed to avoid competition with the corn (Ilnicki & Enache, 1992; Echtenkamp & Moomaw, 1989). Cereal rye has a high C:N ratio, but its N release can be delayed when compared to legumes (Sievers & Cook, 2018). Since 1940, the benefits of using cereal rye as weed suppressor in the U.S. have been reported (Faulkner, 1943), and since then, its use as a cover crop remains significant.

Siller et al. (2016), found cereal rye with kura clover in corn silage, reduced water runoff and soil and nutrient losses. Rorick and Kladivko (2017) observed that cereal rye improved soil aggregate stability, helping with erosion control and improving water infiltration in no-till corn systems. Although crimson clover and cereal rye are commonly grown as cover crops, their use

as LM can decrease the extra costs of termination and incorporation of the forage in the systems before the cash crop. In addition, their use as LM has not been previously assessed.

Living Mulch Grazing

The need to provide sufficient nutritious forage for grazing animals has been recognized since the 1800's by using grass and legume mixtures (Fussel, 1966). The use of legumes for animal consumption leads to better quality forage and, consequently, better quality manure which can assist in the growth of the subsequent row-crop (Fussel, 1967). There are several available white clover cultivars, and all can provide sufficient nutrients to grazing ruminants (Jahufer et al., 2021). In the Southeastern U.S., white clover can withstand persistent grazing due to its regional adaptation (Brink et al., 1999). When grazed by dairy cows, it can result in up to 33% more milk than pastures without clovers (Harris et al., 1997). However, legume species as a monoculture in grazing systems are not common due to potential bloating issues. Therefore, grass and legume mixed pastures are often recommended.

Binary mixtures of white clover with grasses result in positive effects on digestibility and protein components of the overall feed, which benefits the grazing animal (Brink et al., 2015). These benefits are expressed by greater milk production and lower methane emissions (Loza et al., 2021). Gerhards (2018) found the residual white clover LM was dense following corn harvest, which could be ideal for its use in grazing systems. In addition, when grazed in areas previously used for corn production, the cattle also would have access to corn stover for consumption (Franzluebbers & Stuedemann, 2014).

The consumption of LM species, such as cereal rye, can provide sufficient forage very early in the growing season, since it is the most cold-hardy of all forages, with ADG greater than

0.8 kg per day (Mckee et al., 2017). Studies have shown that the grazing of cover crops can positively affect soil health. Schomberg et al. (2021) studied the grazing of cereal rye cover crop in no-till cotton systems and observed that cereal rye increased the C return to the soil organic matter (SOM) pool, improving soil health. Blanco-Canqui et al. (2020) observed that soil fertility and corn silage yield did not differ in grazed and non-grazed paddocks containing a cereal rye cover crop. The grazing of crimson clover-ryegrass cover crops also enhanced soil microbial biomass C under no till systems (Franzluebbers & Stuedemann, 2015).

The average farm size in the Southeastern U.S. is 90 ha, according to 2019 summary of farmland in the U.S. (USDA, 2020). Therefore, the use of strategies that can optimize land use efficiency is warranted, and the grazing of LM can be beneficial. To define LM as a sustainable practice, it has to follow the three dimensions of sustainability, which are social, environmentally, and economic (Purvis et al., 2019). However, the economic assessment of LM system has yet to be performed.

Living mulch economics

The cropland in the U.S. has remained constant in the past 100 years; however, the land shifting of production makes land more vulnerable to erosion and nutrient loss (Lubowski et al., 2006). Every agricultural decision can affect the biodiversity and food security in a system (Kanter et al., 2018). The use of agricultural practices that deviate from monocultures are increasing and are often preferable given the sustainable targets of the 21st century (Jordan et al., 2007). Practices such as intercropping and using cover crops or LM aid the net returns for row crop producers (Schnitkey et al., 2016).

Alexander et al. (2019) studied the use of kura clover LM in corn production and found that partial management costs are greater in LM systems than conventional corn production. The N costs were reduced by \$132 ha⁻¹ in the first year of production. Although, the costs were not different from conventional production in the second year, there are economic returns of stover harvest up to \$318 ha⁻¹ in LM systems.

The use of forage legumes in cropping systems often decrease the reliance on N fertilization due to its BNF (Ledgard et al., 2001). However, to maximize yield in cropping systems such as corn, additional N fertilizer is essential, even when intercropping legumes in the system (Karpenstein-Machan & Stuelpnagel, 2000). This additional N fertilizer can be economically hefty, especially since urea is often the N fertilizer of choice. The use of urea exponentially increased from less than 20% of the total N fertilizer used worldwide to more than 40% in 2005 (Gilbert et al., 2006). According to the USDA Economic Research Service, urea is one of the most utilized N sources for cropping systems, with 6,384,816 metric tons in 2015 (USDA, 2019). Even when commodity prices increase, the high fertilizer prices result in negative net returns (Huang et al., 2009). In a publication by Huang (2009), the author explains that the increase in fertilizer prices since 2008 were due to exponential demand for production, intensifying the N reliance in crop monocultures.

Corn is one of the most valuable commodities in the U.S. in agricultural exports, valued as more than 17 billion dollars in 2021 (USDA¹, 2022). Heavy feeder cattle prices are positively affected by the increase in corn prices (Martinez et al., 2021), yet, according to the USDA agricultural projections, the price of corn in monoculture is expected to decrease from \$4.53 per bushel to \$4.00 per bushels in 10 years (USDA², 2022). In TN, the pastureland asset value in \$ ha⁻¹ increased 225% from 1997 to 2021 (NASS Quick Stats², 2022). The production of corn in

LM systems can be a good strategy compared to purchasing feed for backgrounding or cow-calf operations. Given the added benefit of LM in weed control (Westbrook et al., 2022), the potential decrease in herbicide use can account for further expense reductions. Tillage practices are known to negatively affect weed control. Ilnicki and Enache (1992) observed that minimum tillage or no-tillage practices decreased weed biomass when utilizing subterranean clover as LM. Therefore, the LM system must be assessed not only agronomically, but also economically.

Objectives

The objectives of this study were [1] to assess corn silage production, grain production, and grazing potential in white clover LM and in a crimson clover and cereal rye mixture [2] evaluate N fertilization rate effects on white clover LM systems for corn yield, and [3] determine the economic viability of the LM system in silage and grain. We hypothesized that [1] white clover LM system will be comparable to conventional systems in corn yield, and grazing will be an added economic benefit of the LM system with additional weight gain or maintenance, [2] the use of synthetic N fertilizer in LM system will be reduced while achieving comparable corn productivity to conventional corn systems, and [3] the LM system will confer economic advantages to producers in yield and profit.

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CHAPTER I: CORN PRODUCTION IN LIVING MULCH SYSTEMS IN

THE SOUTHEASTERN U.S.

Abstract

The living mulch (LM) system is a novelty in the southeastern U.S., and its viability and functionality must be studied. The objective of this study was to evaluate the benefits of LM in corn silage and grain production, and to evaluate the potential of LM grazing between the corn growing season. The experiment was conducted in Spring Hill, TN in 2020 and 2021, and consisted of two LM species, white clover (Trifolium repens L. [WC]) and a mixture of crimson clover (Trifolium incarnatum L.) and cereal rye (Secale cereale L. [CCCR]). Cull cows were used for the grazing period of four weeks before planting and after harvest of corn. The study evaluated the botanical composition (BC), LM mass (LMM), nutritive value (NV), corn silage and grain production, and cow average daily gain (ADG). The WC treatment had a greater weed control than CCCR. In 2020, when differences in LMM were observed, CCCR had greater LMM than WC. Meanwhile, in 2021 the LMM did not differ between WC and CCCR, with both treatments showing less mass in spring and early summer. Greater silage and grain production were observed in 2020 for WC paddocks, but, in 2021, no differences were observed. The ADG was greater in WC than CCCR paddocks. It was concluded that WC as LM can lead to greater corn production than CCCR. The LM for grazing is a beneficial strategy if feeding costs are greater than \$2.28 head/day.

Introduction

The inclusion of corn in living mulch (LM) systems are limited in the southeastern U.S., and the system could potentially benefit the environment as well as decrease the need for synthetic fertilization. The use of LM in the Southeastern U.S. has not yet been assessed for its potential for grazing operations to increase land use efficiency. Therefore, the necessity to determine the viability of the system is warranted.

The LM is a system in which the forage species are established before or simultaneously with the row crop and maintained as a living cover throughout the growing season (Hartwig & Ammon, 2002). Living mulch can improve soil structure by reducing soil erosion (Siller et al., 2016), while limiting weed competition in between corn rows (Sanders et al., 2018, Deguchi et al., 2017; Ilnicki & Enache, 1992). Additionally, legume species used as a living mulch can provide N to the corn (Andrews et al., 2018; Brophy & Heichel, 1989; Ebelhar et al., 1984).

The utilization of white clover (*Trifolium repens* L. [WC]) intercropped with grass species has been long determined a good strategy to decrease the use of synthetic N fertilizers (Martin et al., 1999) due to its ability to biologically fix N (Peters et al., 2020). It grows best in the humid regions of the temperate zone (Gibson & Hollowell, 1966); therefore, TN is in a wellsuited region for incorporating WC. Meanwhile, cereal rye (*Secale cereale* L.) and crimson clover (*Trifolium incarnatum* L.) have been traditionally used as cover crops in corn production systems;; however, data in crimson clover or cereal rye as a living mulch do not exist.

To ensure the benefits of LM in the field, LM must be well suppressed to avoid competition with the main crop. Studies examining the use of herbicides (Sanders et al., 2017; Eberlein et al., 1992) or mechanical suppression of LM (Grubinger & Minotti, 1990) have been conducted. Yet, LM grazing has the potential to suppress the LM before corn planting, while

providing sufficient feed for body weight maintenance of dry cows or weight gain of calves in integrated systems (Guy et al., 2020). According to Johansen et al. (2017), the grazing of WC can lead to greater milk production when compared to red clover and grasses. Meanwhile, cereal rye can extend the grazing season and, when mixed with legumes, can generate equal ADG to that from pastures without legumes and with 45 to 67 kg ha⁻¹ of synthetic N fertilizer (Mckee, et al., 2017). Therefore, there is a need to determine the benefits of grazing and corn production in LM production systems.

The objective of this study was to evaluate the benefits of two different LM in corn silage and grain production, and to evaluate the potential of LM grazing before and after the corn growing season in spring and fall. It was hypothesized that the LM systems can successfully increase corn production compared to no-LM systems, and produce sufficient LM mass for grazing operations.

Materials & Methods

The research was conducted at the Middle Tennessee Research and Education Center (MTREC) in Spring Hill, TN ($35^{\circ}72'$ N, $86^{\circ}96'$ W) from October 2018 to April 2021. Initial soil nutrient levels on the experiment site were pH = 5.6, P = 810 kg ha⁻¹, K =415 kg ha⁻¹, Ca = 3904 kg ha⁻¹, and Mg = 300 kg ha⁻¹. The area consisted of several well drained soil types with elevation ranging from 400 to 1200 ft. The soil types were Armour series (fine-silty, mixed, active, thermic Ultic Hapludalfs) silt loam soil complex with 0 to 5% slopes, Braxton series (fine, mixed, active, thermic Typic Paleudalfs) cherty silty clay loam soil complex with 5 to 12% slopes, Braxton series (fine, mixed, active, thermic Typic Paleudalfs), silty clay loam soil complex with 5 to 12% slopes, Huntington series (fine-silty, mixed, active, mesic Fluventic Hapludolls) silt loam soil complex 0 to 6% slopes, Inman series (fine, mixed, active, thermic

Ruptic-Alfic Eutrudepts) and Hampshire series (fine, mixed, active, thermic Ultic Hapludalfs) silty clay loams soil complex with 4 to 12% slopes, and Maury series (fine, mixed, active, mesic Typic Paleudalfs) silt loam with 2 to 5% slopes (NRCS, 2019).

Due to limited available space, the plots were divided into large paddocks and a smaller set of control plots. The large paddocks contained either corn silage or grain, grown with living mulch of either ("Durana" white clover (*Trifolium repens* L.) [WC]or with a mixture of crimson clover ("AU Sunrise" *Trifolium incarnatum* L.) and cereal rye ("Wintergrazer" *Secale cereale* L.) [CCR]). Paddocks were arranged in a complete randomized design (CRD) in triplicate, totaling 12 plots. Each large paddock had on average 0.7 ha⁻¹. The small plots consisted of the control group and were grown in an adjacent area, each measuring approximately 6 x 16 m. In the control group, the two corn types were seeded in seedbeds without LM and replicated four times, totaling eight plots also in a CRD.

Measurements and Management

On Oct 18, 2018, the field was cleaned by spraying 2.25 kg ha⁻¹ glyphosate (N-(phosphonomethyl) glycine; Cornerstone Plus, Agrisolutions, St. Paul, MN) and 0.2 kg ha⁻¹ of Fusilade (FusiladeDX, Syngenta, United Kingdom) in the entire area using a Bestway Field-pro III trailer-mounted sprayer (Janson). On Oct 23, 2018, WC was seeded at 11 kg ha⁻¹ using a Great Plains 38-cm drill (Manufacturing Inc, Salina, KS). To ensure complete establishment of WC, the experimental period started in the fall of 2019.

Crimson clover and cereal rye were seeded on Oct 23, 2019, and on Nov 28, 2020 at 11 kg ha⁻¹ and at 22 kg ha⁻¹, respectively, using the same Great Plains 38-cm drill (Manufacturing Inc, Salina, KS). Lanes for corn seeding measured approximately 90-cm. These lanes were

created each year (May 15, 2020 and May 17, 2021) by spraying 0.45 kg ha⁻¹ glyphosate (N-(phosphonomethyl) glycine; Cornerstone Plus, Agrisolutions, St. Paul, MN) and 0.16 kg ha⁻¹ 2,4-D ammine 4 (Loveland, Greeley, CO) using a 210 Redball 4-row and 8-row hooded sprayer (Wilmar, Benson, MN). The second spraying preceding corn seeding occurred on May 29, 2020 and May 31, 2021, with 0.5 kg ha⁻¹ of paraquat (Paraquat concentrate, Solera, Yuma, AZ), 0.62 kg ha⁻¹ of atrazine (Atrazine 4L, Drexel, Memphis, TN), 0.28 kg ha⁻¹ of s-metolachlor (Charger Max, Agrisolutions, St. Paul, MN), and surfactant (Surf 80, Cannon packaging Co., Inc.) at 35 ml ha⁻¹ using a 210 Redball 4-row and 8-row hooded sprayer (Wilmar, Benson, MN). The entire area with the control plots were sprayed on May 28, 2020 and on May 17, 2021 with 1.3 kg ha⁻¹ of paraquat (Paraquat concentrate, Solera, Yuma, AZ) and surfactant (Surf 80, Cannon packaging Co., Inc.) 35 ml ha⁻¹, using the RM 200 plot sprayer (AgSpray, Hopkinsville, KY).

Different relative maturity (RM) of each corn variety was used to minimize the harvest period between grain (RM 108) and silage (RM 120). On June 1 of 2020 and 2021, corn grain (Croplan 5887VT2P/RIB) and corn silage (Dekalb DKC67-44) were planted at 77,000 seeds per ha⁻¹ with 90-cm spacing using a 4-row planter (John Deere, Lewisburg, TN). Urea (46-0-0) was applied at 36 kg ha⁻¹ using a 3-m drop spreader (Gandy, Owatonna, MN) on June 8 and 26, 2020, and on May 3 and June 30, 2021.

The corn silage was harvested on August 20, 2020, and on August 23, 2021, using a 900 and T100 chopper (New Holland, Racine, WI) and a feeder mixer for material collection (Helm Welding, Lucknow, Ontario). The corn grain was harvested on September 16, 2020, and Oct 13, 2021, using a plot combine (Almaco and Allis-chalmers Gleaner K2), the Ford F600 grain truck, and a service weigh wagon (Par-Kan).

Living mulch botanical composition, mass, and nutritive value

Measurements to determine botanical composition (BC) and living mulch mass (LMM) were conducted simultaneously by clipping samples using a 0.1 m² quadrat in 10 random spots within each paddock, randomly choosing corn lanes, totaling 120 samples. These samples were collected biweekly, from May 28 to Sept 24 in 2020, and from May 25 to Oct 15 in 2021. Each sample was then separated into LM (WC or crimson clover + cereal rye) and weeds and placed in a drier at 60°C to constant weight (~72 hr). The samples were weighed for determination of the dry matter (DM) LMM. All living mulch samples collected were then recombined after separation for BC, and ground in a Wiley Mill Grinder (Thomas-Wiley Laboratory Mill Model 4, Arthur H. Thomas Co., Philadelphia, PA) using a 2-mm screen for CP, NDF, and IVTDMD48 analyses. Samples were then passed through a 1-mm cyclone mill (FOSS Cyclotec, Eden Praire, MN) to decrease particle size (McIntosh et al., 2022). Prior to analysis, the samples were individually placed in foil tins and dried for 30-min in a forced air oven at 55°C to allow consistent moisture content for scanning and decrease variability across results (McIntosh et al., 2019). These samples were scanned in small ring cups on Near-infrared spectroscopy (NIRS) technology (Unity SpectraStar XL-R, Unity Scientific, Milford, MA). Unfermented Corn Silage (UC), Fermented Corn Silage (CS) and Haylage (HL) calibrations were provided and licensed by the NIRS Forage and Feed Consortium (NIRSC, Berea, KY) were used. The global and neighborhood statistical tests were monitored and analyzed for accuracy across all predictions with entire data set fitting the calibrations within the (H < 3.0) limit of fit and reported accordingly (Murray and Cowe, 2004). Units of measurement for nutritive analyses are presented at 100% dry matter (DM) across the entire data set.

Corn production and nutritive value

Corn yield of silage and grain were determined by harvesting the two middle rows of each plot (both large paddocks and control plots). The length of the plot and numbers of rows were determined prior to harvesting, and the weight was recorded for determination of the production per ha⁻¹. In both years, a sample from the harvested material of corn silage was taken from the bulk harvested, then dried and ground in a Wiley Mill Grinder (Thomas-Wiley Laboratory Mill Model 4, Arthur H. Thomas Co., Philadelphia, PA) using a 2-mm screen to be used later for the determination of the silage nutritive value of unfermented corn silage on the NIRS. Fermented silage was made by utilizing 18 x 20 cm air-tight Ziploc[®] bags, containing approximately 100 g of the harvested material, then pressed by hand to remove as much air as possible. Individual bags were placed in an extra-large space bag (Ziploc[®] Space Bag[®]). The bags were airtight and waterproof, and the air was removed by utilizing the vacuum in the oneway valve to maintain an anaerobic environment. These bags were placed in a 55 L plastic bin covered by a 32 kg⁻¹ sandbag to compress the samples and avoid external oxygen contamination. The plastic bin was then closed with a lid for 30 days before opening for determination of the nutritive value after dried. The samples were also ground in a Wiley Mill Grinder (Thomas-Wiley Laboratory Mill Model 4, Arthur H. Thomas Co., Philadelphia, PA) using a 2-mm screen. All samples from both unfermented and fermented silage were scanned using the same methodology described for the LM nutritive value analyses on the NIRS, using the unfermented and fermented corn silage spectra for CP, NDF, Starch, and IVTDMD48. The corn grain was analyzed using the Ingratec Grain Analyser (FOSS North America, Inc., Eden Prairie, MN). The samples were best scanned when the amount averaged 470g. However, some samples, especially

in 2021, did not yield the optimum amount and. Therefore, these were scanned twice for accuracy and the average results were utilized.

Grazing

Grazing was performed in spring and fall of 2020 and 2021 (Table 1.1). The stocking rate was variable among paddocks according to the forage availability of each large paddock. Variable stocking rate was conducted to avoid over or under-grazing and varied from 1 to 4 animals (Table 1.1). Jersey cull cows were used, averaging 513 kg of body weight (BW) per paddock for each 28-d grazing period, and weighed immediately before and after entering the paddocks. Single-wire electric fences were used in each paddock and Bloat Guard[®] blocks (Sweetlix, Mankato, MN) were available to the animals to avoid bloating issues, while water was available *ad libitum*. To determine BC and LMM of the paddocks, ten 0.1 m² quadrats were randomly clipped before and after the grazing period. The BC was determined by separating each LM sample into three categories: LM (WC or crimson clover + cereal rye), broadleaf weeds (BLW), and grass weeds (GW). Each sample was dried at 60°C for 72-h, recombined, and weighed to determine the DM LMM.

Economic analysis

Annual production budgets for each LM treatment were developed based on The University of Tennessee Field Crop Budgets (Smith & Bowling, 2022). The values of seeding and machinery for WC planting were only accounted in the year of establishment, while CCCR costs of machinery and seeding were accounted every year. The cost of production includes the corn and LM seed, the fertilizer rate, herbicides, repair, maintenance, fuel, oil, and filter of the utilized machinery, operating labor and crop insurance as variable expenses, and capital recovery

and management labor as fixed expenses. The budget costs were used to determine the difference between the change in cost per ha of corn in LM and without LM (\$ ha⁻¹). The \$ head⁻¹ was determined by dividing the \$ ha⁻¹ by the stocking density in each plot (Table 1.1). Finally, the \$/head/day was determined by dividing the \$ head⁻¹ by 28 grazing days.

Statistical analysis

Mixed model analyses of variance were performed to determine differences in least square means using the Fisher's LSD. Data were analyzed by using the PROC GLIMMIX procedure in SAS (SAS 9.4, Cary, NC). The dependent variables related to LM were BC, LMM, and CP the fixed effects were sampling month, LM species, corn type, year and its four-way interaction with random effects of replication within LM species, and replication × LM species × sampling month × year, with repeated measures of sampling day.

The corn production fixed effects were LM species, corn type, and its two-way interaction with the random effect of replication. For the grain, dependent variables of NV were starch, oil, and protein, and the fixed effects were LM species with the random effect of replication. For fermented and unfermented silage, dependent variables were CP, NDF, starch, and IVTDMD48; and fixed effects were LM species, year, and its two-way interaction, with the random effect of replication.

The grazing study was separated by year (2020 and 2021) and season (fall and spring), given the differences of each grazing period in production and composition. The sampling dates were considered the beginning and end of the grazing period. The grazing study dependent variables were BC, LMM, and NV (CP, NDF, and IVTDMD48); and fixed effects were sampling date, LM species, and its two-way interaction with the random effect of replication. For

the ADG dependent variable, fixed effects were considered corn type, LM species, and its twoway interactions with random effect of replication within corn type and LM species. All results were evaluated for significance at P < 0.05.

Results and Discussion

Weather

In Oct. 2018, there was 8% less precipitation than the 30-year average; yet the temperature was 22% greater than the 30-year average. Meanwhile, in Oct. 2019, there was nearly 90% greater precipitation than the 30-year average, and 14% greater temperature than the 30-year average. From June to September, in 2020 there was 20% greater precipitation compared to the 30-year average, and temperature was 2% greater than the 30-year average. From June to September 2021, precipitation was 60% greater than the 30-year average, and temperature was 4% less than the 30-year average in 2021 (Table 1.2). Although precipitation was greater than average through the growing season, in June there was a lack of precipitation following planting that greatly affected corn germination (Fig. 1.1). Germination of corn occurs between 2 to 6 days, depending on the soil temperature (Wang & Fields, 1978);). During this time, the only precipitation between day 1 and 6 was a 20-mm on day four after planting. In total, there were only nine days of precipitation throughout the entire month, which affected the germination.

Botanical composition (BC)

There were differences between years for the BC of LM, but no differences between corn type (grain or silage) were found; therefore, results are reported by year while corn type was combined (Table 1.3). In 2020, there was a sampling month \times LM species interaction (*P* < 0.0001; Table 1.3). In May, the LM proportion was greater for CCCR, but it did not differ from

WC in Jun; whereas in the remaining months, WC showed greater LM proportion. Meanwhile, the exact opposite happened for weed proportion, which is expected given that crimson clover and cereal rye are short-lived annual species (Ingels et al., 1998). The mass accumulation of cereal rye can be great until the first week of June. In cover crop practices it is common to terminate crimson clover or cereal rye between May and June prior corn planting (Keene et al., 2017; Vann et al., 2018).

In 2021, the LM proportion did not differ between CCCR and WC in May, likely due to the late establishment of CCCR in 2020, reducing its overall presence in the field. Abdin et al. (1997) also observed that late seeding of grass species can lead to poor establishment when compared to early seeding. Due to the low soil temperatures that can occur in Nov and Dec, late seeding can delay seed germination (Wilson et al., 2013). In addition, water uptake at low temperatures damages seedling development (Mayer & Shain, 1974), which affects its establishment. Meanwhile, in Jun and Jul the LM proportion of CCCR was lower than WC, and in the later months (Aug, Sep and Oct) no differences were observed between LM treatments (Table 1.3).

These later months of the growing season shows a shift in the overall BC of the stand, with predominantly weeds in all treatments. Clovers can be challenging when grown under high temperatures and dry conditions (Kendall et al., 1985); leading to decreases in overall proportion in the field. In a study conducted by Ehret et al. (2015), working with different shading levels to WC in agroforestry systems, it was concluded that WC responded negatively to shade for its productivity. The WC in our study was established in the fall of 2018, and as observed by Guy et al. (2020) the persistence of WC decreases during the third year after establishment, which led to the greater weed competition for WC plots in 2021 as compared to 2020 (Fig. 1.2).

Living mulch mass (LMM)

The years were analyzed separately given the differences. In 2020, there was a LM × sampling date interaction ($P \le 0.0001$). No differences in LMM between WC and CCCR were observed in May and Jun (Table 1.4, Fig. 1.3). The greatest mass production was observed in Aug for CCCR and in May for WC (Table 1.4). As a C3 plant, CCCR and WC grows rapidly in spring and thrive under lower temperatures (Ball et al., 2007). The greater mass in Aug for CCCR reflect the increased proportion of warm-season weeds shown in CCCR treatments (Table 1.3), such as crabgrass [*Digitaria sanguinalis* (L.) Scop] and especially pigweed (*Amaranthus ssp.*) (data not shown), which can reach heights up to 2-m (Steckel, 2007). These results reveal that annual winter species, such as crimson clover and cereal rye are better suited as cover crops rather than LM (Hill, et al., 2021; Xu et al., 2021; Andrews et al., 2020; Sanders et al., 2018).

In 2021, there was only sampling month main effect (P < 0.0001) with less LMM in May and Jun, and greater mass in Sept and Oct (Table 1.4, Fig. 1.3). The lack of differences between CCCR and WC is attributed to the decreased persistence of WC, greatly affected by the intense management of the area and stressors from the previous corn presence. In addition, the CCCR mixture was planted in December 2020, whereas it was the third year after WC establishment, leading to a weakened stand. Meanwhile, in September, cool-season forages grow more vigorously, contributing to the increased LMM (Mullenix & Rouquette Jr, 2018).

Crude Protein value of LM

There was a LM × Year (P = 0.0351) and a LM × sampling month (P = 0.0091) interaction in the CP content of the LM species throughout the corn growing season (Table 1.5). The protein levels could influence the level of N available for corn uptake during WC decomposition. For WC no differences were observed throughout the months, but in 2020 there were greater CP content than 2021, likely due to the greater WC content (Table 1.3). Meanwhile, the CCCR had greater CP in Jul and Aug, likely due to the presence of pigweed, that as a broadleaf weed, is also known to have great CP content, although is not consumed by animals. No differences were observed between 2020 and 2021 in CCCR, likely due to the similar botanical composition (Table 1.3).

Corn production and nutritive value (NV)

In 2020 and 2021, silage production was greater than grain production when they are compared in the same scale (Table 1.6). This occurs because when harvesting corn for silage, the entire plant is harvested and utilized, which increases the final weight of the harvested material. Meanwhile, in grain production, only the kernels are harvested, decreasing the total weight. Conventionally, the planting of corn at MTREC in Spring Hill, TN, occurs at the end of April, but in this study, both years, corn was seeded on June 1st, and delaying planting can lead to a reduction in corn yield (Hoffman et al., 1993). The lower production of 2021 is likely due to the decrease in precipitation after corn seeding compared to 2021 (Table 1.2). Given that precipitation greatly affects germination of seeds (Queiroz et al., 2019), plants were not able to establish and germinate properly since the peak of drought occurred in June (Table 1.2). In addition, the average ear height was 129-cm in 2020 as compared to 76-cm in 2021 (data not shown). Ears were also much smaller and some plants did not produce kernels.

According to the corn silage and grain variety tests in Tennessee (Sykes, et al., 2020 & 2021), the average production in Spring Hill at MTREC had an average corn silage production of 18.3 t ha⁻¹ in 2020 and 11.4 t ha⁻¹ in 2021. The same can be said about grain production, in which

the average production was 13.2 t ha⁻¹ in 2020 and 10.3 t ha⁻¹ in 2021. Therefore, the LM production of corn was lower than conventional systems.

The lack of differences between CCCR, WC, and control in 2020 and 2021 reflects the great presence of weeds in all paddocks, especially after corn was established (Table 1.3, Fig. 1.2). A study conducted by Sanders et al., (2018) showed that WC LM led to less corn production under drought than CCCR cover crops. Although in optimal conditions the WC can supplement the N required for corn growth, its decreased persistence in the third year did not confer any production advantages to the WC when compared to CCCR or control.

The analysis of fermented and unfermented silage was different between years, and due to the lack of kernels in 2021, the samples was analyzed as haylage in 2021, and corn silage in 2020. There was an interaction between LM species \times year (P = 0.0005) for CP content of unfermented silage, with greater CP observed in WC in 2020 (Table 1.7). These results are expected, given the greater proportion of WC (Table 1.3). WC has high CP content, therefore increasing the overall nutritive value of the feed (Javanmard et al., 2009). The control plots did not differ from CCCR, and both treatments were composed mainly of weeds during the course of corn production. Interestingly, there were no differences in the NDF content among treatments, and these results could be attributed to the overall composition of the sward. Most weeds found in both paddocks and control plots were crabgrass and broadleaf weeds, which are known for low NDF content, therefore contributing to the lack of differences in the NDF of unfermented silage. Meanwhile, starch content was greater in 2020 than 2021, but it did not differ among treatments each year. The starch is present in the endosperm of the corn grain (Rooney & Pflugfelder, 1986), and given the greater ear production in 2020, the starch content was expected to be higher. The IVTDMD48 was greater in 2021 than 2020 (P = .0001; Table 1.7), and also no

differences were observed among treatments each year. The WSC (water soluble carbohydrates) are responsible for aiding the fermentation in haylage and silage, and the WSC are greater in haylage than silage (Müller et al., 2016) which affects the digestibility of the feed. For this reason, 2021 had greater digestibility, due to the greater amount of forage material in the silage.

Meanwhile, the fermented silage only showed differences between years for NDF, starch, and IVTDMD48 (Table 1.7). The NDF was greater in 2021 than 2020, likely due to the weed composition in the harvested material, while starch and IVTDMD48 had greater values in 2020, due to greater ear presence in the fermented sample. The endosperm of corn increases the digestibility of the feed (Rooney & Pflugfelder, 1986) and, when fermented, its sugars are utilized during the process of fermentation towards stability (Kung, 2018). The differences in CP content are more difficult to observe since nearly 60% of the protein is utilized during the process of fermentation (Woolford & Pahlow, 1998), and these results are reflected in our study.

The grain nutritive value showed a greater starch content in 2021 than 2020, likely due to the concentration of the component in less kernels. Greater protein content in WC (Table 1.8). A study conducted by Miao et al., (2006) working with N levels in corn production, showed an increase in protein content with increased N, and since legumes are a source of N to the companion crops, these results were expected. The lack of differences of starch and oil showed the stability and consistency of these components. Singh et al., (1996) showed that even locations did not affect the starch content of the kernel. Although the oil content can vary within different hybrids (Singh et al., 2000), in our study there were no differences between corn grain and silage, therefore the lack of differences in starch and oil content was expected.

Grazing

1. Botanical composition (BC)

In spring 2020, the LM proportion was greater in CCCR than WC, and a greater proportion of broadleaf weeds (BLW) were observed in WC (Table 1.9). These results are expected, since WC was slowly starting to grow during spring giving BLW an opportunity to grow, while CCCR (a winter annual mixture) was thriving and outcompeting weeds. In a study conducted by Adhikari et al. (2018), cereal rye affected the seedling establishment of alfalfa due to its allelopathic effects, which could explain why crimson clover had lower proportions (Fig. 1.3). Yet, the BC between the beginning and end of the grazing period did not differ (Table 1.9), likely because of the vigorous spring growth of C₃ species (Ball et al., 2007). Similarly, during spring of 2021 greater LM proportion was observed in CCCR as compared to WC, although there were less LM in CCCR at the end of the grazing period. The CCCR were seeded in December 2020, and WC established in the fall of 2018, which could reflect the weakened stand to support animal pressure. The BLW remained greater in WC similar to results observed in the spring of 2020. An interaction between sampling date and LM species in the grass weeds (GW) proportions was observed. The CCCR had greater GW proportion at the end of the grazing period (Table 1.9), likely because after the annual forages were grazed, the weeds were able to establish and compete for resources.

In the fall of 2020, LM proportion was greater in WC than CCCR (Table 1.9) due to the WC regrowth that occurred in the fall (Mullenix & Rouquette Jr, 2018) after corn harvest. In addition, BLW were greater in WC than CCCR, and the GW proportion was inversely related to the BLW composition in the field (Table 1.9). Meanwhile, in the fall of 2021, no differences were observed for all variables (Table 1.9). In addition, GW grew exponentially in the fall on

WC as compared to the other grazing periods, due to the low competitive strength of WC in the third year of establishment (Guy et al., 2020).

2. Living mulch mass (LMM)

Given the differences observed between corn types and between years, the analyses were conducted separately (Table 1.10). In spring 2020, there were no differences in LMM between the beginning and end of the grazing period in corn grain or in silage paddocks, yet greater LMM was observed in CCCR than WC in silage paddocks (P = 0.0494; Table 1.10), likely due to the greater LM proportion observed in the BC (Table 1.9). In spring 2021, the grain paddocks had greater mass in the beginning of the season than the end (P = 0.0125), and greater LMM was observed in WC (P = 0.0145; Table 1.10), which follows the finding of the BC, where greater BLW proportion was observed in WC, increasing the LMM accumulation (Table 1.10). However, these findings were not seen in the silage paddocks, where no differences were found (Table 1.10). When harvesting the silage paddocks, the entire plant is removed of what would otherwise become organic matter which assists with plant regrowth. A study by Bertora et al. (2009) showed that maize straw can increase the C in the soils, and subsequently forages can remove it for its growth.

In the fall of 2020, there was a main effect of LM species (P = 0.0001, Table 1.10) in grain paddocks, with greater LMM in CCCR, and these results were attributed to the greater GW content (Table 1.9). Meanwhile, there was a sampling date × LM species interaction (P =0.0404) for silage paddocks, with less LMM observed for WC at the end of the grazing period (Table 1.10). Although these results are not a direct reflection of the BC, it is possible to assume that WC was getting ready for dormancy, since the grazing ended in the last week of November.

In the fall of 2021, there was a sampling date × LM species interaction (P = 0.0321) in grain paddocks, with the greatest LMM observed in WC at the beginning of the grazing period. The CCCR paddocks did not differ in the same period and treatment, likely due to the amount of corn residue in the field, which remained constant throughout the grazing period. There was a main effect of sampling date in silage paddocks (P = 0.0171), with a decreased LMM at the end of the grazing period for both CCCR and WC (Table 1.10), which is expected after the forage is consumed by the animals.

3. Nutritive Value (NV)

No differences between grain and silage were observed in spring 2020 and 2021, and fall 2020, therefore the analyses for these three periods were combined (Table 1.12). In spring 2020, the CP was greater in WC both in the beginning and end of the grazing period, results that are expected in legume monocultures. The NDF content showed the opposite pattern to CP concentration, which is expected. For IVTDMD48, there was also greater digestibility in the legume monoculture (WC) as opposed to mixed swards (CCCCR) due to the greater presence of CP.

In spring 2021, no differences in CP were observed (Table 1.12). Most species present in the CCCR paddocks, although weeds, were immature at vegetative stage and had similar CP content as those of legumes at the same stage of maturity. There was a main effect of sampling date (P = 0.0078) and LM species (P = 0.0329) for NDF content (Table 1.12). Greater fiber content is expected in CCCR versus WC since there are greater structural components required for the growth and development of cereal rye (Brink & Fairbrother, 1992). Meanwhile at the end of the grazing period, greater NDF was observed, due to the consumption of leaves, which have lower fiber content as opposed to the stubble left behind (Griggs et al., 2007). Interestingly,

greater IVTDMD48 was observed in CCCR, likely due to the vegetative presence of the weeds as compared to WC.

In the fall of 2020, there was a main effect of sampling date and LM species in CP (sampling date: P = 0.0032; LM: P = 0.0047), NDF (sampling date: P = 0.0004; LM: P = 0.0033), and IVTDMD48 (sampling date: P < 0.0001; LM: P = 0.0024). Greater CP was observed in WC as compared to CCCR, leading to lower NDF and greater IVTDMD48. Also, greater CP, greater IVTDMD48, and lower NDF were observed in the beginning of the grazing period as compared to the end (Table 1.12). The results are expected because when fiber content is reduced, it usually indicates high digestibility (Cherney & Parsons, 2020).

In the fall of 2021, there was the main effect of corn type, therefore the analysis was conducted separately. For grain paddocks, there was a main effect of sampling date (P = 0.0069) with greater CP in the beginning of the grazing period. Also, there was greater CP content for CCCR than WC paddocks (P = 0.0090, Table 1.12). These results reflect the increase in weed proportion in WC paddocks (Table 1.9). The IVTDMD48, was greater in the beginning of the grazing season, which is expected because after removal of leaves by grazing, the stems are left behind, which are less digestible (Nave et al., 2014). For silage in the fall of 2021, there were no differences in CP and NDF content, reflecting the homogeneity of the forage composition in the plot (Table 1.9). The IVTDMD48 showed a sampling date (P = 0.0187) main effect with greater digestibility in the beginning of the grazing period than the end (Table 1.12) similarly to the grain paddocks.

4. Average Daily Gain (ADG)

Differences between corn types and grazing periods were significant; therefore, the analyses were done separately. In spring 2020, there was a LM species \times corn interaction (P =

0.0272), and the ADG in WC was greater in silage than grain, while no differences were observed in CCCR (Table 1.13). A study conducted by Schaefer et al., (2014) observed that WC can positively influence animal performance through its greater NV, which is confirmed by this study. In spring 2021, there was only the main effect of LM species (P = 0.0052) where greater ADG was observed in WC than CCCR due to the greater LMM available (Table 1.10). Mckee et al., (2017) also observed that animals grazing in legume or grass paddocks containing cereal rye led to lower ADG due to legume selectivity. In the fall of 2020, the main effect of LM species (P= 0.0304) also showed greater ADG for WC, although lower LMM was recorded (Table 1.10); the WC had greater CP, and it was more digestible than CCCR (Table 1.12), which affected the ADG. Finally, in the fall of 2021, no differences were observed, which was reflected by the lack of differences in nutritive value of the feed (Tables 1.12, 1.13).

From these observations, it is important to observe that nutritive value plays an important role in ADG during the fall, while LMM plays a greater role in the spring. Cool-season forage species have greater growth in the spring (Ball et al., 2007), and the NDF tends to be lower than in the fall, which affects the digestibility. The lack of differences in ADG in the fall of 2021 showed that the lack of BC differences (Table 1.9) directly affected the weight gain of the animals. Also, the ADG tended to decrease in WC from spring 2020 as compared to later grazing periods. This is likely due to decreased persistence of these species (Guy et al., 2020; Schaefer et al., 2014). In addition, the average NDF had > 70% content in the fall of 2021, which greatly affects the intake and digestibility of the feed (Cherney & Parsons, 2020; Buxton, 1996), and consequently the ADG.

Economic analysis

The WC is a perennial forage; therefore, the cost of production is different from 2020 to 2021. Meanwhile, CCCR and conventional corn production remained the same across the years (Table 1.14). Profits were greater in corn grown in WC LM in 2020, likely due to the added N from WC, leading to a \$683.74 higher profit than corn in conventional systems. However, CCCR did not result in higher profits when compared to conventional systems (\$252.00 less profit in 2020). Although a similar pattern was observed in 2021, the production and outcomes were much lower due to the poor establishment of corn, therefore all treatments were not profitable. For corn grain, similar results were observed, with greater profit in corn with WC LM in 2020, but no profits observed in 2021 (Table 1.14).

The decrease in corn production in 2021 is attributed to the low precipitation after seeding, which affected its germination and development. Except for grain in WC in 2021, the differences in cost by adopting LM are greater than conventional corn systems (Table 1.14), yet it is possible to conclude that the use of LM is beneficial in WC systems rather than CCCR based on the profits. The grazing component diluted the additional costs of production when the system is adopted. Although the \$/head/day costs are dependent on the stocking density adopted, the highest cost of grazing was \$2.22 for WC in the Spring of 2020 (Table 1.15). Therefore, if feeding costs of cull cows are above the calculated values (Table 1.15), it is recommended that LM is adopted for greater returns and land use efficiency.

Conclusions

The use of WC as LM leads to a reduction in weed pressure and increased corn production compared with crimson clover-cereal rye mixtures. Also, the use of a perennial forage decreases the need for annual planting. Although CCCR produced greater LMM in the first week of sampling, the use of cereal rye would be more beneficial as a cover crop, due to its short-lived morphology. In normal weather conditions corn grain and silage produced similar yields, but having WC allowed a greater overall production, likely due to the added N. When irregular precipitation patterns are observed, grain production is more affected than silage; therefore, irrigation might be necessary to ensure germination and development of corn seedlings. WC as a living mulch also showed positive applications in grazing systems with greater LMM and NV in most instances. Although cull cows were utilized for grazing, a slight weight gain was observed in animals grazing WC. Further studies using steers are warranted to help advance the use of LM in the Southeastern U.S. The profit is greater in silage than grain and greater in WC LM systems than CCCR or conventional systems when the weather patterns are favorable for corn growth, otherwise irrigation might be necessary. In addition, when feeding costs are greater than \$2.22 head/day, LM systems are a good strategy for grazing operations.

Appendix

		Forage	Sampling	Grazing	g Period	
Year	Season	Enter	Exit	Enter	Exit	
2020	Spring	30-Mar	1-May	1-Apr	30-Apr	
2020	Fall	26-Oct	24-Nov	28-Oct	24-Nov	
2021	Spring	5-Apr	3-May	5-Apr	3-May	
2021	Fall	18-Oct	19-Nov	20-Oct	17-Nov	
	r of animals					
		2	020	2021		
Treatme	ents	Spring	Fall	Spring	Fall	
		2	3	2	3	
	[†] CCCR	1	3	3	3	
Com main		2	3	2	3	
Corn grain		2	3	3	3	
	WC	1	3	3	3	
		1	3	3	3	
		4	3	3	3	
	CCCR	3	3	3	3	
		3	3	3	3	

Table 1-1: Grazing period, forage sampling dates and number of animals used on paddocks of corn growing with living mulch in 2020 and 2021 in Spring Hill, TN

[†]CCCR, Crimson clover- cereal rye mixture; White clover, WC.

WC

Corn silage

Month	30-year avg.		2018	2019	2020	2021
_]		Precipitat	ion (mm)		
Jan	11	6.3	34	162.6	172.2	77
Feb	129	9.6	273.3	267.5	253	117.3
Mar	130	6.6	171.2	102.1	206.5	315.2
Apr	12.	3.6	160.8	158.8	126.7	23.9
May	129	9.6	90.4	81.3	106.7	142.7
Jun	11.	3.2	116.1	209.3	106.4	93.2
Jul	112	2.5	41.7	48	168.9	127
Aug	92	2.8	56.4	53.1	125.5	260.9
Sep	112	2.7	234.7	19.3	126.7	187.2
Oct	104	4.7	96.8	198.4	127.8	119.9
Nov	10	1.9	105.4	143.8	44.7	44.7
Dec	14'	7.4	180.3	187.7	146.1	59.2
_			Tempera	ture (°C)		
Jan	3.	.2	0.2	3.7	6.1	4.5
Feb	5.	5.1		7.5	6.2	2.9
Mar	7.	7.9		8.7	12.6	7.2
Apr	14	14.6		15.8	13.5	13.6
May	19	19.4		17.8	16.2	19.4
Jun	23	6.6	25.9	23.6	24	23.3
Jul	25	5.6	26.5	26.3	26.9	23.5
Aug	24	.9	26.1	25.8	25.2	24.8
Sep	21	.6	25	25.2	21.2	20.5
Oct	1	4	17.2	15.9	16	16.9
Nov	8.	.1	6.3	6.8	11.3	6.4
Dec	4.	.8	5.3	8	4.5	10
		June 20	21 Precipitatio	on (mm)		
	Day	mm	Day	mm	Day	mm
	1	0.0	11	11.7	21	0.0
	2	50.0	12	0.0	22	3.3
	3	1.8	13	0.0	23	0.0
	4	0.0	14	1.8	24	0.0
	5	1.5	15	0.0	25	0.0
	6	3.0	16	0.0	26	0.0
	7	0.0	17	0.0	27	0.0
	8	9.4	18	0.0	28	0.0
	9	8.9	19	0.0	29	0.0
	10	0.5	20	0.0	30	1.5

Table 1-2: Precipitation (mm) and temperature (°C) from 2018 to 2021 field preparation and growing season, and 30-year average in Spring Hill, TN

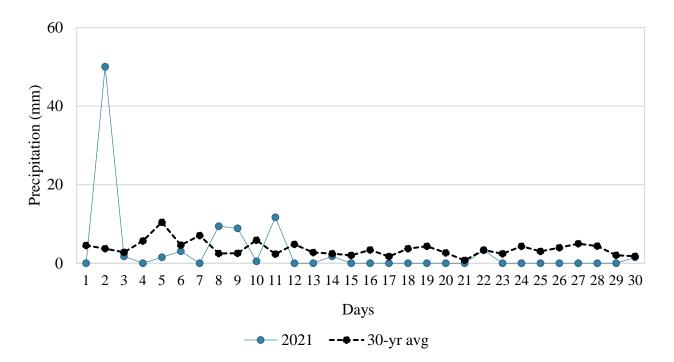


Figure 1-1: Precipitation (mm) of June 2021 growing season and 30-year average in

Spring Hill, TN

	.	2020				
_	L	М	Weed	ds		
	CCCR	WC	CCCR	WC		
May	940.8 ^a	553.4 ^b	59.2 ^b	446.6 ^a		
Jun	440.6 ^a	671.53 ^a	559.4 ^a	328.5 ^a		
Jul	90.3 ^b	622.7 ^a	909.7 ^a	377.3 ^b		
Aug	0.0^{b}	428.7 ^a	1000.0 ^a	571.3 ^b		
Sept	0.0^{b}	324.9 ^a	1000.0 ^a	675.0 ^b		
_		2021				
May	387.9 ^a	534.7 ^a	612.0 ^a	465.3 ^a		
Jun	191.9 ^b	673.6 ^a	808.0^{a}	326.4 ^b		
Jul	17.6 ^b	459.9 ^a	982.4 ^a	540.0 ^b		
Aug	0.0^{a}	178.2 ^a	1000.0 ^a	821.8 ^a		
Sept	0.0^{a}	25.9 ^a	1000.0^{a}	974.0 ^a		
Oct	0.0^{a}	35.8 ^a	1000.0 ^a	964.2 ^a		
_	ANOVA					
	L	М	Weeds			
Sampling month (S)	<.0		<.0001			
LM Species (LM)		594	0.0594			
$S \times LM$	<.0		<.0001			
Corn (C)		456	0.4456			
$\mathbf{S} \times \mathbf{C}$		580	0.258			
$C \times LM$		689 184	0.1689			
$S \times LM \times C$		184	0.7184 <.0001			
Year (Y) $S \times Y$	<.0	915	<.000			
$S \times I$ LM × Y	0.6		0.191			
$S \times LM \times Y$	<.0		<.0001			
$C \times Y$		622	0.9622			
$\mathbf{C} \times \mathbf{S} \times \mathbf{Y}$	0.20		0.2697			
$LM \times C \times Y$	0.9		0.956			
$LM \times C \times Y \times S$	0.9		0.9763			

Table 1-3: Botanical composition (g kg⁻¹) of living mulch (LM) (crimson clover/cereal rye, CCCR; and white clover, WC) and its differences between beginning and end of the corn growing season of two consecutive years in Spring Hill, TN.

Means without a common superscript letter differ within a row. *P*-values are significant at $\alpha \leq$.05 using Fisher's LSD test.

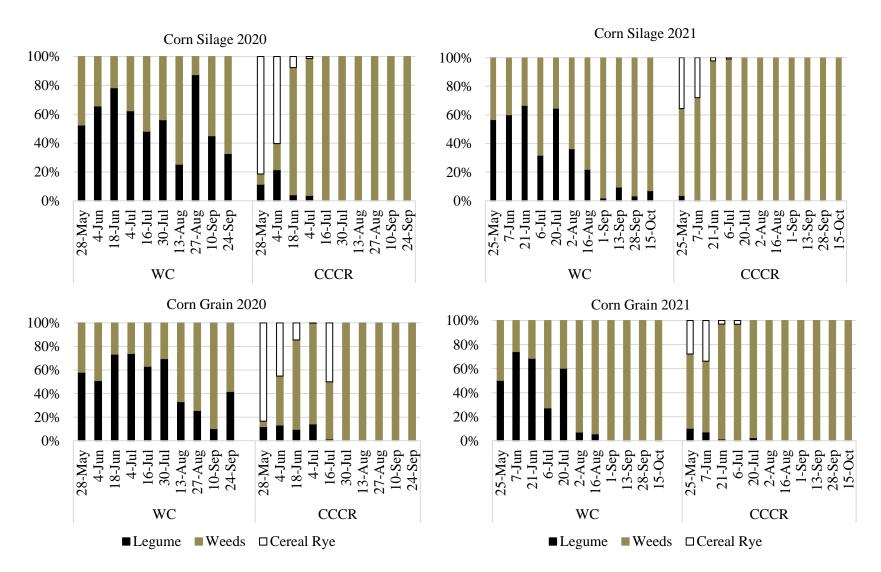


Figure 1-2: Botanical composition (BC) of living mulch (LM) (crimson clover/cereal rye, CCCR; and white clover, WC) paddocks during the 2020 and 2021 corn growing season in Spring Hill, TN.

				2020		
	May	Jun	Jul	Aug	Sept	Oct
CCCR	1330 ^c	1176 ^c	2415 ^b	3502 ^a	1580 ^c	-
WC	1653 ^a	1127 ^b	819 ^b	979 ^b	938 ^b	-
-			,	2021		
CCCR	808	1122	1585	1969	2362	2138
WC	967	996	1453	1512	2097	1980
x	887 ^d	1059 ^d	1519 ^c	1740 ^{bc}	2229 ^a	2059 ^{ab}
			Al	NOVA		
Sampling month (S)			<	.0001		
LM Species (LM)			0	.0221		
$S \times LM$			<	.0001		
Corn (C)			0	.4381		
$\mathbf{S} \times \mathbf{C}$			0	.3317		
$\mathbf{C} \times \mathbf{L}\mathbf{M}$			0	.3681		
$S \times LM \times C$			0	.9106		
Year (Y)			0	.5058		
$\mathbf{S} imes \mathbf{Y}$			<	.0001		
$LM \times Y$			0	.0008		
$S \times LM \times Y$			<	.0001		
$\mathbf{C} \times \mathbf{Y}$			0	.5251		
$C\times S\times Y$			0	.5927		
$LM \times C \times Y$			(0.217		
$LM \times C \times Y \times S$			0	.3172		

Table 1-4: Living mulch mass (LMM) (kg ha⁻¹) of white clover (WC) and a crimson clovercereal rye mixture (CCCR) during the corn growing season of two consecutive years (2020 and 2021) in Spring Hill, TN

Means without a common subscript letter differ within rows. *P*-values are significant at $\alpha \le .05$ using Fisher's LSD test.

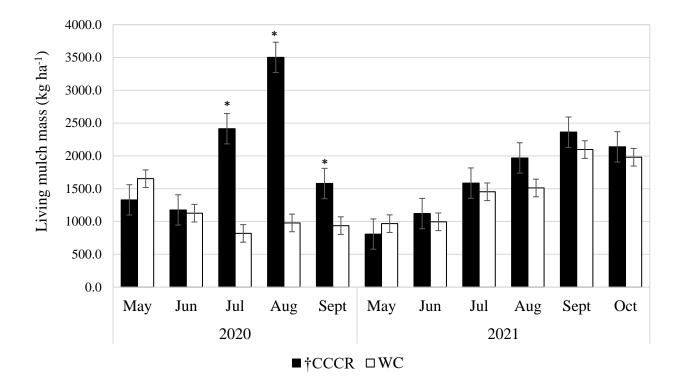


Figure 1-3: Living mulch mass (LMM) (kg ha⁻¹) of CCCR and WC during the growing season of 2020 and 2021 in Spring Hill, TN. *Differences between LM treatments at $\alpha \le 0.05$; † CCCR, crimson clover-cereal rye mixture, white clover, WC.

<u>(2020 und 2021) in Sprin</u>	5,		Cru	ude protein	(g kg ⁻¹)		
	Year	May	Jun	Jul	Aug	Sept	Ā
	2020	338.5	250.4	168.9	180.5	184.5	224.6 ^A
WC	2021	204.7	238.9	276.5	222.4	300.2	248.5 ^B
		271.6 ^a	244.6 ^a	222.7 ^a	201.4 ^a	242.3 ^a	
	2020	215.6	187.4	401.4	523.1	222.4	224.6 ^A
CCCR	2021	130.2	193.1	233.5	246.7	286.9	248.5 ^A
	Ā	172.9 ^c	190.2 ^c	317.4 ^{ab}	384.9 ^a	254.6 ^{bc}	
				ANOV	A		
Year (Y)				0.2127	,		
Sampling month (S)				0.3394			
$\mathbf{S} imes \mathbf{Y}$				0.0971			
LM species (LM)				0.3427	,		
$LM \times Y$				0.0351			
$S \times LM$				0.0091			
$S \times LM \times Y$				0.0993			
Corn (C)				0.4554			
$\mathbf{C} imes \mathbf{Y}$				0.9413			
$S \times C$				0.9043			
$S \times C \times Y$				0.9656			
$LM \times C$				0.4059	1		
$LM \times C \times Y$				0.5163			
$S \times LM \times C$				0.9980			
$S \times LM \times C \times Y$				0.8918			

Table 1-5: Crude protein levels (g kg⁻¹) of white clover (WC) and a crimson clover-cereal rye mixture (CCCR) as a living mulch during the corn growing season of two consecutive years (2020 and 2021) in Spring Hill, TN

Means without a common uppercase superscript letter differ within a column. Means without a common superscript letter differ within a row at $\alpha \leq 0.05$ using Fisher's LSD test.

Treatment		2020		2021					
		t ha-1t ha-1							
	Grain	Silage	x	Grain	Silage	x			
CCCR	6	8	10	1	б	3			
WC	9	13	15	1	6	3			
Control	6	11	11	2	6	4			
x	7^{b}	10 ^a		1^{b}	6 ^a				
			ANC						
LM Species (LM)			0.2	073					
Corn (C)			<.0	001					
$LM \times C$			0.6	025					
Year (Y)			<.0	001					
$LM \times Y$			0.0	021					
$\mathbf{Y} \times \mathbf{C}$			0.04	468					
$LM \times Y \times C$			0.04	474					

Table 1-6: Corn grain and silage production (DM t ha⁻¹) grown in living mulch systems (crimson clover and cereal mixture, CCCR; white clover, WC) of two consecutive growing seasons (2020 and 2021) in Spring Hill, TN

Means without a common superscript letter differ within a row at $\alpha \le 0.05$ using Fisher's LSD test.

Year	Treatment	CP	NDF	Starch	IVTDMD48			
			g k	kg ⁻¹				
		Unfermented						
	[†] CCCR	82 ^{bc}	514	175 ^a	801 ^b			
2020	WC	104 ^a	503	186 ^a	801 ^b			
	Control	72 ^c	522	208 ^a	806 ^b			
	CCCR	80^{bc}	545	49 ^b	839 ^a			
2021	WC	77 ^c	537	75 ^b	843 ^a			
	Control	90 ^b	534	61 ^b	879 ^a			
	LM species (LM)	0.0548	0.8142	0.4358	0.1025			
ANOVA	Year (Y)	0.3286	0.0704	<.0001	0.0001			
	$LM \times Y$	0.0005	0.7368	0.6003	0.2415			
	_		Ferm	ented				
	CCCR	83	494 ^b	200^{a}	844 ^a			
2020	WC	96	449 ^b	246 ^a	852 ^a			
	Control	83	497 ^b	211 ^a	829 ^a			
	CCCR	95	594 ^a	37 ^b	759 ^b			
2021	WC	94	602 ^a	24 ^b	756 ^b			
	Control	92	600 ^a	33 ^b	745 ^b			
	LM species (LM)	0.3292	0.4444	0.5315	0.1391			
ANOVA	Year (Y)	0.1578	<.0001	<.0001	<.0001			
	$LM \times Y$	0.4061	0.3210	0.1726	0.7820			

Table 1-7: Nutritive value of unfermented and fermented silage grown in living mulch systems during two consecutive growing seasons (2020 and 2021) in Spring Hill, TN

[†]WC, white clover; CCCR, crimson clover-cereal rye mixture. [‡]N.S. not significant Means without a common superscript letter differ within a column at $\alpha \le 0.05$ using Fisher's LSD test.

Treatment	Starch	Oil	Protein
		2020	
-		g kg ⁻¹	
†CCCR	620 ^b	41 ^a	67 ^b
WC	619 ^b	39 ^a	76 ^a
Control	624 ^b	41 ^a	65 ^b
		2021	
CCCR	727 ^a	38 ^b	82 ^b
WC	724 ^a	42 ^a	92 ^a
Control	723 ^a	39 ^b	82 ^b
		ANOVA	
	0.7685	0.5874	0.0006
Year (Y)	<.0001	0.3456	<.0001
$LM \times Y$	0.3632	0.0153	0.7607

Table 1-8: Nutritive value analysis of corn grain grown in living mulch systems during two consecutive growing seasons (2020 and 2021) in Spring Hill, TN

[†]WC, white clover; CCCR, crimson clover-cereal rye mixture. [‡]N.S. not significant Means without a common superscript letter differ within a column at $\alpha \le 0.05$ using Fisher's LSD test.

		LM			BLW			GW	
Spring 2020	Beginning	End	x	Beginning	End	x	Beginning	End	x
[†] CCCR	961	994	977 ^a	40	6	23 ^b	-	-	-
WC	268	307	288 ^b	732	692	712 ^a	-	-	-
x	615	651		385	349				
Sampling date (D)		0.5029			0.5029			-	
LM Species (LM)		0.0059			0.0059			-	
$D \times LM$		0.9539			0.9539			-	
Spring 2021									
CCCR	748	378	562 ^a	251	259	255 ^b	1 ^b	364 ^a	182
WC	344	321	333 ^b	654	648	651 ^a	2 ^b	31 ^b	16
x	546 ^A	349 ^B		452	453		1	197	
Sampling date (D)		0.0494			0.9921			0.0072	
LM Species (LM)		0.0242			0.0009			0.0189	
$D \times LM$		0.0793			0.9482			0.0184	
Fall 2020									
CCCR	3	0	1^{b}	29	56	43 ^b	968	944	955 ^a
WC	343	191	267 ^a	377	394	386 ^a	279	415	347 ^b
x	173	95		203	225		644	673	
Sampling date (D)		0.4875			0.7404			0.5320	
LM Species (LM)		0.0272			< 0.0001			< 0.0001	
$D \times LM$		0.5029			0.9442			0.3751	

Table 1-9: Botanical composition (g kg⁻¹) of living mulch (LM) (crimson clover/cereal rye, CCCR; and white clover, WC) and its differences between beginning and end of each 28-d grazing period during two consecutive years (2020 and 2021) in Spring Hill, TN.

Table 1.9 continued: Botanical composition (g kg⁻¹) of living mulch (LM) (crimson clover/cereal rye, CCCR; and white clover, WC) and its differences between beginning and end of each 28-d grazing period during two consecutive years (2020 and 2021) in Spring Hill, TN.

	LM			BLW			GW		
Fall 2021	Beginning	End	Ā	Beginning	End	x	Beginning	End	x
CCCR	56	0	28	17	11	14	927	989	958
WC	2	1	1.5	22	34	28	976	964	970
x	29	0.5		20	23		951	976	
Sampling date (D)	0.3298			0.7937			0.4425		
LM Species (LM)		0.3615			0.2174			0.7084	
$D \times LM$		0.3354			0.4197			0.2660	

Means without a common superscript letter differ within each grazing season at $\alpha \le 0.05$ using Fisher's LSD test. [†]BLW, broadleaf weeds; GW, grass weeds.



Figure 1-4: Botanical composition (BC) of living mulch (LM) (crimson clover/cereal rye, CCCR; and white clover, WC) paddocks during each 28-d grazing period on spring and fall of 2020 and 2021 in Spring Hill, TN (BLW, broadleaf weed; GW, grass weed).

		Spring	g 2020			
		Grain			Silage	
	Beginning	End	x	Beginning	End	x
[†] CCCR	1608	1428	1518	2493	2364	2429 ^a
WC	983	1098	1040	1171	1605	1388 ^b
x	1295	1263		1832	1985	
Sampling date (S)		0.9102			0.7311	
LM Species (LM)		0.1324			0.0494	
$\mathbf{S} imes \mathbf{L} \mathbf{M}$		0.6100			0.5306	
		Spring				
CCCR	388	111	249 ^b	724	505	615
WC	582	379	481 ^a	805	537	671
x	485 ^A	245 ^B		764	521	
Sampling date (S)		0.0125			0.0825	
LM Species (LM)		0.0145			0.6474	
$\mathbf{S} imes \mathbf{L} \mathbf{M}$		0.6010			0.8410	
		Fall	2020			
CCCR	1299	1764	1531 ^a	1181 ^a	1137 ^a	1159
WC	407	391	399 ^b	1155 ^a	549 ^b	852
x	853	1078		1168	843	
Sampling date (S)		0.1232			0.0236	
LM Species (LM)		0.0001			0.0294	
$\mathbf{S} imes \mathbf{L} \mathbf{M}$		0.1040			0.0404	
		Fall	2021			
CCCR	1964 ^b	1593 ^b	1778	1755	855.	1305
WC	2758 ^a	1394 ^b	2076	1759	1081	1419
x	2361	1494		1757 ^A	968 ^B	
Sampling date (S)		0.0029			0.0171	
LM Species (LM)		0.1473			0.6517	
$\mathbf{S} imes \mathbf{L} \mathbf{M}$		0.0321			0.6629	

Table 1-10: Living mulch mass (LMM, kg ha⁻¹) at the beginning and end of each 28-d grazing period during two consecutive years in Spring Hill, TN.

[†]WC, white clover; CCCR, crimson clover-cereal rye mixture. [‡]N.S. not significant Means without a common superscript letter differ within each grazing season at $\alpha \le 0.05$ using Fisher's LSD test.

		Spring 2020	
	СР	NDF	IVTDMD48
Sampling month (S)	<.0001	<.0001	<.0001
LM species (LM)	0.0345	0.0004	0.0014
$S \times LM$	0.3771	0.0027	<.0001
Corn (C)	0.6544	0.1657	0.7045
$S \times C$	0.9162	0.663	0.0574
$LM \times C$	0.0393	0.0996	0.7400
$S \times LM \times C$	0.7909	0.6641	0.0876
		Spring 2021	
Sampling month (S)	0.1586	0.0078	0.0002
LM species (LM)	0.3478	0.0329	0.1276
$S \times LM$	0.1692	0.6081	0.8595
Corn (C)	0.4873	0.6153	0.9672
$S \times C$	0.0373	0.3488	0.4430
$LM \times C$	0.0465	0.0503	0.0385
$S \times LM \times C$	0.1360	0.128	0.0764
		Fall 2020	
Sampling month (S)	0.0047	0.0004	<.0001
LM species (LM)	0.0032	0.0033	0.0024
$S \times LM$	0.0671	0.3708	0.9716
Corn (C)	0.4447	0.8687	0.9534
$S \times C$	0.7079	0.3417	0.2703
$LM \times C$	0.0129	0.0046	0.0092
$S \times LM \times C$	0.4839	0.6862	0.2022
		Fall 2021	
Sampling month (S)	0.0101	0.0114	<.0001
LM species (LM)	0.0093	0.1952	0.0122
$S \times LM$	0.1802	0.4477	0.2064
Corn (C)	0.0076	0.0136	0.0075
$S \times C$	0.9370	0.1250	0.9110
$LM \times C$	0.7827	0.9901	0.5605
$S \times LM \times C$	0.8160	0.7653	0.6762

Table 1-11: Crude Protein (CP), Neutral Detergent Fiber (NDF), and In-Vitro Dry Matter Digestibility - 48 hours (IVTDMD48) ANOVA for the grazing LM nutritive values of CP, NDF, and IVTDMD48, during the grazing seasons in 2020 and 2021 in Spring Hill, TN

		СР			NDF		IVTDMD48		
				Sprir	ng 2020				
	Beginning	End	x	Beginning	End	x	Beginning	End	ā
[†] CCCR	164 ^b	101 ^b	132	526 ^a	674 ^a	600	792 ^b	557 ^b	675
WC	224 ^a	177 ^a	200	330 ^b	366 ^b	348	882 ^a	863 ^a	872
x	194	139		428	520		837	710	
				Fall	1 2020				
CCCR	132	117	125 ^b	657	764	710 ^a	631	461	546 ^b
WC	117	176	205 ^a	382	540	461 ^b	842	673	758 ^a
x	183 ^a	147 ^b		519 ^b	652 ^a		736 ^a	567 ^b	
				Sprir	ng 2021				
CCCR	163	199	181	369	411	190 ^a	883	837	893 ^a
WC	197	197	197	195	353	324 ^b	904	855	846 ^b
x	180	198		332 ^b	382 ^a		894	846	
				Fall	1 2021				
				G	rain				
CCCR	104	97	115 ^a	783	797	790	583	540	562
WC	127	103	99 ^b	751	781	766	627	546	587
x	100 ^b	115 ^a		767	789		605 ^a	543 ^b	
				Si	lage				
CCCR	120	110	115	711	771	741	614	564	638
WC	144	121	132	668	766	717	662	592	578
x	132	116		690	769		638 ^a	578 ^b	

Table 1-12: Crude Protein (CP), Neutral Detergent Fiber (NDF), and In-Vitro Dry Matter Digestibility - 48 hours (IVTDMD48) for grain and silage paddocks at the beginning and end of grazing period of 30-days in Spring Hill, TN

[†]CCCR, crimson clover-cereal rye mixture; WC, white clover.

Different superscript letters mean differences within a row and column at $\alpha \le 0.05$ using Fisher's LSD test.

		Spring 2020				
	Grain	Silage		x		
[†] CCCR	0.2^{bc}	-0.2 ^c		0		
WC	0.8^{b}	1.8 ^a		1.3		
		Fall 2020				
CCCR	0.2	0.2		0.2 ^b		
WC	0.6	0.7		0.6^{a}		
CCCR	-0.5	-0.5		-0.5 ^b		
WC	0	0.3		0.1 ^a		
		Fall 2021				
CCCR	0.4	0.3		0.3		
WC	0	0.4		0.2		
	ANOVA					
	Spring 2020	Fall 2020	Spring 2021	Fall 2021		
LM Species (LM)	0.0013	0.0304	0.0052	0.5801		
Corn (C)	0.3098	0.6371	0.4295	0.5019		
$LM \times C$	0.0272	0.5936	0.3984	0.3104		

Table 1-13: Average Daily Gain (ADG, kg) of cows in living mulch (LM) (crimson clover/cereal rye, CCCR; and white clover, WC) paddocks at the beginning and end of each 28-d grazing period during two consecutive years in Spring Hill, TN.

[†]CCCR, crimson clover-cereal rye mixture; WC, white clover. [‡]N.S. not significant

Different superscript letter means differences within a row and column. *P*-values are significant at $\alpha \leq .05$ using Fisher's LSD test.

				Silage		
		Cost of production (ha ⁻¹)	Production (t ha ⁻¹)	Gross revenue (\$ ha ⁻¹)	Profit (\$ ha ⁻¹)	[†] Δ profit (\$)
	No-LM	\$ 967.29	33.0	\$ 1583.74	\$ 616.45	
2020	CCCR	\$ 1085.71	30.2	\$ 1450.16	\$ 364.45	\$ 252.00
	WC	\$ 1056.03	49.0	\$ 2356.22	\$ 1300.19	\$ 683.74
	No-LM	\$ 967.29	14.2	\$ 619.12	\$-348.17	
2021	CCCR	\$ 1085.71	17.1	\$ 745.56	\$-340.15	\$ 8.02
	WC	\$ 967.34	17.2	\$ 749.92	\$-306.11	\$ 42.06
				Grain		
	No-LM	\$ 844.23	6.1	\$ 1237.44	\$ 393.21	
2020	CCCR	\$ 935.27	6.4	\$ 1298.30	\$ 363.03	\$ 30.18
	WC	\$ 957.83	9.1	\$ 1846.02	\$ 888.19	\$ 494.98
	No-LM	\$ 844.23	1.6	\$ 324.58	\$-519.65	
2021	CCCR	\$ 935.27	1.0	\$ 202.86	\$-732.41	\$ 212.76
	WC	\$ 817.00	1.0	\$ 202.86	\$ -614.14	\$ 94.49

Table 1-14: Cost of production in 65% moisture (\$ ha⁻¹), corn yield (t ha⁻¹), revenue (\$ ha⁻¹), and profit (\$ ha⁻¹) of corn production of silage and grain in no-LM (living mulch) systems compared to CCCR (crimson clover-cereal rye mixtures) and white clover (WC) LM in Spring Hill, TN during 2020 and 2021 growing seasons.

[†]Difference in profit in \$ ha⁻¹ between no-LM and LM (CCCR or WC)

			Spring			Fall		
					Silage			
		$^{\ddagger}\Delta$ \$ ha ⁻¹	ha head ⁻¹	†\$ head	\$/head/day	ha head ⁻¹	\$ head	\$/head/day
2020	CCCR	\$ 118.42	0.21	\$ 24.87	\$ 0.89	0.23	\$ 27.71	\$ 0.74
2020	WC	\$ 88.74	0.70	\$ 62.12	\$ 2.22	0.23	\$ 20.71	\$ 0.74
2021	CCCR	\$118.42	0.23	\$ 27.63	\$ 0.99	0.23	\$ 27.63	\$ 0.99
2021	WC	\$ 0.05	0.23	\$ 0.01	\$ 0.00	0.23	\$ 0.01	\$ 0.00
					Grain			
2020	CCCR	\$ 91.04	0.40	\$ 38.24	\$ 1.37	0.23	\$ 21.24	\$ 0.76
2020	WC	\$ 113.60	0.52	\$ 59.64	\$ 2.13	0.23	\$ 26.51	\$ 0.88
2021	CCCR	\$ 91.04	0.30	\$ 27.31	\$ 0.98	0.23	\$ 21.24	\$ 0.76
2021	WC	\$ 27.23	0.23	\$ 6.35	\$ 0.23	0.23	\$ 6.35	\$ 0.23

Table 1-15: Difference in cost of production (\$ ha⁻¹) between no-LM (living mulch) and LM systems (CCCR, crimson clover-cereal rye; white clover, WC), \$ per head, and \$ per head per day in Spring and Fall grazing in 2020 and 2021 growing seasons in Spring Hill, TN.

[‡]The difference was determined based on the cost of production of corn in no-LM vs LM treatments

[†]Stocking rate was determined based on the number of animals in 2.10 ha⁻¹ which represents 3 paddocks per treatment

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CHAPTER II

NITROGEN REQUIREMENTS FOR CORN PRODUCTION IN WHITE

CLOVER LIVING MULCH SYSTEMS

Abstract

The use of legume living mulch (LM) on increasing corn (Zea mays L.) production must be evaluated with different N fertilization levels. The objective of this study was to determine the optimum amount of N fertilization in white clover (Trifolium repens L. [WC]) LM in grain and silage production. The study was conducted in Spring Hill, TN from Mar 2020 to Sept 2021. The treatments were [C-0] no LM + no N, [C-135] no LM + 135 kg ha⁻¹ N, [CLM-0] LM + no N, [CLM-45] LM + 45 kg ha⁻¹ N, [CLM-90] LM + 90 kg ha⁻¹ N, and [CLM-135] LM + 135 kg ha⁻¹ N; all treatments had corn silage or grain hybrids seeded in the first week of June. The projected measurements included corn yield at harvest, botanical composition, and LM mass throughout the corn growing season. The results showed that the lack of precipitation after seeding affected corn development, especially due to the interspecies competition between LM and corn (Silage -2020 \bar{x} : 6.8 t ha⁻¹; 2021 \bar{x} : 2.8 t ha⁻¹; Grain - 2020 \bar{x} : 1.8 t ha⁻¹; 2021 \bar{x} : 0.1 t ha⁻¹). The weed suppression by WC was greater in the second year after establishment; therefore, it is important to suppress WC growth to decrease its competition with corn. The N rate did not affect the nutritive value of the LM, and from May to Sept the CP decreased and fiber content increased, likely due to maturity and species composition. In conclusion, although adding WC did not lead to similar corn productivity as in conventional systems, adding LM to the field can aid in weed control and corn nutritive value.

Introduction

Nitrogen is an important nutrient for plant growth, and the suggested N requirement for corn (*Zea mays* L.) can be as high as 280 kg ha⁻¹, depending on the yield goal (Vanotti & Bundy, 2006). The use of legumes intercropped with corn can reduce the reliance on synthetic N fertilization (Martin et al., 1999) due to the N release from these legumes, such as white clover (WC) (*Trifolium repens* L.) (Peters et al., 2020). The living mulch (LM) system is a strategy in which forages are grown simultaneously with the main crop, serving as a living cover throughout the growing season (Hartwig & Ammon, 2002), and generally the use of legumes in this system increases its benefits. The adoption of LM systems has shown a significant reduction in water runoff (Siller, et al., 2016), which can decrease NO₃ leaching and overall N losses as compared to conventional systems. The use of WC as a legume LM species can not only confer good soil coverage due to its stolons and rhizomes, but also provide sufficient biological N fixation.

White clover also has the potential to increase total soil N availability (Sanders et al., 2018), which can result in increased grain and whole plant corn yield (Sheaffer et al., 2006). Corn is commonly fertilized at establishment and at the V6 and V7 stage of growth (Sripada, et al., 2006), when it is growing rapidly. Legume LM systems can provide additional N, but the timing of N availability is unknown. Also, it is likely that legume LM alone will not provide sufficient N necessary for optimum productivity in corn (Reberg-Horton et al., 2012). Affeldt et al (2004) applied N at 45 kg ha⁻¹ between the V3 and V5 stage to avoid resource limitation for corn growth, and Zemenchick et al. (2000) observed that side dressing N to LM grown with corn increased whole-plant corn yield.

Although legumes could provide up to 10% of their symbiotically fixed N to the root zone (Brophy & Heichel, 1989), the addition of synthetic N can suppress persistence of WC in

the field (Guy et al., 2020); therefore, no more than 120 kg ha⁻¹ of N should be applied to fields containing WC (Enriquez-Hidalgo et al., 2016). Therefore, the level of N fertilization in systems containing WC must be assessed to avoid competition between species. The objectives of this study were to determine the amount of synthetic N fertilization in WC LM to sustain grain and silage production as compared to conventional systems. The hypothesis is that adding 90 kg ha⁻¹ of N to WC LM can be as productive as corn with no-LM and 135 kg ha⁻¹ of N.

Materials & Methods

The research was conducted at the Middle Tennessee Research and Education Center (MTREC) in Spring Hill, TN ($35^{\circ}71'$ N, $86^{\circ}94'$ W) from March 2020 to Apr. 2021. Initial soil levels on the experiment site were pH = 6.5, P = 30 kg ha⁻¹, K = 86 kg ha⁻¹, Ca = 2564 kg ha⁻¹, and Mg = 216 kg ha⁻¹. The area consisted of two well drained soil types with elevation ranging from 540 to 1200 ft. The soil types were Braxton series (fine, mixed, active, thermic Typic Paleudalfs) silt loam soil complex with 0 to 5% slopes, and Maury series (fine, mixed, active, mesic Typic Paleudalfs) silt loam with 2 to 5% slopes (NRCS, 2022). Following the soil test recommendations, on Apr. 22, 2020 and May 25, 2021 K₂O was applied at 157 kg ha⁻¹, and superphosphate was applied on Apr.l 27, 2020 at 82 kg ha⁻¹ and on May 28, 2021 at 76 kg ha⁻¹.

Two experiments were performed, experiment one with corn silage and experiment two with corn grain. In both experiments, treatments were as followed: 1) corn only + 0 N (C-0), 2) corn only + 135 kg ha⁻¹ N (C-135), 3) corn and LM + 0 N (CLM-0), 4) corn and LM + 45 kg ha⁻¹ N (CLM-45), 5) corn and LM + 90 kg ha⁻¹ N (CLM-90), and 6) corn and LM + 135 kg ha⁻¹ N (CLM-135). Both C-0 and C-135 are considered control plots. In both experiments, all treatments were replicated three times in a randomized complete block design (RCBD), totaling 18 experimental units per experiment. Each experimental unit measured 25 by 32-m.

Measurements and Management

On March 7, 2020, the field was sprayed with 0.84 kg ha⁻¹ a.i. of paraquat (Gramoxone SL 2.0, Syngenta, United Kingdom) and surfactant (Surf 80, Cannon packaging Co., Inc.) 35 ml ha⁻¹ using a 210 Redball 8-row hooded sprayer (Wilmar, Benson, MN). On March 9, 2020, "Durana" WC (*Trifolium repens* L.) was planted at 2.2 kg ha⁻¹ with a Great Plains 38-cm drill (Manufacturing Inc, Salina, KS).

On May 15, 2020, and May 18, 2021, 90-cm lanes were created for corn seeding by spraying 0.5 kg ha⁻¹ a.i. of glyphosate (N-(phosphonomethyl) glycine; Cornerstone Plus, Agrisolutions, St. Paul, MN) and 0.16 kg ha⁻¹ a.i. of 2,4-D ammine 4 (Loveland, Greeley, CO) using a 210 Redball 4-row and 8-row hooded sprayer (Wilmar, Benson, MN). The second spraying was conducted on May 29, 2020, and May 31, 2021, with 0.5 kg ha⁻¹ a.i. of paraquat (Paraquat concentrate, Solera, Yuma, AZ), 0.62 kg ha⁻¹ a.i. of atrazine (Atrazine 4L, Drexel, Memphis, TN), 0.3 kg ha⁻¹ a.i. of s-metolachlor (Charger Max, Agrisolutions, St. Paul, MN), and surfactant (Surf 80, Cannon packaging Co., Inc.) at 35 ml ha⁻¹ using a 210 Redball 4-row and 8-row hooded sprayer (Wilmar, Benson, MN). Meanwhile, the control plots were sprayed on May 28, 2020, with 1.3 kg ha⁻¹ a.i. of paraquat (Paraquat concentrate, Solera, Yuma, AZ) and surfactant (Surf 80, Cannon packaging Co., Inc.) 35 ml ha⁻¹, and on May 20, 2021, 0.5 kg ha⁻¹ of paraquat (Paraquat concentrate, Solera, Yuma, AZ) and surfactant (Surf 80, Cannon packaging Co., Inc.) 35 ml ha⁻¹, and on May 20, 2021, 0.5 kg ha⁻¹ of paraquat (Paraquat concentrate, Solera, Yuma, AZ) and surfactant (Surf 80, Cannon packaging Co., Inc.) 35 ml ha⁻¹, and on May 20, 2021, 0.5 kg ha⁻¹ of paraquat (Paraquat concentrate, Solera, Yuma, AZ) and surfactant (Surf 80, Cannon packaging Co., Inc.) 35 ml ha⁻¹, and on May 20, 2021, 0.5 kg ha⁻¹ of paraquat (Paraquat concentrate, Solera, Yuma, AZ) and surfactant (Surf 80, Cannon packaging Co., Inc.) 35 ml ha⁻¹, and on May 20, 2021, 0.5 kg ha⁻¹ of paraquat (Paraquat concentrate, Solera, Yuma, AZ) and surfactant (Surf 80, Cannon packaging Co., Inc.) 35 ml ha⁻¹, and on May 20, 2021, 0.5 kg ha⁻¹ of paraquat (Paraquat concentrate, Solera, Yuma, AZ) and surfactant (Surf 80, Cannon packaging Co., Inc.) 35 ml ha⁻¹ ha⁻¹

On June 1 of both years (2020 and 2021), corn grain (Croplan 5887VT2P/RIB) and corn silage (Dekalb DKC67-44) were planted at 77,000 seeds per ha⁻¹ with 90-cm spacing using 4-row planter (John Deere, Lewisburg, TN). Due to wildlife animal damage, grain and silage hybrids were replanted in affected areas on June 10, 2020. Split applications of urea (46-0-0)

were applied by hand on 9 June and 10 July 2020; and on 3 June and 1 July 2021, where half of each treatment rate (45 kg ha⁻¹, 90 kg ha⁻¹, and 135 kg ha⁻¹) was applied each day.

The corn silage was harvested on Aug. 19, 2020, and Aug 25, 2021, using a T100 chopper (New Holland, Racine, WI) and a feeder mixer (Helm Welding, Lucknow, Ontario). The corn grain was harvested on Oct 14, 2020 and 2021, with a plot combine (Almaco and Allis-chalmers Gleaner K2), and Ford F600 grain truck.

LM botanical composition, mass, and nutritive value

In both years, LM samples were collected monthly from May 28 to Sept 28. Samples were collected by clipping the forage using two randomly placed 0.1 m² quadrats within each experiment unit within random corn lanes; samples were then separated into botanical components (WC LM or weeds) and placed in a drier until constant weight at 60°C (~72 hr). After drying, samples were weighed to determine the total dry matter (DM) LM mass and the botanical composition (BC). After BC and LM mass were determined, samples were then recombined for the determination of the nutritive value (NV).

Samples were ground in a Wiley Mill Grinder (Thomas-Wiley Laboratory Mill Model 4, Arthur H. Thomas Co., Philadelphia, PA) using a 2-mm screen, and then passed through a 1-mm cyclone mill (FOSS Cyclotec, Eden Praire, MN) to decrease particle size (McIntosh et al., 2022). Prior to analysis, the samples were individually placed in foil tins and dried for 30-min in a forced air oven at 55°C to allow consistent moisture content for scanning and decrease variability across results (McIntosh et al., 2019). These samples were scanned in small ring cups on Nearinfrared spectroscopy (NIRS) technology (Unity SpectraStar XL-R, Unity Scientific, Milford, MA) for determination of crude protein (CP), neutral detergent fiber (NDF), and *in vitro* true dry matter digestibility 48h (IVTDMD48), by utilizing Mixed Hay (MH), Legume Hay (LH), and Grass Hay (GH) calibrations, provided and licensed by the NIRS Forage and Feed Consortium (NIRSC, Berea, KY). The global and neighborhood statistical tests were monitored and analyzed for accuracy across all predictions with entire data set fitting the calibrations within the (H < 3.0) limit of fit and reported accordingly (Murray and Cowe, 2004). Units of measurement for nutritive analyses are presented at 100% dry matter (DM) across entire data set.

Corn production and nutritive value

The corn yield of silage and grain was determined by harvesting the two middle rows of each plot. The length of the plot and the number of plants per harvested row were measured before harvesting, and the weight was recorded for determination of the yield per ha⁻¹. In both years, a sample from the harvest of corn silage was taken from the bulk harvested, then dried and ground in a Wiley Mill Grinder (Thomas-Wiley Laboratory Mill Model 4, Arthur H. Thomas Co., Philadelphia, PA) using a 2-mm screen to determine the silage NV of unfermented corn silage. Fermented silage was made by utilizing 18 x 20 cm air-tight Ziploc[®] bags, containing approximately 100 g of the harvested material, then pressed by hand to remove as much air as possible. Individual bags were placed in an extra-large space bag (Ziploc[®] Space Bag[®]). The bags are airtight, waterproof, and reusable, with the air removed by utilizing the vacuum in the one-way valve that maintained an anaerobic environment. These bags were placed in a 55 L plastic bin covered by a 32 kg⁻¹ sandbag over it to compress the samples and avoid external oxygen contact. The plastic bin was then closed with a lid for 30 days before opening for further processing.

These samples were ground in a Wiley Mill Grinder (Thomas-Wiley Laboratory Mill Model 4, Arthur H. Thomas Co., Philadelphia, PA) using a 2-mm screen. All samples from both fermented and unfermented silage were scanned using the same instrument and methods from the LM nutritive value analyses (CP, NDF, IVTDMD48, starch) on the NIRS, with the Unfermented Corn Silage (UC), Fermented Corn Silage (CS), and Haylage (HL) calibrations. The corn grain was analyzed using the Ingratec Grain Analyser (FOSS North America, Inc., Eden Prairie, MN) for determination of the oil, starch, and CP. The samples were best scanned when the amount averaged 470g. However, some samples, especially in 2021, did not yield the optimum amount; therefore, it was scanned twice for accuracy.

Statistical analysis

Mixed model analyses of variance were performed using the GLIMMIX procedure in SAS 9.4 (Cary, NC). For corn production and corn nutritive value (CP, NDF, IVTDMD48, Starch, Oil), the fixed effects were considered N rates and year, with replication as random effects. Given the differences in year (2020 and 2021), the analyses were performed separately for LM mass, CP, IVTDMD48, NDF, and botanical composition (weed and WC). The fixed effects were N rates, sampling month, and their two-way interactions with sampling month as the repeated measures, and random effects as the replications.

Results & Discussion

Weather

The mean air temperature during the 2020 growing season (May-Sept) was 2% lower than the 30-year average, and 3% lower in 2021 (Fig 2.1). In 2020, precipitation was 15% above the 30-year average, and in 2021, 50% above the 30-year average (Fig 2.1). However, in June

2021, precipitation was 17% lower than the 30-year average, and although both years had greater total precipitation, the total precipitation distribution in 2021 (especially in June) affected corn germination, establishment and further development.

Experiment I

Living mulch botanical composition

There was a month × treatment interaction between botanical components (LM and weed) in 2020 (P < 0.0001, Table 2.1). Among plots containing LM, the presence of LM did not differ among treatments in May; however, this pattern shifted as the growing season progressed, reducing the amount of LM across treatments (Table 2.1). This the result of corn shading the LM as it grew in the field (Mercier et al., 2020), likely decreasing the competitive ability of LM and allowing weed species to grow. Meanwhile, the weed proportion in control plots (C-0 and C-135) was greater than in all remaining treatments early in July and remained high until September (Table 2.1). In the plots with no N application (CLM-0), the LM had to rely solely on its N fixing ability, which gives a competitive advantage against other species. In 2020, the weed proportion in the plots tended to increase with the growing season, as also observed by Nakamoto & Tsukamoto (2006), in which weed biomass increased at the same rate as the WC LM.

In 2021, there was the main effect of month and treatment (P < 0.0001; Table 2.1) for the LM. The LM proportion remained constant until Aug, with reduced LM proportion observed in Sept (Table 2.1). Since shading decreases the LM mass, it is likely that, after harvest, WC was still competing for space and resources with the weeds, leading to decreased mass. Meanwhile, there was a month × treatment interaction (Table 2.1) in the weed proportion. The weed

proportion did not differ among LM treatments, and greater weed proportion was observed in Sept. Overall, less weeds were observed in 2021 than 2020 (Table 2.1). Nakamoto & Tsukamoto (2006) found that in the second year, the WC LM suppressed weeds more effectively likely due to the shift in the microbial composition in soil. In addition, Osipitan et al. (2018) observed that in cover crops, weed control is greater when greater plant residue is present, confirming the fact that during the second year after LM establishment, the greater LM accumulation can reduce weed biomass.

Living mulch total mass accumulation

In 2020, there was a month \times treatment interaction (*P* = 0.0430; Table 2.2). The LM mass was variable among treatments in each month, but overall, the LM mass increased in Aug for all treatments. These findings are associated with the greater weed proportion during the same period (Table 2.1). Most of the weeds present in the field were pigweed (genus *Amaranthus*), which are considered very invasive species that can grow up to 2 m height (Steckel, 2007), therefore affecting the overall mass accumulation observed. Meanwhile, the lowest LM mass was observed in May and June, likely because 2020 is the WC establishment year, and it can take up to a year for its complete establishment in the field (Brock et al., 2005).

In 2021, there was a month × treatment interaction (P < 0.0001; Table 2.2). In May and June there were no LM mass differences among the LM treatments, whereas the control plots were not active before corn planting. After corn planting (July), there was an increase in mass of non-LM (Table 2.2) due to the documented increase in weed competition at the same time (Table 2.1). Most weeds in these plots were *Amaranthus* and *Digitaria* genus, which are annual summer weeds that have a great competitive ability against perennial cool-season forages, such as WC (Steckel, 2007; Ball et al., 2005). The increase in weed biomass and consequent senescence of

the WC LM can be beneficial to the corn because the N fixed by the legume becomes available as organic matter to the corn; however, the N also becomes available to the weeds, which actively compete with the corn. By the end of the season, in Aug and Sept, the total mass was greater in non-LM treatments, due to the continued increase in weed proportion, as opposed to LM treatments. Although LM mass decreases as corn grows due to shading on WC (Sanders et al., 2018; Woledge, 1986), WC was still effective in reducing weed competition.

Living mulch crude protein (CP)

In 2020 there was the main effect of sampling month (P < .0001; Table 2.3), with the greatest CP content in Aug due to the increase in forage mass. The values are reflecting the amount of CP within each treatment, and the greater production in Aug allowed for greater CP content. In 2021, there was a treatment × sampling month interaction (P = 0.0296; Table 2.3), and although the amount of CP within each treatment and month showed statistical differences, the amount was balanced due to the stability in the WC botanical composition (Table 2.1).

Corn silage production

There was a difference between years (P < 0.0001) and among treatments (P = 0.0025). Greater silage production was observed in 2020 than in 2021 (Table 2.4). Corn was planted in the first week of June in both years; however, in 2021, there was lower precipitation in June (Fig 2.1). This lack of precipitation in early June directly affected corn germination and further development. According to the corn silage and grain variety tests in Tennessee (Sykes, et al., 2020 & 2021), the average production in Spring Hill at MTREC had an average corn silage production of 18.3 t ha⁻¹ in 2020 and 11.4 t ha⁻¹ in 2021, and they are often planted in the last week of April. The corn production in the conventional plots have qual spacing and density than corn with LM, and among LM treatments there was greater corn silage production in the CLM- 135 than CLM-0, and both were not different from CLM-45 and CLM-90. In addition, there was no difference between all LM treatments and C-0 (Table 2.4). This occurred because the presence of the WC LM led to an increased competition for soil resources that can affect the emergence and development of corn (Martin et al., 1999), and interspecies competition can be as detrimental as the lack of synthetic N fertilizer.

The choice of forage grown in intercropping systems is important to avoid this reduction in corn production (Borghi et al., 2013). Zhang et al. (2011), studying the intercropping of alfalfa with corn, showed that alfalfa is more competitive than corn, therefore recommending two lanes of corn and five of alfalfa for a more stable yield advantage. The use of WC as a LM between the corn lanes could have impacted corn silage production. However, the LM can significantly reduce weed competition, decreasing the reliance on herbicides and increasing the overall sustainability of the system.

The use of LM can be considered a more sustainable production practice, with a documented greater water use efficiency, decreased use of synthetic fertilizer, and increased biodiversity (Westbrook et al., 2022; Sanders et al., 2018; Andrews et al., 2018). Although no decrease in the use of synthetic fertilizer was observed in our study, the use of LM in the systems should be evaluated as a whole, not only as a way to increase crop production.

Corn silage nutritive value

Nutritive value analyses of unfermented silage showed a year \times treatment interaction (*P* = 0.0295) for CP (Table 2.5). In 2020, C-0 had less CP than C-135, while LM treatments did not differ within and from C-135, confirming the effect of N in CP levels. The increase in silage CP associated with the increased N fertilization rate and legume presence was also observed by Jahanzad et al (2014). Overall, CP was greater in 2021 than 2020, except on C-135 (Table 2.5).

Corn did not develop well in 2021; therefore, the increase in CP content in silage is probably due to the presence of WC LM in the harvested material (Table 2.2). Meanwhile, the year × treatment interaction (P = 0.0081) for NDF showed that the added N led to less fiber content (Table 2.5). Almodares et al. (2009) also found that corn has less fiber and more CP when N fertilization is increased. The NDF was lower in 2020 than 2021, except for CLM-45 and CLM-90, which remained similar in both years (Table 2.5). The greater NDF in 2021 is likely due to the WC LM being harvested with the corn. For IVTDMD48 there were only differences between years (P < 0.0001), with greater IVTDMD48 in 2020 than 2021, likely due to the greater presence of kernels that increased digestibility. These results can also be confirmed by the greater amount of starch in 2020 as compared to 2021 (Table 2.5). Starch is present in the endosperm of the corn grain and leads to greater digestibility (Rooney & Pflugfelder, 1986). The year × treatment interaction (P < 0.0001) in the starch content showed greater starch in the C-135 of both years, although not different than C-0 in 2021 (Table 2.5). This is likely due to the lack of competitiveness with the LM.

Meanwhile for the fermented silage, the year × treatment interaction (P = 0.0003) in the CP showed greater content for CLM-135 in 2020, likely due to the greater available N provided by LM and the fertilizer; while in 2021, the treatments with LM had greater CP than the controls (Table 2.5). Also, C-0 showed lesser CP content in both years due to the lack of N in the soil. The CP values increased from 2020 to 2021 for CLM-0, CLM-45, and CLM-90, while it remained unchanged for the control treatments and for CLM-135 (Table 2.5). In 2021, the corn ears did not develop as well as in 2020, thus the CP content in treatments where LM is present is increased due to LM being harvested during the corn silage harvesting process. The NDF content showed a difference between years (P < 0.0001), with greater NDF in 2020 than 2021. The

greater fiber content in 2020 is associated with the increased structural components of the crop, as compared to the crop in 2021 that remained undeveloped. The microbial and enzymatic activity that uses carbohydrates for fermentation (Weiss and Hall, 2020) could explain the lack of differences within treatments in 2021.

There was a year × treatment interaction (P = 0.0430) in the 2020 IVTDMD48 content, with greater digestibility observed for CLM-0 (although not different from C-0 and CLM-45). In the remaining treatments, the greater N availability allowed for greater corn growth, resulting in more structural components that led to less digestibility. Meanwhile, no differences in digestibility were observed in 2021, likely due to the stunted corn growth. Also, IVTDMD48 was greater in 2021 than 2020, since there was a greater amount of WC LM harvested with the silage (Table 2.5). There was a year (P < 0.0001) and treatment (P = 0.0100) effect in the starch content of the fermented silage, with greater starch in 2020 than 2021 due to greater number of kernels present in the sample. Among treatments, there was greater starch content in the control treatments, although C-0 did not differ from any other treatment (Table 2.5). These results show that the LM can be highly competitive with corn. Since corn growth rate directly affects the kernel number and ear growth rate (Westgate et al., 2004) due to LM competition for soil moisture and nutrients, reduced growth and ear development can occur.

Experiment II

Living mulch botanical composition

There was a month × treatment interaction in both years for LM and weed components (Table 2.6). In 2020, from May through July the LM proportion was greater for LM treatments (as expected). However, this pattern shifted in Aug, with a lack of differences between control

treatments and CLM-90 or CLM-135, due to a significant reduction in the LM proportion. In Sept the LM proportion did not differ among all treatments, and this reduction in the proportion of WC is expected by the end of the season. These results are also attributed to the increased shading from corn (Mercier et al., 2020), therefore reducing the percentage of LM in the plots. The exact opposite is true about the weed proportion in the same period, where weed proportion increased with the decreased LM in the plots (Table 2.6), which further explains the similarities between control and LM treatments.

In 2021, the LM proportion was similar among all LM treatments from May to Aug. However, in Sept the LM proportion decreased (Table 2.6), as it had in the previous year. At the same time, there was an increase in weed proportion (Table 2.6). Therewere no plants growing in the control plots in May in June, since herbicide was applied preceding corn planting.

Living mulch total mass accumulation

An interaction between month × treatment was observed in both years (Table 2.7). In 2020, the LM mass did not differ among LM treatments in May and Jun (Table 2.7). However, in July the LM mass was the same among all treatments, including controls. This is expected since the cool-season legume WC slows down productivity in summer, which coincides with the appearance of summer weeds in the control treatments (Table 2.6). In Aug, greater LM mass was observed in the CLM-90 and CLM-135, while less mass was observed in the remaining treatments. In Sept, a similar response was observed with the greatest LM mass in the CLM-90 and CLM-135 did not differ from the remaining treatments (Table 2.7). Among LM treatments, the LM mass was generally lower in May, except for CLM-0 that showed constant LM mass throughout the growing season (Table 2.7), likely due to reduced competition for resources as compared to the other treatments. It is known that excessive

amounts of N decrease the potential for BNF (Simms & Taylor, 2002), and it is likely that the added synthetic N fertilizer contributed to the growth of WC, resulting in greater competition between the LM and the corn.

In 2021, the greatest LM mass was observed in May for CLM-135, while in June, July, Aug, and Sept there were no differences among treatments. According to our results, although the addition of synthetic N does not affect the presence of the LM as much as expected, the second year after establishing, the LM appears to be more uniform; therefore, WC growth should be controlled to reduce the competition between LM and corn.

Living mulch crude protein (CP)

In 2020, there was a treatment × sampling month interaction (P = 0.0387; Table 2.8). Lower CP content was observed in Aug in corn with LM with 0 and 45 kg ha⁻¹ and in Sept at 45 kg ha⁻¹ compared to the remaining treatments within a month (Table 2.8). Since the results reflect the amount of CP in each plot, it is expected that greater CP are observed in plots with N and LM later in the season. In 2021, there were differences among treatments, however, the standard error values (50.1) did not allow for mean letter group separation. Therefore, no differences were observed in 2021 due to the stability of the WC botanical composition within each plot (2.6).

Corn grain production

There was a treatment × year interaction and greater production was observed in no LM treatments in 2020, while in 2021 no differences among treatments were observed (Table 2.9). These results are expected given the low precipitation in early June in 2021 (Fig 2.1). Drought greatly affects grain yield, because it delays silk emergence and can cause embryo sac abortion (Moss & Downey, 1971), which is responsible for the pollination and fertilization process. Also, the corn grain was a relatively early maturity hybrid, and Larson & Clegg (1999) found that

when using early maturing hybrids, it is important to increase plant population to have equivalent yields as the late-maturing hybrids. In addition, Byers et al. (2019) found that WC can improve corn production if suppressed with herbicides to decrease interspecies competition. The importance of WC suppression as LM was also observed by Sanders et al. (2017). In 2021, the total mass of WC LM was greater than 2020; therefore, combining lower precipitation and greater proportion of WC LM, the competition was too great for an optimum interspecies development. According to the corn silage and grain tests in Tennessee (Sykes, et al., 2020 & 2021), the average production in Spring Hill at MTREC had an average corn grain of 13.2 t ha⁻¹ in 2020 and 10.3 t ha⁻¹ in 2021. Therefore, the LM production of corn was lower than conventional systems.

Corn grain nutritive value

There was a year × treatment interaction in oil (P = 0.0104) and starch (P = 0.0499) content, but for CP only the main effects of year (P < 0.0001) and treatment (P < 0.0001; Table 2.7) were found. The CLM-135 could not be evaluated in 2021 due to the low quality and quantity of grains. The oil content was greater in 2020 than 2021 for all treatments except C-0. Given that ears were normally developed in 2020, the oil content of grains was expectedly greater than in 2021. About 10-12% of the kernel is the germ which comprises the oil, which is likely the reason for the greater content in 2020 (Smith et al., 2004).

The CP content was greater in LM treatments than in controls (Table 2.10), could be attributed to the BNF directly affecting overall protein content. In addition, it appears that synthetic N fertilization alone is not sufficient to increase overall CP content of corn grains. Also, the CP content was greater in 2021 than 2020, when the presence of legume on the field was greater (Table 2.6). Jahangirlou et al. (2020) studied the effect of planting date, irrigation,

and N stress in corn nutritive value and found that the increase in N availability in the soil increases the CP content of corn grain. Similar results were observed in Song et al (2021) in which corn in intercropping with legume had greater CP content than corn in monoculture.

The starch content was also greater in 2021 than 2020 for all treatments (Table 2.10). In 2020 the starch content was somewhat consistent among treatments, but in 2021, greater N in LM led to less starch content (Table 2.10), likely due to the increase in WC content, which increases the LM to corn competition, decreasing the amount and size of the kernels where the starch is present (Rooney & Pflugfelder, 1986).

Conclusion

White clover LM does not increase corn silage or grain production when compared to conventional systems. Irrigation might be necessary in LM programs especially in the first weeks after corn planting to ensure corn germination and development. Although the LM did not increase corn yield, more studies on the benefits of LM in soil must be assessed. Nutritive value is not greatly affected by the amount of N added, but, as the growing season progresses, the fiber content increases in LM. Weed pressure increases as the growing season progresses, but it is higher in the establishment year. Therefore, the use of WC as LM reduced weed competition, which is considered a good strategy against noxious weeds that negatively affect corn growth and development.



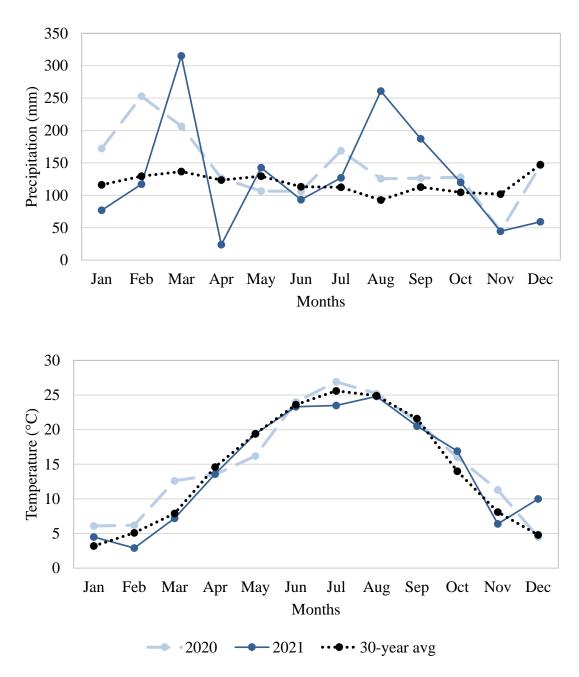


Figure 2-1: Precipitation (mm) and temperature (°C) from 2018 to 2021 field preparation and growing season, and 30-year average in Spring Hill, TN

			202	0				
			Living mulo	$ch (g kg^{-1})$				
	May	Jun	Jul	Aug	Sept	x		
[†] C-0	$0.0^{b,A}$	$0.0^{d,A}$	$0.0^{d,A}$	$0.0^{b,A}$	$0.0^{b,A}$	0		
C-135	0.0 ^{b,A}	$0.0^{d,A}$	$0.0^{d,A}$	0.0 ^{b,A}	$0.0^{b,A}$	0		
CLM-0	1000.0 ^{a,A}	847.2 ^{ab,B}	494.2 ^{a,C}	235.4 ^{a,D}	165.1 ^{a,D}	548.4		
CLM-45	991.7 ^{a,A}	900.3 ^{a,A}	465.1 ^{ab,B}	16.9 ^{b,D}	187.1 ^{a,C}	512.2		
CLM-90	933.5 ^{a,A}	743.8 ^{bc,B}	353.1 ^{bc,C}	24.8 ^{b,D}	101.2 ^{ab,D}	431.3		
CLM-135	986.3 ^{a,A}	681.8 ^{c,B}	252.0 ^{c,C}	63.7 ^{b,D}	125.3 ^{ab,CD}	421.8		
x	651.9	528.8	260.7	56.8	96.4			
Sampling month (M)			< 0.0001					
Treatment (T)			< 0.0001					
$\mathbf{M} imes \mathbf{T}$	< 0.0001							
	Weed (g kg ⁻¹)							
C-0	0.0 ^{a,B}	0.0 ^{d,B}	1000.0 ^{a,A}	1000.0 ^{a,A}	1000.0 ^{a,A}	600		
C-135	0.0 ^{a,B}	$0.0^{d,B}$	1000.0 ^{a,A}	1000.0 ^{a,A}	1000.0 ^{a,A}	600		
CLM-0	0.0 ^{a,D}	152.8 ^{bc,C}	505.8 ^{d,B}	764.6 ^{b,A}	834.9 ^{b,A}	451.6		
CLM-45	8.3 ^{a,D}	99.7 ^{cd,D}	534.8 ^{cd,C}	983.0 ^{a,A}	812.9 ^{b,B}	487.8		
CLM-90	66.5 ^{a,D}	256.2 ^{ab,C}	646.8 ^{bc,B}	975.2 ^{a,A}	898.8 ^{ab,A}	568.7		
CLM-135	13.7 ^{a,D}	318.2 ^{a,C}	748.0 ^{b,B}	936.3 ^{a,A}	874.7 ^{ab,AB}	524.2		
x	14.7	137.8	694.2	943.2	903.5			
Sampling month (M)			0.0002					
Treatment (T)			< 0.0001					
$\mathbf{M} imes \mathbf{T}$			< 0.0001					

Table 2-1: Living mulch (LM) botanical composition (DM g kg⁻¹) grown with silage corn during two consecutive growing seasons (2020 and 2021) in Spring Hill, TN.

			202	.1				
		Living mulch (g kg ⁻¹)						
C-0	0	0	0	0	0	0.0^{b}		
C-135	0	0	0	0	0	0.0^{b}		
CLM-0	1000	1000	882.8	916.7	616.3	883.2ª		
CLM-45	1000	1000	818.2	980.4	374.5	834.6 ^a		
CLM-90	1000	1000	878.4	913.4	457.8	849.9 ^a		
CLM-135	1000	1000	1000	791.3	538.5	865.9ª		
$\bar{\mathbf{x}}$	666.7 ^A	666.7 ^A	596.6 ^A	600.3 ^A	331.2 ^B			
Sampling month (M)			<.0001					
Treatment (T)			<.0001					
$\mathbf{M} imes \mathbf{T}$			0.3363					
	Weed $(g kg^{-1})$							
C-0	0.0 ^{a,B}	0.0 ^{a,B}	1000.0 ^{a,A}	1000.0 ^{a,A}	1000.0 ^{a,A}	600		
C-135	$0.0^{\mathrm{a,B}}$	$0.0^{a,B}$	1000.0 ^{a,A}	1000.0 ^{a,A}	1000.0 ^{a,A}	600		
CLM-0	$0.0^{a,B}$	$0.0^{a,B}$	117.2 ^{b,AB}	83.3 ^{b,AB}	383.7 ^{b,A}	116.8		
CLM-45	$0.0^{a,B}$	$0.0^{\mathrm{a,B}}$	181.8 ^{b,B}	19.6 ^{b,B}	625.5 ^{b,A}	165.4		
CLM-90	$0.0^{\mathrm{a,C}}$	$0.0^{\mathrm{a,C}}$	121.6 ^{b,BC}	86.6 ^{b,BC}	542.2 ^{b,A}	150.1		
CLM-135	$0.0^{\mathrm{a,B}}$	$0.0^{\mathrm{a,B}}$	$0.0^{b,B}$	208.7 ^{b,AB}	461.5 ^{b,A}	134		
x	0	0	403.4	399.7	668.8			
Sampling month (M)			<.0001					
Treatment (T)			<.0001					
$\mathbf{M} imes \mathbf{T}$			<.0001					

Table 2.1 (continued): Living mulch (LM) botanical composition (DM g kg⁻¹) grown with silage corn during two consecutive growing seasons (2020 and 2021) in Spring Hill, TN.

[†]C-0, corn + no N; C-135, corn + 135 kg ha⁻¹ of N; CLM-0, corn in living mulch (LM) + no N; CLM-45, corn in living mulch + 45 kg ha⁻¹ of N; CLM-90, corn in living mulch + 90 kg ha⁻¹ of N; CLM-135, corn in living mulch + 135 kg ha⁻¹ of N. Means without a common lowercase letter differ within a column. Means without a common uppercase letter differ within rows. *P*-values are significant at $\alpha \le 0.05$ using Fisher's LSD test.

	May	June	July	August	September	x
			kg ha⁻¹			
			2020			
[†] C-0	0.0 ^{a,C}	0.0 ^{b,C}	223.3 ^{b,B}	2510.0 ^{ab,A}	835.0 ^{ab,B}	713.7
C-135	$0.0^{a,B}$	$0.0^{b,B}$	578.3 ^{ab,B}	2163.3 ^{bc,A}	1525.0 ^{a,A}	853.3
CLM-0	371.7 ^{a,B}	513.3 ^{ab,B}	$881.7^{ab,B}$	1653.3 ^{c,A}	926.7 ^{ab,B}	869.3
CLM-45	381.7 ^{a,C}	806.7 ^{a,BC}	1131.7 ^{a,B}	$2806.7^{ab,A}$	1043.3 ^{ab,BC}	1234.0
CLM-90	425.0 ^{a,B}	781.7 ^{a,B}	873.3 ^{ab,B}	2950.0 ^{a,A}	708.3 ^{b,B}	1147.7
CLM-135	418.3 ^{a,C}	146.7 ^{ab,C}	1173.3 ^{a,B}	2776.7 ^{ab,A}	$895.0^{ab,BC}$	1082.0
x	266.1	374.7	810.3	2476.7	988.9	
			2021			
C-0	0.0 ^{b,B}	$0.0^{b,B}$	456.6 ^{b,B}	1423.3 ^{a,A}	1843.3 ^{a,A}	744.7
C-135	$0.0^{b,C}$	$0.0^{b,C}$	873.3 ^{ab,B}	1513.3 ^{a,AB}	1690.0 ^{a,A}	815.3
CLM-0	1776.7 ^{a,A}	1126.7 ^{a,AB}	1170.0 ^{a,AB}	530.0 ^{b,BC}	293.3 ^{b,C}	979.3
CLM-45	1726.7 ^{a,A}	970.0 ^{a,B}	823.3 ^{ab,B}	586.6 ^{b,B}	546.6 ^{b,B}	930.7
CLM-90	1903.3 ^{a,A}	1030.0 ^{a,B}	853.3 ^{ab,B}	736.6 ^{b,B}	736.6 ^{b,B}	1052.0
CLM-135	1770.0 ^{a,A}	1003.3 ^{a,B}	1046.7 ^{ab,B}	781.6 ^{b,BC}	336.6 ^{b,C}	987.7
x	1196.1	688.3	870.5	928.6	907.8	
		AN	IOVA			
		2020			2021	
Sampling month (M)		<.0001			0.1543	
Treatment (T)		0.0606			0.0107	
$M \times T$		0.0430			<.0001	

Table 2-2: Total living mulch (LM) mass (LM and weeds) (DM kg ha⁻¹) grown with silage corn during two consecutive growing seasons (2020 and 2021) in Spring Hill, TN.

Means without a common lowercase letter differ within a column. Means without a common uppercase letter differ within rows. *P*-values are significant at $\alpha \le 0.05$ using Fisher's LSD test.

	Crude protein (g kg ⁻¹)						
	May	Jun	Jul	Aug	Sep		
			2020				
CLM-0	90	133	159	242	125		
CLM-45	90	210	202	367	143		
CLM-90	99	198	164	387	109		
CLM-135	99	54	241	432	104		
	94 ^C	149 ^{BC}	192 ^B	357 ^A	120 ^C		
	2021						
CLM-0	431 ^a	303 ^a	241 ^a	77 ^a	57 ^a		
CLM-45	447 ^a	199 ^{ab}	171 ^{ab}	122 ^a	101 ^a		
CLM-90	352 ^{ab}	155 ^b	97 ^b	149 ^a	162 ^a		
CLM-135	234 ^b	294 ^a	211 ^{ab}	131 ^a	69 ^a		
	ANOVA						
	20	020		20	21		
Treatment (T)	0.1	659		0.5	506		
Sampling month (S)	<.0	001	<.0001				
$T \times S$	0.1124			0.02	296		

Table 2-3: Living Mulch (LM) crude protein (CP), grown with silage corn during two consecutive growing seasons (2020 and 2021) in Spring Hill, TN.

[†]CLM-0, corn in living mulch (LM) + no N; CLM-45, corn in living mulch + 45 kg ha⁻¹ of N; CLM-90, corn in living mulch + 90 kg ha⁻¹ of N; CLM-135, corn in living mulch + 135 kg ha⁻¹ of N. Means without a common uppercase letter differ within rows. Means without a common lowercase letter differ within a column *P*-values are significant at $\alpha \le 0.05$ using Fisher's LSD test.

	2020	2021	x				
		DM Ton ha ⁻¹					
[†] C-0	5.3	2.3	3.8 ^{bc}				
C-135	10.0	5.5	7.8^{a}				
CLM-0	3.8	2.4	3.1 ^c				
CLM-45	6.1	2.2	4.1 ^{bc}				
CLM-90	7.1	2.4	4.7 ^{bc}				
CLM-135	8.6	2.1	5.3 ^b				
x	6.8 ^A	2.8 ^B					
	ANOVA						
Year (Y)		< 0.0001					
Treatment (T)		0.0025					
$\mathbf{Y} \times \mathbf{T}$		0.2488					

Table 2-4: Corn silage production (DM ton ha⁻¹) during two consecutive growing seasons (2020 and 2021) in Spring Hill, TN.

[†]C-0, corn + no N; C-135, corn + 135 kg ha⁻¹ of N; CLM-0, corn in living mulch (LM) + no N; CLM-45, corn in living mulch + 45 kg ha⁻¹ of N; CLM-90, corn in living mulch + 90 kg ha⁻¹ of N; CLM-135, corn in living mulch + 135 kg ha⁻¹ of N. Means without a common lowercase letter differ within a column. Means without a common uppercase letter differ within rows. *P*-values are significant at $\alpha \leq 0.05$ using Fisher's LSD test.

growing seasons (2020 and 2021) in Spring Hill, TN.								
	Unfei	mented silag		Fermented silage				
	2020	2021	x	2020	2021	$\bar{\mathbf{x}}$		
		Cr	ude prote	in (CP g kg ⁻¹)			
[†] C-0	67.5 ^{b,B}	81.4 ^{c,A}	74.4	56.3 ^{d,A}	53.0 ^{d,A}	54.6		
C-135	88.8 ^{a,A}	95.0 ^{bc,A}	88.9	83.9 ^{b,A}	74.4 ^{c,A}	79.1		
CLM-0	77.1 ^{ab,B}	101.3 ^{b,A}	89.2	70.9 ^{c,B}	106.9 ^{ab,A}	88.9		
CLM-45	79.4 ^{ab,B}	108.1 ^{b,A}	93.8	74.4 ^{bc,B}	100.2 ^{b,A}	87.3		
CLM-90	84.7 ^{a,B}	124.9 ^{a,A}	105.8	84.1 ^{b,B}	109.8 ^{ab,A}	96.9		
CLM-135	89.4 ^{a,B}	126.2 ^{a,A}	107.8	108.8 ^{a,A}	112.3 ^{a,A}	110.6		
$\bar{\mathbf{x}}$	80.1	106.15		79.7	92.8			
Year (Y)	< 0.0	001		< 0.0	001			
Treatment (T)	< 0.0	001		< 0.0	001			
$\mathbf{Y} imes \mathbf{T}$	0.02	295		0.00	003			
		Neutral	detergent	fiber (NDF	g kg ⁻¹)			
C-0	580.1 ^{a,B}	645.9 ^{a,A}	613	624.7	563.7	594.2		
C-135	515.7 ^{c,B}	612.1 ^{b,A}	563.9	593.0	549.4	571.2		
CLM-0	571.5 ^{ab,B}	$608.0^{bc,A}$	589.8	648.2	548.7	598.4		
CLM-45	571.6 ^{ab,A}	589.8 ^{bc,A}	580.7	637.7	543.5	590.6		
CLM-90	541.9 ^{bc,A}	554.6 ^{d,A}	548.3	657.9	541.5	599.7		
CLM-135	533.3 ^{c,B}	580.3 ^{cd,A}	556.8	631.7	556.0	593.8		
$\overline{\mathbf{x}}$	552.3	598.4		632.2 ^A	550.5 ^B			
Year (Y)	< 0.0			< 0.0001				
Treatment (T)	< 0.0	001		0.2474				
$\mathbf{Y} \times \mathbf{T}$	0.00			0.0				
	In	- <i>vitro</i> dry ma	tter diges		(IVTDMD48))		
C-0	819.4	740.1	780.1	804.4 ^{ab,B}	859.9 ^{a,A}	818.3		
C-135	823.3	748.5	785.9	784.8 ^{cd,B}	851.7 ^{a,A}	811.5		
CLM-0	828.2	755.1	791.6	814.1 ^{a,B}	858.3 ^{a,A}	836.2		
CLM-45	817.4	764.5	790.9	800.4 ^{abc,B}	864.5 ^{a,A}	832.5		
CLM-90	824.8	779.5	801.9	778.5 ^{d,B}	867.6 ^{a,A}	823.1		
CLM-135	828.8	769.5	799.2	788.0 ^{bcd,B}	852.1 ^{a,A}	820.0		
x	823.6 ^A	759.1 ^B		795.0	859.0			
Year (Y)	< 0.0			<0.0001				
Treatment (T)	0.34			0.05				
$\mathbf{Y} \times \mathbf{T}$	0.53	377		0.04	430			

Table 2-5: Nutritive value (g kg⁻¹) of fermented and unfermented silage during two consecutive growing seasons (2020 and 2021) in Spring Hill, TN.

consecutive growing see		· · · · ·					
	Unfer	Unfermented silage			Fermented silage		
	2020	2021	x	2020	2021	x	
			Starcl	h g kg ⁻¹			
C-0	101.0 ^{cd,A}	27.1 ^{ab,B}	64.1	111.0	34.2	72.5 ^{ab}	
C-135	168.1 ^{a,A}	33.5 ^{a,B}	100.8	149.6	44.9	97.2 ^a	
CLM-0	97.6 ^{cd,A}	13.7 ^{bc,B}	55.6	69.7	21.3	45.5 ^b	
CLM-45	92.2 ^{d,A}	10.6 ^{bc,B}	51.4	96.1	25.1	60.6 ^b	
CLM-90	123.6 ^{b,A}	4.1 ^{c,B}	63.8	64.5	16.9	40.7 ^b	
CLM-135	113.4 ^{bc,A}	4.8 ^{c,B}	59.1	65.7	22.1	43.9 ^b	
$\bar{\mathbf{x}}$	115.9	15.6		92.8 ^A	27.4 ^B		
Year (Y)	< 0.0	< 0.0001		< 0.0001			
Treatment (T)	< 0.0	< 0.0001		0.0100			
$\mathbf{Y} \times \mathbf{T}$	< 0.0	001		0.3313			
4		. 1					

Table 2.5 (continued): Nutritive value (g kg⁻¹) of fermented and unfermented silage during two consecutive growing seasons (2020 and 2021) in Spring Hill, TN.

[†]C-0, corn + no N; C-135, corn + 135 kg ha⁻¹ of N; CLM-0, corn in living mulch (LM) + no N; CLM-45, corn in living mulch + 45 kg ha⁻¹ of N; CLM-90, corn in living mulch + 90 kg ha⁻¹ of N; CLM-135, corn in living mulch + 135 kg ha⁻¹ of N. Means without a common lowercase letter differ within a column. Means without a common uppercase letter differ within rows. *P*-values are significant at $\alpha \le 0.05$ using Fisher's LSD test.

			2020				
	Legume $(g kg^{-1})$						
	May	Jun	Jul	Aug	Sept	x	
[†] C-0	$0.0^{b,A}$	$0.0^{b,A}$	$0.0^{c,A}$	$0.0^{c,A}$	$0.0^{\mathrm{a,A}}$	0	
C-135	$0.0^{b,A}$	$0.0^{b,A}$	$0.0^{c,A}$	$0.0^{c,A}$	$0.0^{\mathrm{a,A}}$	0	
CLM-0	735.7 ^{a,A}	946.3 ^{a,A}	702.0 ^{ab,A}	426.2 ^{a,B}	249.3 ^{a,B}	611.9	
CLM-45	692.5 ^{a,AB}	927.3 ^{a,A}	648.3 ^{ab,BC}	385.4 ^{ab,CD}	212.6 ^{a,D}	573.2	
CLM-90	942.7 ^{a,A}	699.2 ^{a,AB}	444.6 ^{b,B}	120.4 ^{bc,C}	39.7 ^{a,C}	449.3	
CLM-135	946.2 ^{a,A}	919.6 ^{a,A}	872.7 ^{a,A}	79.5 ^{c,B}	100.2 ^{a,B}	583.6	
x	552.9	582	444.6	168.6	100.3		
Sampling month (M)			<.0001				
Treatment (T)			<.0001				
$M \times T$			<.0001				
			Weed (g k	(g ⁻¹)			
C-0	0.0 ^{b,B}	$0.0^{b,B}$	1000.0 ^{a,A}	1000.0 ^{a,A}	1000.0 ^{a,A}	600	
C-135	0.0 ^{b, B}	$0.0^{b,B}$	1000.0 ^{a,A}	1000.0 ^{a,A}	1000.0 ^{a,A}	600	
CLM-0	264.3 ^{ab,BC}	53.6 ^{ab,B}	298.0 ^{bc,BC}	573.8 ^{c,A}	750.7 ^{a,A}	388.1	
CLM-45	307.4 ^{a,CD}	72.7 ^{ab,D}	351.7 ^{bc,BC}	614.5 ^{bc,AB}	787.4 ^{a,A}	426.8	
CLM-90	57.3 ^{ab,C}	301.0 ^{a,BC}	555.4 ^{b,B}	879.6 ^{ab,A}	960.3 ^{a,A}	550.7	
CLM-135	53.7 ^{ab,B}	80.4 ^{ab,B}	127.3 ^{c,B}	920.5 ^{a,A}	899.8 ^{a,A}	416.4	
x	113.8	84.7	555.4	831.3	899.7		
Sampling month (M)			< 0.0001				
Treatment (T)			0.0107				
M imes T			< 0.0001				

Table 2-6: Living mulch (LM) botanical composition (DM g kg⁻¹) grown with grain corn during two consecutive growing seasons (2020 and 2021) in Spring Hill, TN.

			2021				
	Legume (g kg ⁻¹)						
C-0	$0.0^{b,A}$	0.0 ^{b,A}	0.0 ^{b,A}	0.0 ^{b,A}	0.0 ^{c,A}	0	
C-135	$0.0^{b,A}$	$0.0^{b,A}$	$0.0^{b,A}$	$0.0^{b,A}$	$0.0^{c,A}$	0	
CLM-0	1000.0 ^{a,A}	1000.0 ^{a,A}	1000.0 ^{a,A}	1000.0 ^{a,A}	767.7 ^{a,B}	953.5	
CLM-45	1000.0 ^{a,A}	1000.0 ^{b,A}	1000.0 ^{a,A}	1000.0 ^{a,A}	472.0 ^{b,B}	894.4	
CLM-90	1000.0 ^{a,A}	1000.0 ^{a,A}	897.4 ^{a,A}	1000.0 ^{a,A}	604.7 ^{ab,B}	900.4	
CLM-135	1000.0 ^{a,A}	1000.0 ^{a,A}	849.0 ^{a,A}	993.0 ^{a,A}	639.0 ^{ab,B}	896.2	
x	666.7	666.7	624.4	665.5	413.9		
Sampling month (M)			<.0001				
Treatment (T)			<.0001				
$\mathbf{M} imes \mathbf{T}$			0.033				
			Weed (g k	(g ⁻¹)			
C-0	0.0 ^{a,B}	0.0 ^{a,B}	1000.0 ^{a,A}	1000.0 ^{a,A}	1000.0 ^{a,A}	600	
C-135	$0.0^{a,B}$	0.0 ^{a,B}	1000.0 ^{a,A}	1000.0 ^{a,A}	1000.0 ^{a,A}	600	
CLM-0	$0.0^{a,B}$	0.0 ^{a,B}	$0.0^{b,B}$	$0.0^{b,B}$	232.3 ^{c,A}	46.5	
CLM-45	0.0 ^{a,B}	0.0 ^{a,B}	0.0 ^{b,B}	0.0 ^{b,B}	528.0 ^{b,A}	105.6	
CLM-90	0.0 ^{a,B}	$0.0^{\mathrm{a,B}}$	102.5 ^{b,B}	$0.0^{b,B}$	395.3 ^{bc,A}	99.6	
CLM-135	0.0 ^{a,B}	0.0 ^{a,B}	151.1 ^{b,B}	$0.7^{b,B}$	361.0 ^{bcA}	103.8	
x	0	0	375.6	334.5	586.1		
Sampling month (M)			<.0001				
Treatment (T)			<.0001				
$M \times T$			<.0001				

Table 2.6 (continued): Living mulch (LM) botanical composition (DM g kg⁻¹) grown with grain corn during two consecutive growing seasons (2020 and 2021) in Spring Hill, TN.

[†]C-0, corn + no N; C-135, corn + 135 kg ha⁻¹ of N; CLM-0, corn in living mulch (LM) + no N; CLM-45, corn in living mulch + 45 kg ha⁻¹ of N; CLM-90, corn in living mulch + 90 kg ha⁻¹ of N; CLM-135, corn in living mulch + 135 kg ha⁻¹ of N. Means without a common lowercase letter differ within a column. Means without a common uppercase letter differ within rows. *P*-values are significant at $\alpha \le 0.05$ using Fisher's LSD test.

during two consecutive						_		
	May	Jun	Jul	Aug	Sept	x		
	kg ha ⁻¹							
	2020							
[†] C-0	0.0 ^{c,C}	0.0 ^{b,C}	179.3 ^{a,C}	2086.7 ^{bc,A}	1120.0 ^{b,B}	677.2		
C-135	$0.0^{\mathrm{a,C}}$	$0.0^{b,C}$	211.7 ^{a,BC}	1750.0 ^{c,A}	880.0 ^{b,B}	568.3		
CLM-0	691.7 ^{a,A}	933.3 ^{a,A}	746.7 ^{a,A}	1400.0 ^{c,A}	836.7 ^{b,A}	921.7		
CLM-45	570.0 ^{a,B}	941.7 ^{a,AB}	781.7 ^{a,B}	1663.3 ^{c,A}	871.7 ^{b,AB}	965.7		
CLM-90	348.3 ^{a,B}	876.7 ^{a,B}	711.7 ^{a,B}	2873.3 ^{ab,A}	2086.7 ^{a,A}	1379.3		
CLM-135	628.3 ^{a,C}	693.3 ^{ab,BC}	626.7 ^{a,C}	3586.7 ^{a,A}	1516.7 ^{ab,B}	1410.3		
x	373.1	574.2	542.9	2226.7	1218.6	-		
			20	021				
C-0	0.0 ^{c,A}	$0.0^{a,A}$	416.7 ^{a,A}	1426.7 ^{a,A}	1266.7 ^{a,A}	622.0		
C-135	$0.0^{c,B}$	$0.0^{a,B}$	616.7 ^{a,AB}	1696.7 ^{a,A}	1253.3 ^{a,AB}	713.3		
CLM-0	1813.3 ^{b,A}	906.7 ^{a,A}	770.0 ^{a,A}	553.3 ^{a,A}	790.0 ^{a,A}	966.7		
CLM-45	1373.3 ^{bc,A}	647.0 ^{a,A}	803.3 ^{a,A}	740.0 ^{a,A}	1036.7 ^{a,A}	920.1		
CLM-90	1276.7 ^{bc,A}	1070.0 ^{a,A}	643.3 ^{a,A}	743.3 ^{a,A}	730.0 ^{a,A}	892.7		
CLM-135	4160.0 ^{a,A}	1040.0 ^{a,B}	776.7 ^{a,B}	596.7 ^{a,B}	726.7 ^{a,B}	1460.0		
x	1437.2	610.6	671.1	959.4	967.2			
		AN	OVA					
		2020			2021			
Sampling month (M)		<.0001			0.0984			
Treatment (T)		0.0027			0.2435			
$M \times T$		0.0381			0.0337			

Table 2-7: Total living mulch (LM) mass (LM and weeds) (DM kg ha-1) grown with grain corn during two consecutive growing seasons (2020 and 2021) in Spring Hill, TN.

[†]C-0, corn + no N; C-135, corn + 135 kg ha⁻¹ of N; CLM-0, corn in living mulch (LM) + no N; CLM-45, corn in living mulch + 45 kg ha⁻¹ of N; CLM-90, corn in living mulch + 90 kg ha⁻¹ of N; CLM-135, corn in living mulch + 135 kg ha⁻¹ of N. Means without a common lowercase letter differ within a column. Means without a common uppercase letter differ within rows. *P*-values are significant at $\alpha \leq 0.05$ using Fisher's LSD test.

	,	Crude protein (g kg ⁻¹)						
	May	Jun	Jul	Aug	Sep			
			2020					
[†] CLM-0	159 ^a	233 ^a	156 ^a	250 ^b	133 ^{ab}			
CLM-45	149 ^a	208 ^a	169 ^a	279 ^b	113 ^b			
CLM-90	79 ^a	203 ^a	151 ^a	444 ^a	268 ^a			
CLM-135	155 ^a	165 ^a	132 ^a	528 ^a	196 ^{ab}			
			2021					
CLM-0	462	240	175	129	244			
CLM-45	270	146	185	160	270			
CLM-90	308	290	159	156	183			
CLM-135	355	271	203	131	81			
			ANOVA					
	20	20		2	021			
Treatment (T)	<.0	001	-	<.(0001			
Sampling month (S)	0.2	213		0.3982				
$T \times S$	0.0	387	0.0626					

Table 2-8: Living Mulch (LM) crude protein (CP), grown with grain corn during two consecutive growing seasons (2020 and 2021) in Spring Hill, TN.

[†]CLM -0, corn in living mulch (LM) + no N; CLM-45, corn in living mulch + 45 kg ha⁻¹ of N; CLM-90, corn in living mulch + 90 kg ha⁻¹ of N; CLM-135, corn in living mulch + 135 kg ha⁻¹ of N. Means without a common lowercase letter differ within a column. *P*-values are significant at $\alpha \le 0.05$ using Fisher's LSD test.

	2020	2021				
	Ton ha ⁻¹					
[†] C-0	3.4 ^b	0.1 ^a				
C-135	4.7^{a}	0.2^{a}				
CLM-0	1.1 ^c	0.1 ^a				
CLM-45	1.4 ^c	0.3ª				
CLM-90	1.5°	0.1 ^a				
CLM-135	1.8°	0.1 ^a				
	ANG	DVA				
Year (Y)	<0.0	0001				
Treatment (T)	<0.0	0001				
$\mathbf{Y} \times \mathbf{T}$	<0.0	0001				

Table 2-9: Corn grain production (Ton ha⁻¹) during two consecutive growing seasons (2020 and 2021) in Spring Hill, TN.

[†]C-0, corn + no N; C-135, corn + 135 kg ha⁻¹ of N; CLM-0, corn in living mulch (LM) + no N; CLM-45, corn in living mulch + 45 kg ha⁻¹ of N; CLM-90, corn in living mulch + 90 kg ha⁻¹ of N; CLM-135, corn in living mulch + 135 kg ha⁻¹ of N. Means without a common uppercase letter differ within rows. *P*-values are significant at $\alpha \le 0.05$ using Fisher's LSD test.

	2020	2021	x
_		Oil	
-		g kg ⁻¹	
[†] C-0	40.4 ^{b,A}	38.9 ^{a,A}	39.6
C-135	43.8 ^{a,A}	39.6 ^{a,B}	41.7
CLM-0	45.1 ^{a,A}	39.3 ^{a,B}	42.2
CLM-45	45.3 ^{a,A}	39.6 ^{a,B}	42.4
CLM-90	45.7 ^{a,A}	39.2 ^{a,B}	42.4
CLM-135	44.0^{a}	-	-
x	44.1	39.3	
Year (Y)		<.0001	
Treatment (T)		0.0045	
Y×T		0.0104	
		Crude protein (CP)	
C-0	62.0	85.9	73.9 ^b
C-135	69.0	88.1	78.5 ^b
CLM-0	80.0	95.4	87.7 ^a
CLM-45	76.0	103.6	89.8 ^a
CLM-90	77.0	109.4	93.2 ^a
CLM-135	77.3	-	-
x	73.6 ^B	96.5 ^A	
Year (Y)		<.0001	
Treatment (T)		<.0001	
$Y \times T$		0.1044	
		Starch	
C-0	628.0 ^{a,B}	715.2 ^{ab,A}	671.6
C-135	618.5 ^{ab,B}	721.5 ^{a,A}	670.0
CLM-0	614.0 ^{b,B}	706.5 ^{bc,A}	660.2
CLM-45	618.0 ^{ab,B}	703.8 ^{c,A}	660.9
CLM-90	612.3 ^{b,B}	692.1 ^{d,A}	652.2
CLM-135	614.7 ^b	-	-
x	617.6	707.8	
Year (Y)		0.0005	
Treatment (T)		<.0001	
$\mathbf{Y} \times \mathbf{T}$		0.0499	

Table 2-10: Nutritive value (g kg⁻¹) of corn grain during two consecutive growing seasons (2020 and 2021) in Spring Hill, TN.

[†]C-0, corn + no N; C-135, corn + 135 kg ha⁻¹ of N; CLM-0, corn in living mulch (LM) + no N; CLM-45, corn in living mulch + 45 kg ha⁻¹ of N; CLM-90, corn in living mulch + 90 kg ha⁻¹ of N; CLM-135, corn in living mulch + 135 kg ha⁻¹ of N. Means without a common lowercase letter differ within a column. Means without a common uppercase letter differ within rows. *P*-values are significant at $\alpha \le 0.05$ using Fisher's LSD test.

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CHAPTER III: ASSESSMENT OF YIELD AND PARTIAL NET RETURNS OF CORN PRODUCTION IN LM SYSTEMS IN DIFFERENT N LEVELS

Abstract

Living mulch (LM) is a practice in which forages are grown simultaneously with the main crop, serving as a living cover throughout the growing season and, when legumes are used, can decrease the reliance on N fertilizer. The objective of this study was to determine the yield and net returns of corn silage and grain in LM systems with different N fertilizer rates. The study was conducted in Spring Hill, TN in 2020 and 2021. The plots consisted of white clover (Trifolium repens L.) [WC] living mulch (LM) and no LM, with six nitrogen treatments, and three replications in a randomized complete block design (RCBD). Corn silage and grain was seeded to the field and the six treatments were assigned to each experimental unit: [1] no LM and no N, [2] no LM + 120 lbs ac⁻¹ N, [3] LM + no N, [4] LM + 40 lbs ac⁻¹ N, [5] LM + 80 lbs ac⁻¹ N, and [6] $LM + 120 lbs ac^{-1} N$. The partial net returns in 2020 were greater in treatment 2, while treatments 1 and 6 did not differ. The positive partial net returns were only observed in 2021 in treatment 2. For the grain production, there was a year main effect (P < 0.0001) with 2020 having lower partial net return losses than 2021; and in both years, the net returns were negative for all treatments. It was concluded that LM requires greater investments and generates lower returns than conventional systems.

Introduction

Corn producers are facing historically high fertilizer prices, and there is a need to find innovative ways to lower fertilizer costs. A growing strategy for the issue is the use of living mulch (LM) systems. The LM is the cultivation of forages simultaneously with the main crop, serving as a living cover throughout the growing season (Hartwig & Ammon, 2002). Often legumes are adopted in these systems, which could decrease N costs due to its biological N fixation (BNF) and aid its transfer to neighboring species (Enriquez-Hidalgo et al., 2016). The maximum yield that could be acquired from cropping systems, such as corn, cannot be achieved without additional N fertilizer, even with the presence of legume in intercropping (Karpenstein-Machan & Stuelpnagel, 2000); however, LM systems have environmental benefits to the system.

Besides the environmental advantages of LM, such as soil erosion control and weed control (Siller et al., 2016; Sanders et al., 2018), the LM could potentially maintain corn production as in conventional systems. In Tennessee, it is expected a return over costs of \$45.43 per acre of non-irrigated, no-till corn production in 2020 (Smith & Bowling, 2022) in monoculture. Adopting LM would increase costs of planting the LM and potentially lower revenue from reduced yields. However, the LM could potentially lower fertilizer costs relative to conventional systems. An additional benefit would be forage for grazing in the fall and spring when corn is harvested and not yet planted.

Gerhards (2018) studied the use of white clover as LM in corn production, and found that after corn harvest the LM produced a dense canopy which can be utilized for grazing cattle. In addition, when grazed in areas previously used for corn production, the cattle have access to corn stover for consumption (Franzluebbers & Stuedemann, 2014). Hay production is another prospect of LM systems that has yet to be assessed.

112

Alfalfa is commonly utilized for hay; however, given its high price establishment and the risks associated with its cultivation, there is an interest in utilizing white clover as hay. It is cheaper than alfalfa and has great nutritive value for dairy consumption, increasing milk yields (Harris et al., 1997). White clover grazing can provide 33% higher milk production than pastures without clover (Harris et al., 1997). Studies have also observed the use of crimson-clover (*Trifolium incarnatum*) and cereal rye (*Secale cereale*) cover crop (Kumwenda et al., 1993; Andrews et al., 2018) where it led to greater corn yields than white clover LM. Therefore, the analysis of these forages in corn growth must be evaluated for better understanding of its economic effects. The objective of the study is to assess the partial net returns in \$ ac⁻¹ of corn production in LM treatments with different N rates. It was hypothesized that the LM system can bring positive revenues for farmers.

Material & Methods

The research was conducted at the Middle Tennessee Research and Education Center (MTREC) in Spring Hill, TN ($35^{\circ}71'$ N, $86^{\circ}94'$ W) from March 2020 to Sept 2021 (Tables 3.1 and 3.2). Initial soil nutrient levels on the experiment site were pH = 6.5, P = 27 lbs ac⁻¹, K = 77 lbs ac⁻¹, Ca = 2287 lbs ac⁻¹, and Mg = 193 lbs ac⁻¹. The area consists of two well drained soil types with elevation ranging from 540 to 1200 ft. The soil types were Braxton series (fine, mixed, active, thermic Typic Paleudalfs) silt loam soil complex with 0 to 5% slopes, and Maury series (fine, mixed, active, mesic Typic Paleudalfs) silt loam with 2 to 5% slopes (NRCS, 2022).

The plots consisted of white clover (*Trifolium repens* L.) [WC] living mulch (LM) and no LM, with six nitrogen treatments, and three replications, totaling 18 experimental units in a randomized complete block design (RCBD). Each experimental unit was 82 ft by 105 ft. Silage corn and grain were seeded to the field, and the six treatments were assigned to each

experimental unit prior to corn seeding: [1] no LM and no N, [2] no LM + 120 lbs ac⁻¹ N, [3] LM + no N, [4] LM + 40 lbs ac⁻¹ N, [5] LM + 80 lbs ac⁻¹ N, and [6] LM + 120 lbs ac⁻¹ N (Table 1 and 2). The corn production of silage and grain was determined by harvesting the two middle rows of each plot; the length of the plot and numbers of plants per harvested row were counted prior to harvesting, and the weight was recorded for determination of the production ac^{-1} .

Economic analysis

Annual production budgets for each of the six treatments were developed based on The University of Tennessee Field Crop Budgets (Smith & Bowling, 2022), the values of white clover seeds were prorated for 2 years, which is the length of the experiment. The cost of production includes the corn and white clover seed; the fertilizer rate for each treatment; herbicides repair, maintenance, fuel, oil, and filter of the utilized machinery; operating labor and crop insurance as variable expenses; and capital recovery and management labor as fixed expenses (Tables 3 and 4). The partial net returns were determined by assuming a price of silage of \$ 43.60 per ac⁻¹ (Edwards, W., 2018) and the price of grain at \$5.45 per bu ac⁻¹ (NASS¹, Quick Stats, 2022). The corn yield was determined by the experiment conducted in 2020 and 2021 in Spring Hill, TN.

Statistical analysis

Mixed model analyses were performed using the GLIMMIX procedure on SAS 9.4 (Cary, NC). For corn production and partial net revenues, the fixed effects were considered N rates, year and its two-way interaction, with replication as random effects.

114

Results & Discussion

Weather

The mean air temperature during the 2020 growing season (May-Sept) was 2% lower than the 30-year average, and 3% lower in 2021 (Fig. 3.1). In 2020, precipitation was 15% above the 30-year average, and in 2021, 50% above the 30-year average (Fig 1). In June 2021, precipitation was 17% lower than the 30-year average, and although both years had greater total precipitation, its distribution in 2021 affected the corn germination and further development.

Corn production

There was a treatment × year interaction (P = 0.0010) of corn silage yield (Table 3.3). The greatest corn silage production was observed in corn with 120 lbs N ac⁻¹ in 2020 and 2021. In 2020, adding WC LM with 40 or 80 lbs N ac⁻¹ did not differ from the corn with no N and no LM. Meanwhile, N rates and LM addition to the system did not differ in 2021 (Table 3.3). Martin et al. (1999) studying the competition between corn and WC LM observed that LM greatly delayed corn emergence. Therefore, the lack of differences between corn production with N and LM likely shows the interspecies competition.

There was a treatment × year interaction (P < 0.0001) of corn grain yield (Table 3.3). No differences were observed among treatments in 2021; yet in 2020, the greatest yield was observed in Corn + 120 lbs N ac⁻¹, and all the LM treatments were lower than the non-LM treatments whilst not different among themselves (Table 3). According to Moss & Downey (1971), drought also delays silk emergence and can cause embryosac abortion in corn. The result of grain in 2021 reflects the lack of precipitation after planting in June that affected the germination and development of both corn hybrids. Meanwhile, in 2020 it is possible to observe

that the lack of WC can lead to greater corn grain yield; however, the benefits of LM in the plots are lost.

Partial net returns and implications of the LM system

The partial net returns for corn silage presented a treatment × year interaction (P = 0.0010, Table 3.3). Following the yield analysis, the partial net returns in 2020 were greater in corn with 120 lbs N ac⁻¹, while corn with no N and corn with LM + 120 lbs N ac⁻¹ did not differ. In addition, the only positive partial net returns in 2020 were observed in corn with 120 lbs N ac⁻¹. In 2021, there was no partial net return for all the treatments, yet greater losses were observed when corn not only is grown in LM, but uses 120 lbs N ac⁻¹ due to the excessive expenses of the system. For the grain, there was a treatment × year interaction (P < 0.0001). In 2020, lower losses were observed in corn without LM due to lower expenses compared to LM adoption and greater production. In 2021, less differences were observed due to the lack of difference in corn production, yet in both years, corn grain net returns for grain production are not positive.

The use of legume forages in cropping systems often decrease the reliance on N fertilization due to its BNF (Ledgard et al., 2001). However, the maximum yield that could be acquired from cropping systems, such as corn, cannot be achieved without additional N fertilizer, even with the presence of legume in intercropping (Karpenstein-Machan & Stuelpnagel, 2000). Therefore, the utilization of N fertilization for cropping systems is essential, yet economically hefty, especially since urea is often the N source of choice. The silage production showed better results when weather patterns are considered normal, but it is likely that with the high fertilizer prices, producers would still have a negative net return even if the commodity price increases (Huang et al., 2009).

116

Corn is one of the most valuable commodities in the U.S. in agricultural exports, valuing more than 17 billion dollars only in 2021 (USDA¹, 2022), yet according to the USDA agricultural projections, the price of corn is expected to decrease from \$4.53 per bushel to \$4.00 per bushels in 10 years (USDA², 2022). A study conducted by Plastina et al., (2020) studied the use of cover crops in corn-soybean rotations, and it was found that the net returns of the practice in Midwestern states was negative. The LM practices are slightly different from cover crops, but the sustainability goals of the system intersect. Therefore, it is possible to infer that adding LM to the system might increase the sustainability of the system (Lu et al., 2000; Teasdale, 1996); yet, studies about the monetary value of sustainability of the system must be conducted.

Conclusions

It was not possible to accept the hypothesis that LM generates positive revenues in corn production. However, silage production generates lower losses than grain production, yet greater returns are observed for corn grown without LM, likely due to species competition for resources on the field which affects yield and increased price of fertilizer in addition to forage seed. Although no sustainability returns were assessed, the use of LM are known for increasing soil health and decreasing weed biomass on the field. Further studies should assess the possibility of hay production out of the LM before corn planting for selling or feeding to animals within the farming operation.

Appendix

Table 3-1: Variety, plating date, and seeding rates of LM grown in 2020 and 202	1 in Spring Hill,
TN.	

Seed	Variety	Planting date	Rate	Machinery	Harvest	Machinery
White clover (<i>Trifolium</i> <i>repens</i> L.)	Durana	March 7, 2020	2.0 lbs ac ⁻¹	Great Plains 38-cm drill (Manufacturing Inc, Salina, KS)		
Corn silage	Dekalb DKC67-44	June 1, 2020 and June 1, 2021	31,000 kernels	4-row planter (John Deere, Lewisburg,	Aug 19, 2020, and Aug 25, 2021	T100 chopper (New Holland, Racine, WI); feeder mixer (Helm Welding, Lucknow, Ontario). Combine
Corn grain	Croplan 5887VT2P/RIB		per ac ⁻¹	TN)	Oct 14, 2020 and 2021	(Almaco and Allis- chalmers Gleaner K2); Ford F600 grain truck; weigh wagon (Par- Kan).

Chemicals					
Purpose	Date	Name	[†] Rate	Machinery	
Burndown	March 7, 2020	paraquat (Gramoxone SL 2.0, Syngenta, United Kingdom) Surfactant (Surf 80, Cannon packaging Co., Inc.)	0.75 lbs ac ⁻¹ 1.2 oz ac ⁻¹	RM 200 plot sprayer	
3- in lane	May 15, 2020 and May 18,	glyphosate (Cornerstone Plus, Agrisolutions, St. Paul, MN)	0.4 lbs ac^{-1}		
(First spray)	2021	2,4-D ammine 4 (Loveland, Greeley, CO)	0.14 lbs ac^{-1}		
36-in lanes (Second spray)	May 29, 2020 and May 31, 2021	(Paraquat concentrate, Solera, Yuma, AZ),	0.4 lbs ac^{-1}	210 Redball 4-row and 8-	
		atrazine (Atrazine 4L, Drexel, Memphis, TN)	0.5 lbs ac ⁻¹	row hooded sprayer (Wilmar, Benson, MN)	
		s-metolachlor (Charger Max, Agrisolutions, St. Paul, MN)	0.3 lbs ac^{-1}		
		Surfactant (Surf 80, Cannon packaging Co., Inc.)	1.2 oz ac ⁻¹		
Control plots	May 28, 2020	paraquat (Paraquat concentrate, Solera, Yuma, AZ)	1.1 lbs ac ⁻¹		
		Surfactant (Surf 80, Cannon packaging Co., Inc.)	35 ml ha ⁻¹	RM 200 plot sprayer	
	Mara 20, 2021	paraquat (Paraquat concentrate, Solera, Yuma, AZ)	0.4 lbs ac ⁻¹	(AgSpray, Hopkinsville, KY	
	May 20, 2021	Surfactant (Surf 80, Cannon packaging Co., Inc.)	1.2 oz ac ⁻¹		
Rates in active	ingredients				

Table 3-2: Variety, planting date, and seeding rates of LM grown in 2020 and 2021 in Spring Hill, TN.

Table 3.2 (continued): Variety, planting date, and seeding rates of LM grown in 2020 and 2021 in Spring Hill, TN.

Fertilizer Purpose	Date	Name	[†] Rate	Machinery
Ν	June 8 and June 26, 2020, and May 3 and June 30, 2021	Urea	40 lbs ac ⁻¹ , 80 lbs ac ⁻¹ , and 120 lbs ac ⁻¹	Manual application
Р	April 27,2020 May 28, 2021	Superphosphate	73 lbs ac ⁻¹ 68 lbs ac ⁻¹	3-m drop spreader
К	April 22, 2020 and May 25, 2021	K ₂ O	140 lbs ac ⁻¹	(Gandy, Owatonna, MN)

[†]Rates in active ingredients

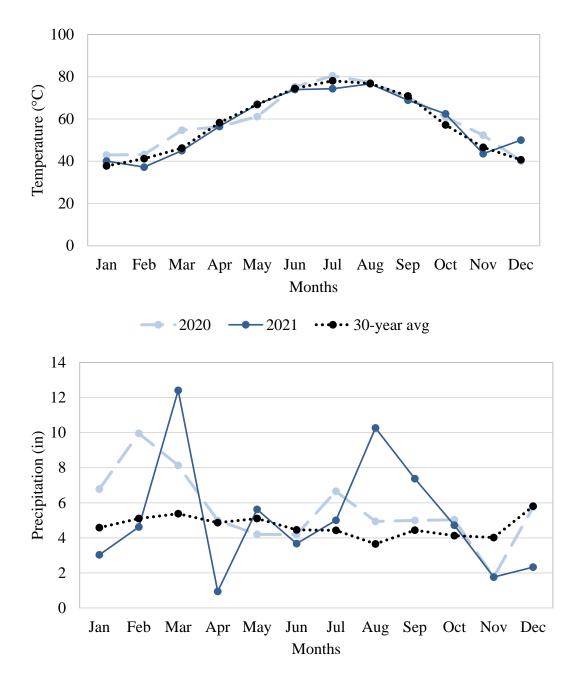


Figure 3-1: Precipitation (in) and temperature (°F) from 2018 to 2021 field preparation and growing season, and 30-year average in Spring Hill, TN

Treatments	Corn + No N	Corn + 120 lbs N	LM with no N	LM with 40 lbs of N	LM with 80 lbs of N	LM with 120 lbs of N	
	Total ($\$$ ac ⁻¹)						
Variable Expenses							
Corn Seed	113.15	113.15	113.15	113.15	113.15	113.15	
[†] White clover seed	-	-	3.06	3.06	3.06	3.06	
Fertilizer	132.48	246.48	132.48	170.48	208.48	246.48	
Chemical	70.07	70.07	70.07	70.07	70.07	70.07	
Repair & Maintenance	25.76	25.76	32.53	32.53	32.53	32.53	
Fuel, Oil, & Filter	7.48	7.48	25.85	25.85	25.85	25.85	
Operator Labor	7.12	7.12	7.47	7.47	7.47	7.47	
Crop Insurance	-	-	-	-	-	-	
Operating interest	5.36	7.84	5.98	6.81	7.64	8.46	
Total variable							
expenses	361.42	477.90	390.60	429.42	468.25	507.08	
Fixed Expenses							
Machinery							
Capital recovery	80.61	80.61	80.61	80.61	80.61	80.61	
Property taxes	-	-	-	-	-	-	
Management labor	7.12	7.12	7.47	7.47	7.47	7.47	
Total fixed expenses	87.73	87.73	87.73	88.08	88.08	88.08	
Total expenses (\$ ac ⁻¹)	449.15	565.63	478.33	517.50	556.33	595.16	

Table 3-3: Annual budget of corn silage production within each assigned treatment with and without living mulch (LM) at different N fertilizer rates in Spring Hill, TN

[†] Cost of white clover seed prorated in 2 years.

	Corn + No	Corn +120 lbs	LM with no	LM with 40 lbs of	LM with 80 lbs of	LM with 120 lbs of
Treatments	Ν	Ν	Ν	Ν	Ν	Ν
Variable Expenses				Total (\$ ac ⁻¹)		
Corn Seed	113.15	113.15	113.15	113.15	113.15	113.15
[†] White clover seed	-	-	3.06	3.06	3.06	3.06
Fertilizer	132.48	246.48	132.48	170.48	208.48	246.48
Chemical	70.07	70.07	70.07	70.07	70.07	70.07
Repair &						
Maintenance	6.69	6.69	13.46	13.46	13.46	13.46
Fuel, Oil, & Filter	5.78	5.78	24.15	24.15	24.15	24.15
Operator Labor	4.72	4.72	5.07	5.07	5.07	5.07
Crop Insurance	0.00	0.00	0.00	0.00	0.00	0.00
Operating interest	4.86	7.34	5.48	6.31	7.13	7.96
Total variable						
expenses	337.75	454.23	366.92	405.75	444.57	483.40
Fixed Expenses						
Machinery						
Capital recovery	45.80	45.80	45.80	45.80	45.80	45.80
Property taxes	-	-	-	-	-	-
Management labor	4.72	4.72	5.07	5.07	5.07	5.07
Total fixed expenses	50.52	50.52	50.87	50.87	50.87	50.87
Total expenses	388.27	504.75	417.79	456.62	495.44	534.27

Table 3-4: Annual budget for corn grain production within each assigned treatment with and without living mulch (LM) at different N fertilizer rates in Spring Hill, TN

[†] Cost of white clover seed prorated in 2 years.

	Corn Silage				
	Yield (US t ac ⁻¹) [‡] Partial net retu		eturns (\$ ac ⁻¹)		
Treatment	2020	2021	2020	2021	
Corn + No N	8.1 ^c	2.8 ^b	-95.11 ^{bc}	-326.06 ^{ab}	
Corn + 120 lbs N	14.5 ^a	7.3 ^a	68.21 ^a	-249.10 ^a	
Corn in [†] LM with no N	4.7 ^d	4.0 ^b	-274.13 ^d	-302.82 ^{ab}	
Corn in LM with 40 lbs of N	7.5 ^c	3.6 ^b	-190.36 ^{cd}	-359.23 ^{bc}	
Corn in LM with 80 lbs of N	7.8 ^c	4.0 ^b	-216.78 ^d	-380.47 ^{bc}	
Corn in LM with 120 lbs of N	12.1 ^b	3.6 ^b	-68.10 ^b	-436.89 ^c	
SEM	0.8		34.5		
<i>P</i> -value	0.001 0.001			001	
		Corn Grain			
	Yield (bu ac ⁻¹)	u ac ⁻¹) Partial net returns (\$ ac		
Corn + No N	53.6 ^b	1.2 ^a	-96.23 ^a	-384.19 ^a	
Corn + 120 lbs N	75.3 ^a	3.5 ^a	-94.1 ^a	-459.65 ^b	
Corn in LM with no N	16.9 ^c	1.0 ^a	-325.84 ^b	-411.17 ^{ab}	
Corn in LM with 40 lbs of N	22.0 ^c	5.3 ^a	-336.51 ^b	-449.21 ^{ab}	
Corn in LM with 80 lbs of N	24.7 ^c	1.8 ^a	-360.64 ^b	-488.63 ^b	
Corn in LM with 120 lbs of N	28.5 ^c	1.3 ^a	-378.83 ^b	-578.29 ^b	
SEM	4.8		28.5		
<i>P</i> -value	< 0.0001		< 0.0001		

Table 3-5: Yield of corn silage and grain in 2020 and 2021 growing seasons, and partial net returns within treatments in Spring Hill, TN

Lowercase superscript letter means difference within a column at $\alpha < 0.05$. [†]LM, living mulch; SEM, standard error of the mean. [‡]Cost of silage per ac⁻¹ established at \$43.60 and cost of grain in bu ac⁻¹ at \$5.45.

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CONCLUSIONS & FUTURE DIRECTIONS

The study of LM in the Southeastern U.S. is a novelty that can improve land use efficiency by incorporating grazing into the system. WC is a more successful LM in weed suppression than CCCR, and overall net returns due to the decrease in the necessity for annual planting. Therefore, CCCR would be best suitable in cover crops systems. In normal weather conditions corn grain and silage produced similar yields, but having WC allowed a greater overall production, likely due to the added N. Although cull cows were utilized for grazing, a slight weight gain was observed in cows grazing in WC pastures.

When assessing the N requirements of WC in LM systems, WC LM does not increase corn silage or grain production when compared to conventional systems. Nutritive value is not greatly affected by the amount of N added, but as the growing seasons progresses the fiber content increases in LM due to the increase in maturity of the forage. The WC LM systems showed greater weed suppression in corn fields, especially in the second year of WC establishment. Yet, it is important to observe that WC LM mass decreases during the growing season because of the shading by corn growsresults incauses LM - senescence. When irregular precipitation patterns are observed, irrigation might be necessary in LM programs especially in the first weeks after corn planting to ensure corn germination and development. Although economic analysis showed benefits of LM use in grazing operations, corn grain production resulted inmore losses than silage corn production.

128

Future studies should focus on evaluation of the sustainability and non-monetary value of the system. It is known that LM can increase biodiversity and soil health in the field, as well as decreasing the reliance on chemical fertilizers and herbicides. Therefore, the use of LM should be evaluated as an holistic approach to sustainable practices in long term production.

VITA

Marcia Quinby was born in Sao Paulo, Brazil. From 2010 to 2015, she attended the Sao Paulo State University and graduated with a Bachelor of Science degree in Animal Science, in which she received the Rotary Club award as best student in the department of animal sciences. In 2018, she completed her master's degree in crop sciences from The University of Tennessee with the mentoring of Dr. Renata Oakes. In 2019, she started her doctoral program at The University of Tennessee under Dr. Renata Oakes. Upon getting her degrees, Marcia participated in the Gamma Beta Phi honor society from 2017 to 2022, and Gamma Sigma Delta since 2020, as well as in efforts to improve the graduate student experience at the Plant Sciences Department by participating as an officer at the Plant Sciences Graduate Student Association.