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Legume Green Manure and Intercropping for High Biomass *Sorghum* Production

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Abstract

Before the advent of cheap, synthetic fertilizers, legumes were commonly used as green manure crops for their ability to fix atmospheric nitrogen (N). A three-year study at Overton, TX examined legume integration into high-biomass *sorghum* (*Sorghum bicolor* L.) production systems on a Lilbert loamy fine sand recently cultivated after a fertilized bermudagrass [*Cynodon dactylon* (L.) Pers.] pasture. In this split-split plot design, 'Dixie' crimson clover (*Trifolium incarnatum* L.) and 'Iron and Clay' cowpea (*Vigna unguiculata* L.) were integrated into a high-biomass *sorghum* production system to evaluate impacts on N concentration, C concentration, and yield of high-biomass *sorghum* and their impacts on soil total N and soil organic carbon (SOC). Main plots were split into crimson clover green manure (CLGM) and winter fallow (FALL) followed by three sub-plots split into warm-season crop rotations: cowpea green manure (CPGM), cowpea-*sorghum* intercrop (CPSR), and *sorghum* monocrop (SORG). Three N fertilizer treatments (0, 45, 90 kg $N \cdot ha^{-1}$) were randomized and applied as sub-sub plots. The CLGM increased ($P < 0.01$) *sorghum* biomass yield (16.5 t $DM \cdot ha^{-1}$) 28% in year three but had no effect in the first two years. The CPSR treatment reduced *sorghum* yield up to 62% compared to SORG; whereas CPGM increased *sorghum* yield 56% and 18% the two years following cowpea incorporation. Rate of N fertilizer had no effect on *sorghum* biomass yield. Decrease in SOC and soil N over time indicated mineralization of organic N and may explain why no N fertilizer response was observed in *sorghum* biomass yield. Cowpea showed strong potential as a green manure crop but proved to be too competitive for successful intercropping in high-biomass *sorghum* production systems.

Keywords

High-Biomass *Sorghum*, Legumes, Green Manure, Intercrop, Cowpea, Crimson Clover, Soil Organic Carbon, Soil Nitrogen

1. Introduction

Bioenergy crops have attracted much attention in recent years in the U.S. in an attempt to replace or supplement the supply of fossil fuels. Dependency on foreign oil supplies and rising oil prices has fueled the search for alternative energy sources. Cellulosic bioethanol, for example, has been one of many options investigated [1] [2] [3], and as a result, bioenergy crops are becoming more present in US cropping systems. The remarkable genetic diversity of *sorghum* has been utilized by plant breeders in recent years [4] [5], and this crop has received considerable attention as a potential biofuel feedstock. When grown in higher latitudes, photoperiod-sensitive cultivars extend vegetative growth and maximize biomass yield. These high-biomass producing *sorghum*s are under advanced evaluation for improved yield with current biomass production exceeding 30 t $DM{\cdot}ha^{-1}$ [6] [7]. High dry matter (DM) yields using limited inputs under warm, dry climates make high-biomass *sorghum* an attractive bioenergy crop for East Texas and the humid Southeastern USA.

Several soil problems may arise with the removal of plant biomass for bioethanol production. In general, removing the majority of the plant biomass disrupts known mass balances of C and plant nutrients compared to traditional cropping systems. Essential nutrients contained within the plant material, which would otherwise be returned to the soil and slowly released during decomposition, are removed from the system [8]. Similarly, fixed plant C is removed, which is detrimental to the maintenance of soil organic C (SOC). Studies by Havlin *et al.* [9] and Mazzoncini *et al.* [10] showed a direct link between the addition of plant residue and SOC. The inclusion of green manure or intercrops into rotations might mitigate SOC losses by returning additional plant biomass to the soil.

Legumes can serve as viable green manures and intercrops because they not only add additional C to the soil but have the added benefit of contributing fixed N to the cropping systems [11]. With the help of Rhizobium bacteria, symbiotically fixed N can provide the majority of legume N requirements. Legume N can then be transferred directly to the soil through root exudates and root senescence, or legume biomass can be incorporated into the soil as a green manure and reduce input costs by replacing N fertilizer requirements of non-leguminous crops [12] [13] [14] [15].

Cool-season annual legumes planted in the fall can contribute organic N prior to typical planting dates of summer crops [16] [17] [18] [19]. Crimson clover is a cool-season legume that was shown to replace up to 120 kg $N{\cdot}ha^{-1}$ of fertilizer for grain *sorghum* on a sandy loam soil in Georgia [20]. In a study conducted in North Carolina, Wagger [21] reported that a subsequent corn (*Zea mays* L.) crop used approximately one third of the N contributed by crimson clover and hairy vetch (*Vicia villosa* Roth). Though N use efficiency may appear small in the first year, up to a third of legume N may be stored in more recalcitrant organic matter [13] and mineralized in later growing seasons, thereby building long-term soil N status.

Warm-season annual legumes can contribute large amounts of biomass and N [22] [23]; however, they must be rotated for green manure or intercropped with primary warm-season crops. Production limitations can occur for primary crops due to the loss of cropping seasons when rotated or through competition for limited water and nutrient resources when intercropped [24]. Neely *et al.* [11] found that clover green manure was a more suitable choice when followed by grain *sorghum* (*Sorghum bicolor* L.) compared to intercropping with cowpeas from both a yield and soil perspective. In fact, cowpea produced deeper roots, maintained higher transpiration rates, and performed better under low soil fertility than maize when intercropped together on a sandy loam soil in Brazil [24].

Due to the N and C contributions of legumes, the primary goal of this research was to identify the best method for legume integration into high-biomass *sorghum* production systems to enhance sustainability of biomass production. Based on previous research by Evers *et al.* [25] and Rouquette *et al.* [26], crimson clover and cowpea were identified as the best adapted crops to represent cool- and warm-season annual legumes, respectively, for the Pineywoods Vegetative Region of East Texas as described by Gould [27]. Research objectives were to 1) maximize yield of high-biomass *sorghum* at reduced inorganic N fertilizer rates using leguminous N sources; and 2) generate a net increase in soil total N and SOC in the top 60 cm of the soil profile over three cropping years using crimson clover green manure and cowpea intercrop or green manure treatments.

2. Methods and Materials

2.1. Site

This experiment was conducted at the Texas A&M AgriLife Research and Extension Center at Overton, TX (32˚17'N 94˚58'W) on a Lilbert loamy fine sand (loamy, siliceous, semiactive, thermic Arenic Plinthic Paleudult). Land was previously managed as a fertilized, permanent bermudagrass pasture until cultivation in preparation for this study. Initial soil pH was 5.02 and 4.18 at 0 to 15 and 15 to 30 cm depths, respectively. Based on initial soil samples taken August 14, 2009, soil was limed (9.2 $t \cdot ha^{-1}$ ECCE 100 limestone) and fertilized with 337 $kg \cdot ha^{-1}$ of broadcast applied 0-60-60 N-P_2O_5-K_2O to meet nutrient requirements prior to initiating the study in fall 2009. Seven months following lime application, pH was 6.93 in the top 0 to 15 cm. Weather data showed below normal rainfall for part or all of the growing season in 2010 and 2011, while 2012 received near normal precipitation (**Figure 1**). The first year appeared to have plentiful rainfall; however, approximately 50% of the precipitation from May through August occurred in only two rain events and was not well distributed through the summer growing season. Temperatures were much above normal during a severe drought in 2011 (**Figure 2**).

2.2. Field Design

The field design was a four replicate, split-split plot design. The main plot effect was split into a cool-season legume ("Dixie" crimson clover) used as a green manure (CLGM) or winter fallow (FALL) and was followed by a rotation effect involving a warm-season legume ("Iron and Clay" cowpea) as the sub-plot. During 2010, the cropping rotation treatments consisted of a cowpea green manure crop (CPGM), a cowpea-*sorghum* ("ES 5200") intercrop (CPSR), and

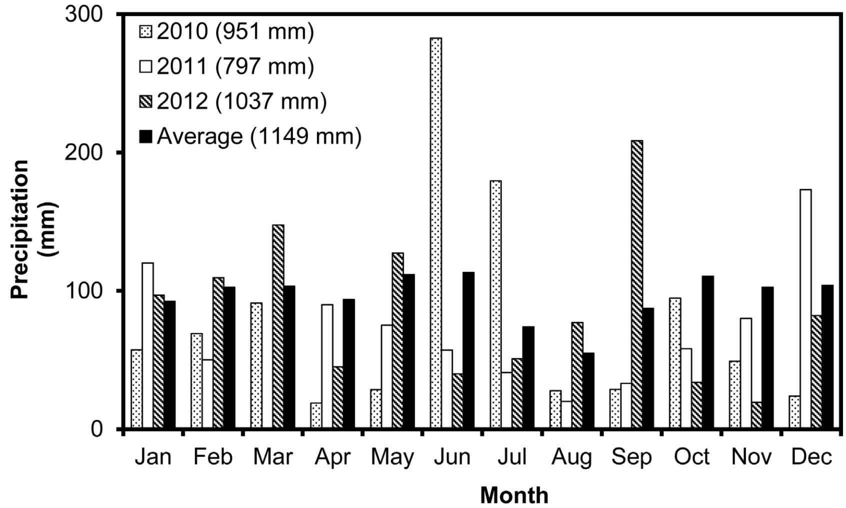

Figure 1. Monthly precipitation for Overton, TX for the three years of the study and the 30-year precipitation average.

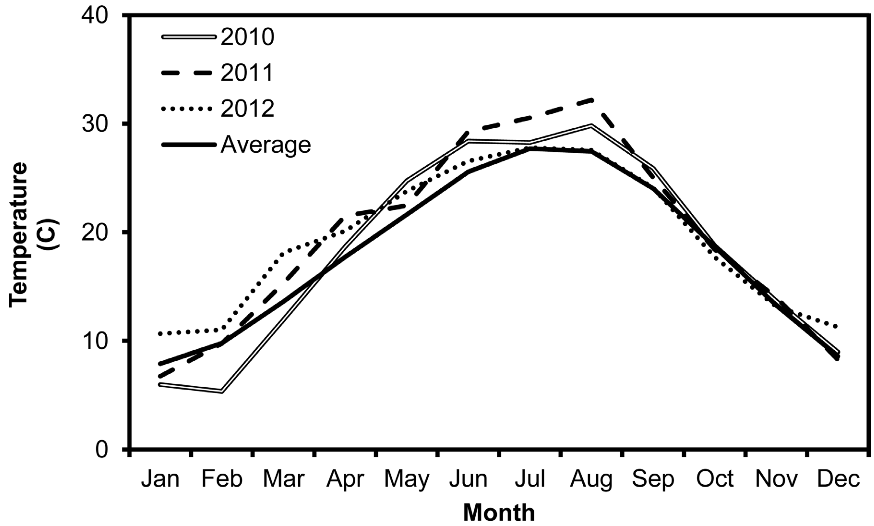

Figure 2. Average monthly temperature for Overton, TX for the three years of the study and the 30-year average monthly temperature.

sole-cropped *sorghum* (SORG). In subsequent years, the previous CPGM plots were planted to sole-cropped *sorghum*, while the initial CPSR and SORG treatments remained the same. Three inorganic N fertilizer rates (0, 45, 90 kg·ha^{-1}) were randomly applied over SORG and CPSR treatments using a granular urea/ammonium sulfate blend (33-0-0 N-P-K). Cowpea green manure treatment did not receive N fertilizer in 2010 but received all three N treatments in subsequent years. Nitrogen fertilizer was hand-broadcasted 15, 19, and 30 days after *sorghum* planting in 2010, 2011, and 2012, respectively. Plot size was 3 m by 3 m.

All crops were planted using a 3 m wide double-disk planter. Crimson clover was planted on a prepared seedbed at 28 kg·ha^{-1} on November 12, November 11, and October 31 in 2009, 2010, and 2011, respectively. The clover was incorporated for green manure impact on April 22, April 13, and March 23 in 2010, 2011, and 2012, respectively, using a rototiller and followed by a roller-packer in soil preparation of *sorghum* and cowpea planting. Prior to incorporation in the spring, a 0.09 m^2 quadrat of clover shoot biomass was hand clipped from each plot each year, and in 2012, roots were dug by shovel (approximately 30 cm depth) to estimate root:shoot ratios. Plant samples were dried at 60ºC to determine DM yield, and later used for C and N concentration analyses.

Sorghum was planted at 5 kg seed·ha^{-1} (160,550 seed·ha^{-1}), and cowpea was planted at 56 kg seed·ha^{-1} as soon as soil moisture was sufficient each year on June 14, 2010, May 6, 2011, and April 9, 2012. *Sorghum* and cowpea plots were terminated on October 21, September 22, and October 3, respectively, in 2010, 2011, and 2012. A 0.46 m^2 quadrat of *sorghum* and cowpea above-ground biomass was hand-harvested from each plot to determine DM mass. *Sorghum* and cowpea root samples were dug (approximately 30 cm depth) in nine selected plots to estimate root:shoot ratios and C and N concentration, but not analyzed for treatment effects. All plant samples were ground to pass through a 1-mm sieve and combustion analyzed [28] for C and N. Stand counts were determined for *sorghum* and cowpea by averaging the number of plants in a linear meter of the two center rows of each plot. All above-ground biomass was removed from plots following sampling except for the annual clover and one-time cowpea green manure treatments, which were mechanically shredded and rototilled into the top 15 cm of soil.

2.3. Soil N and C

Four soil cores (22-mm diameter) were taken from each treatment plot each December. Soil samples were divided into 0 to 15, 15 to 30, and 30 to 60 cm depths, air dried (60˚C), ground (840 μm), and processed using combustion analysis [28] to track total N and total C levels in the soil throughout the duration of the project. Total C was assumed to be equivalent to SOC due to the highly weathered nature of the soil and depletion of carbonates. A fizz test using 10% HCl was negative and confirmed inorganic C in the soil was absent or below detectable levels. Four soil cores were taken and separated by depth in the same manner on March 23, 2012 to estimate soil moisture. Samples were weighed, air dried at 60˚C, and reweighed to calculate gravimetric water content and then converted into volumetric water content using bulk density.

2.4. Data Analysis

All data were analyzed using GLIMMIX model of SAS 9.2 [29]. Cool-season crop treatments, summer crop rotation treatments, and N rate treatments were considered fixed effects with year and replication designated as random effects. Proc Univariate indicated data did not meet the normality assumption, so data were analyzed by year using a square root transformation. All individual treatment comparisons ($P < 0.05$) were made using LSD mean separation.

3. Results

3.1. Cool-Season Green Manure

Dixie crimson clover had the highest yield (4.59 t DM·ha^{-1}) in 2012 but produced only half as much (2.30 t DM·ha^{-1}) in 2010, and less than a third (1.38 t DM·ha^{-1}) of that during an extreme drought in 2011 (**Table 1**). Recovered roots in 2012 indicated belowground biomass production was equivalent to only 5 to 6% of aboveground biomass with approximately 21.7 g N·kg^{-1} and 432 g C·kg^{-1} root tissue.

The CLGM increased ($P = 0.01$) *sorghum* biomass yield one out of three years in this study (**Table 2**). This coincided with the year (2012) that exhibited a rainfall distribution closest to the average for Overton, TX and the highest clover yield. It is possible that the effect of CLGM on *sorghum* biomass yield simply required three years to manifest and is part of a long-term trend. *Sorghum* biomass yield was not impacted by the CLGM in lower rainfall years despite visual appearance of moisture depletion at the time of *sorghum* planting. In some cases, cool-season green manure crops will decrease yield of primary crops because of its effect on soil moisture. For instance, Ewing *et al.* [30] observed a significant decrease in soil moisture following a crimson clover green manure compared to

Table 1. Biomass yield, nitrogen (N) concentration, carbon (C) concentration, nitrogen yield, and carbon yield of crimson clover green manure crop in Overton, TX.

Year	Biomass		N Concentration		C Concentration		Total N Yield		Total C Yield	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
	t DM·ha^{-1}		g·kg^{-1}		g·kg^{-1}		kg·ha^{-1}		kg·ha^{-1}	
2010	2.30b*	--	24.8a	--	415.9b	--	57b	--	957b	--
2011	1.38c	--	25.4b	--	415.6b	--	35c	--	574c	--
2012	4.59a	0.26	31.2c	21.7	428.6a	431.9	143a	6	1967a	112

*Different letters represent significant differences ($P < 0.01$) between years for each component.

Table 2. Total *Sorghum* and Cowpea yields over three years by three treatments.

Year	Treatment	Biomass Yield (t DM·ha^{-1})			N Concentration (g N·kg^{-1})		C Concentration (g C·kg^{-1})		Total N Yield (kg N·ha^{-1})		Total C Yield (kg C·ha^{-1})	
		Sorghum	Cowpea	Total	*Sorghum*	Cowpea	*Sorghum*	Cowpea	*Sorghum*	Cowpea	*Sorghum*	Cowpea
Warm-season crop												
2010	SORG†	17.6 a*	---	17.6 a	8.8 a	---	452 b	---	152 a	---	7940 a	---
	CPSR	7.3 b	5.6 a	12.9 b	8.5 a	17.9 a	454 a	440 a	61 b	102 a	3307 b	2465 a
	CPGM	---	5.9 a	5.9 c	---	18.9 a	---	438 a	---	112 a	---	2568 a
2011	SORG	5.9 b	---	5.9 b	18.2 a	---	425 ab	---	104 b	---	2485 b	---
	CPSR	3.6 c	0.8	4.3 c	18.7 a	21.9	419 b	420	64 c	17	1489 c	319
	CPGM	9.2 a	---	9.2 a	19.0 a	---	428 a	---	172 a	---	3952 a	---
2012	SORG	17.3 b	---	17.3 b	9.0 b	---	458 b	---	153 a	---	7936 a	---
	CPSR	6.5 c	4.8	11.3 c	10.9 a	23.3	466 a	449	69 b	113	3004 b	2169
	CPGM	20.4 a	---	20.4 a	8.7 b	---	457 b	---	177 a	---	9351 a	---
Winter crop incorporation												
2010	FALL‡	12.4 a	5.7 a	15.2 a	8.5 a	17.8 a	453 a	440 a	104 a	104 a	5598 a	2503 a
	CLGM	12.5 a	5.8 a	15.3 a	8.8 a	19.0 a	453 a	439 a	109 a	110 a	5649 a	2529 a
2011	FALL	6.5 a	0.8 a	6.7 a	17.7 b	21.8 a	425 a	420 a	111 a	19 a	2747 a	340 a
	CLGM	6.0 a	0.7 a	6.2 a	19.6 a	22.0 a	423 a	419 a	115 a	16 a	2537 a	298 a
2012	FALL	12.9 b	4.9 a	14.5 b	8.4 b	23.3 a	463 a	449 a	100 b	114 a	5958 b	2209 a
	CLGM	16.5 a	4.8 a	18.1 a	10.6 a	23.2 a	458 a	449 a	166 a	112 a	7569 a	2129 a
Level of N fertilizer												
2010	0N§	11.7 a	5.9 a	14.7 a	8.4 a	18.6 a	452 a	439 a	98 a	112 a	5289 a	2595 a
	45N	13.2 a	4.7 a	15.6 a	8.6 a	17.5 a	453 a	440 a	113 a	84 a	5971 a	2080 a
	90N	12.4 a	6.0 a	15.4 a	8.8 a	18.4 a	452 a	440 a	110 a	111 a	5611 a	2638 a
2011	0N	7.3 a	0.9 a	7.6 a	15.4 c	21.7 a	428 a	418 a	115 a	20 a	3116 a	363 a
	45N	5.4 b	0.6 a	5.7 b	18.9 b	21.1 a	423 a	420 a	103 a	13 a	2308 b	336 a
	90N	5.9 b	0.8 a	6.2 b	21.6 a	23.0 a	421 a	421 a	122 a	19 a	2501 b	258 a
2012	0N	14.9 a	4.8 a	16.5 a	9.2 b	23.3 a	459 a	450 a	127 a	107 a	6844 a	2145 a
	45N	13.9 a	4.6 a	15.5 a	9.7 a	23.5 a	460 a	448 a	129 a	108 a	6377 a	2061 a
	90N	15.3 a	5.1 a	17.0 a	9.7 a	23.0 a	461 a	449 a	143 a	122 a	7070 a	2301 a

†Cropping rotation treatments of *sorghum* monocrop (SORG), cowpea-*sorghum* intercrop (CPSR), and cowpea green manure (CPGM) were averaged over cool-season crop treatments and nitrogen rates, except CPGM 2010, which only received 0 kg·ha^{-1} of nitrogen fertilizer. ‡Cool-season crop treatments of fallow (FALL) and clover green manure (CLGM) were averaged over all crop rotations and nitrogen rates except in 2010 when they were averaged over SORG and CPSR only. §Nitrogen fertilizer rates of 0, 45, 90 kg N·ha^{-1} were averaged over all crop rotations and cool-season crop treatments except in 2010 when they were averaged over SORG and CPSR only. *Different letters represent significant differences ($P < 0.05$) between treatments within each year.

fallow ground on a loamy sand soil, which resulted in 27% yield suppression in corn.

Soil moisture was measured in 2012 at the time of clover incorporation (March 23) and revealed higher ($P < 0.01$) moisture content in CLGM plots than FALL. This was likely due to a recent rain event the previous day. Clover roots enable preferential flow and reduce run off, while foliage slows evaporative losses from the soil surface as described in reviews by Dabney [31] and Unger and Vigil [32]. Temporal proximity of the soil sampling to the rain event did not allow enough time to reflect moisture loss through clover transpiration and resulted in a 2.5%-unit (4 mm) increase ($P < 0.01$) in volumetric soil moisture in the top 15 cm of the soil profile. No differences were observed at the 15 to 30 cm depth.

Compared to FALL, CLGM decreased initial stand counts of *sorghum* (7.8 plants linear·m^{-1}; $P < 0.01$) and cowpea (16.6 plants linear·m^{-1}; $P = 0.04$) in 2012 by 45% and 44%, respectively, at 37 days after planting; however, this stand reduction did not negatively impact overall biomass yields. Because soil moisture was near field capacity, the decrease in stand may have resulted from uneven seed bed conditions due to the large amount of clover biomass incorporated into the soil. Alternatively, the CLGM may have increased the incidence of pests and diseases that attack emerging seedlings and reduce stands of primary crops as illustrated by Dabney *et al.* [33] However, detection of pests and disease was beyond the scope of this study and no documentation was made insofar as effects on stand

3.2. Warm-Season Crop Rotation

Cowpea biomass was 5.55 t DM·ha^{-1} in 2010 and 4.83 t DM·ha^{-1} in 2012 under the CPSR rotation (**Table 2**). Rotation treatment did not affect cowpea biomass production in 2010. Comparable biomass yields were found for Iron and Clay by Harrison *et al.* [34], Muir *et al* [28], and in a previous study on Lilbert soils at Overton by Rouquette *et al* [26]. Cowpea root biomass was approximately equal to 18% and 11% of aboveground biomass in 2010 and 2011, respectively, and contained 19.4 g N·kg^{-1} and 433 g C·kg^{-1} on average. Drought conditions lowered biomass yield to 0.76 t DM·ha^{-1} in 2011. Adding 90 kg of N fertilizer in 2012 increased ($P = 0.05$) cowpea biomass 56% compared to 45 N following FALL but was not significantly different from the control (0 N). Nitrogen fertilizer rates had no significant effect on cowpea biomass following CLGM in 2012, suggesting mineralized N from clover and from symbiotic N fixation was meeting cowpea growth requirements. Biomass response in cowpeas based on N fertilizer varies widely throughout the literature and is dependent on both environment and cultivar [35] [36]. Cowpeas are unable to symbiotically fix N in their roots during the seedling stage, and small amounts of N fertilizer at planting can enhance yield [37]. However, cowpea yield may also positively respond to N fertilizer as late as early-bloom [38] [39]. When available, legumes may assimilate soil N instead of sacrificing energy to fix N symbiotically. In some cases, N fertilizer has no effect on biomass yield [40].

Cowpea N and C concentrations of plant material did not differ for CLGM or N fertilizer treatments unlike findings by Hasan *et al.* [37] where cowpea protein concentration increased with only 25 $kg \cdot ha^{-1}$ of N fertilizer. However, cowpea plant N concentration was higher under drought conditions in 2011 (21.9 $g \cdot kg^{-1}$) than 2010 (18.2 $g \cdot kg^{-1}$) (**Table 2**). Rouquette *et al.* [41] found a similar N concentration in cowpea biomass equivalent to 19.7 $g \cdot kg^{-1}$. Crops often accumulate nitrate under drought stress. Cowpeas were likely obtaining all or most of their N in 2011 from the applied fertilizer and not symbiotic fixation with root nodules, which is inhibited under drought conditions [42] [43]. Regardless of N concentration, cowpea C concentration was lower ($P < 0.0001$) in 2011 (419.5 g kg) than in 2010 (439.4 g kg), suggesting the dry conditions affected processes related to photosynthesis and production of carbohydrates (**Table 2**).

On average, SORG yielded 58% more ($P < 0.01$) *sorghum* biomass than CPSR each year (**Table 2**). The additional biomass contributed by the cowpea in CPSR did not compensate for the reduction of *sorghum* yield and resulted in a net loss of total biomass yield. Neely *et al.* [16] in a comparison study reported that intercropped cowpea with grain *sorghum* resulted in a 77% reduction in grain yield compared to mono-cropped grain *sorghum*. Research by Riday and Albrecht [44] and Reda *et al.* [45], however, at their locations showed no effect on biomass yield of maize or *sorghum*, respectively, when intercropped with warm-season legumes, and Geren *et al.* [46] found no advantage in total biomass production of a maize-cowpea intercrop. Aboveground cowpea biomass from the CPGM rotation in 2010 contributed 105 kg $N \cdot ha^{-1}$ when incorporated. In the CPGM rotation, sole-*sorghum* yield following the cowpea green manure was 58% and 18% higher in 2011 and 2012, respectively, compared to SORG. Legume green manure crops commonly enhance yield of subsequent non-legume crops [47] [48] [49]. During the drought of 2011, CLGM increased N concentration of *sorghum* (19.6 $g \cdot kg^{-1}$, $P = 0.03$) compared to FALL (17.7 $g \cdot kg^{-1}$), as did CPGM (16.7 $g \cdot kg^{-1}$, $P = 0.02$) compared to the other rotation treatments (14.6 $g \cdot kg^{-1}$) at the 0 N rate. Average *sorghum* root production (2.5 t $DM \cdot ha^{-1}$) was estimated at 22% and 39% of above-ground biomass production in 2010 and 2011, respectively, which contributed 251% more total root biomass to the soil than cowpea.

3.3. Nitrogen Fertilizer

Interestingly, N fertilizer had no effect on *sorghum* biomass production throughout this study. In comparison, Hons *et al.* [8] reported a 40% increase in high-biomass *sorghum* yield with the application of 86 kg $N \cdot ha^{-1}$ in College Station, TX. Several reasons may have contributed to the lack of N response including field history, weather, and timing of N application [50]. Prior to this study, soil tests of the fertilized bermudagrass pasture revealed SOC and total soil N levels of 14.3 g·kg^{-1} and 1.4 g·kg^{-1}, respectively, in the top 0 to 15 cm of soil. The initial soil test also revealed nitrate-N levels of only 8 ppm, which suggested most of the soil N was present in unavailable organic compounds. The soil was tilled twice each year after cool-season and warm-season crops, which aerated the soil and accelerated SOC mineralization which released organically bound N. In Lubbock, TX, high-biomass *sorghum* did not respond to N fertilizer in the first of a two-year study due to elevated residual NO_3-N (140 kg N·ha^{-1} in top 0.9 m soil profile) from a previous cotton crop [51].

The single N application at the beginning of the growing season could also have led to large environmental losses through leaching and volatilization. Urea-based fertilizers routinely lose approximately 32% - 35% of total N [52] [53] and up to 47% under certain conditions [54]. In 2010, Overton received 200 mm of rain in the week immediately following N application which could have resulted in large N losses from leaching. The opposite problem in 2011 may have led to large losses from volatility due to broadcast application of the urea blended fertilizer combined with extremely hot, dry conditions.

3.4. Soil N and C

Prior to this study, field management consisted of a permanent, fertilized bermudagrass pasture for several decades. Under this management SOC was 14.3 and 3.2 g C kg·soil^{-1}, and total soil N was 1.4 and 0.3 g N kg·soil^{-1} at 0 to 15 and 15 to 30 cm depths (**Figure 3** and **Figure 4**), respectively. As a result, soil total N and SOC both dropped during the first year of the study due to tillage. The decrease was more severe in the 0 to15 cm depth (**Figure 3**) where the highest SOC was located and the most soil disturbance occurred. Franzluebbers and Stuedemann [55] showed a similar drop in SOC following tillage of a bermudagrass pasture. However, no significant changes were observed at this depth for SOC (11.3 g·kg^{-1}) or soil N (1.0 g·kg^{-1}) from 2010 to 2011. In 2010, SORG rotation following CLGM appeared to decrease both SOC ($P = 0.08$) and soil N ($P = 0.12$) by 8% compared to SORG after FALL.

At 15 to 30 cm depth, SOC was 21% higher for SORG (3.0 g·kg^{-1}, $P = 0.05$) over CPGM (2.4 g·kg^{-1}) when comparing treatments at 0 N after the 2011 growing season (**Figure 4**). *Sorghum* produces more root biomass than cowpea and therefore contributes more organic C to the SOC pool. Less root biomass production may explain the lower SOC at this depth for the CPGM rotation in 2011. Havlin *et al.* [9] also correlated an increase in SOC to higher biomass contributions under a *sorghum*-only rotation compared to a soybean-only rotation. However, CPGM contributed approximately 2498 kg C·ha^{-1} to the top 0 to15 cm of soil in 2010 and still had no significant impact on SOC. Cowpea biomass did have a relatively low C:N ratio (23:1) in 2010, and much of its biomass was likely decomposed quickly by soil organisms and lost as CO_2. Often, green manure crops take many years to improve SOC.

Average SOC continued to drop ($P = 0.03$) after two years at the 30 to 60 cm

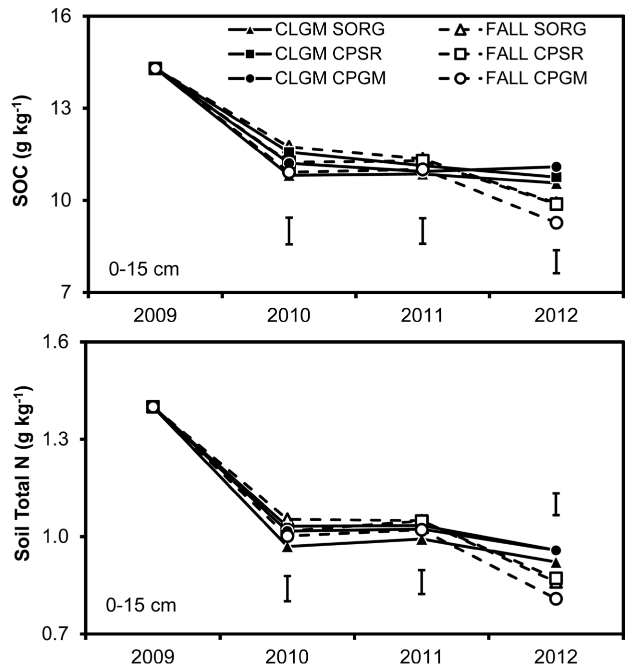

Figure 3. Effect of cool-season crop treatment (clover green manure—CLGM; fallow—FALL) and summer crop rotation (cowpea green manure—CPGM; cowpea-*sorghum* intercrop—CPSR; *sorghum* monocrops—SORG) on soil organic C (SOC) and soil total N at soil depth of 0 - 15 cm. Bars represent LSD values. CPGM did not receive the same N treatments as CPSR and SORG in 2010, and thus the LSD does not apply to those data points.

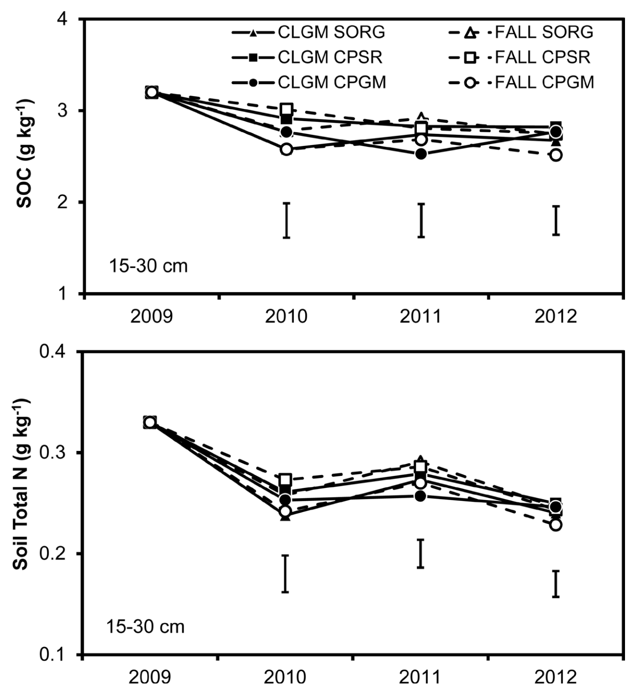

Figure 4. Effect of cool-season crop treatment (clover green manure—CLGM; fallow—FALL) and summer crop rotation (cowpea green manure—CPGM; cowpea-*sorghum* intercrop—CPSR; *sorghum* monocrops—SORG) on soil organic C (SOC) and soil total N at soil depth of 15 - 30 cm. Bars represent LSD values. CPGM did not receive the same N treatments as CPSR and SORG in 2010, and thus the LSD does not apply to those data points.

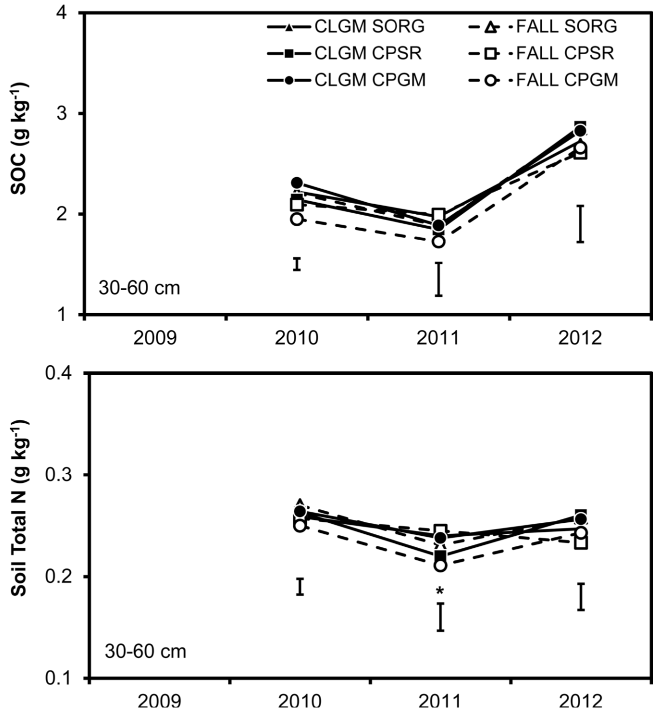

Figure 5. Effect of cool-season crop treatment (clover green manure—CLGM; fallow—FALL) and summer crop rotation (cowpea green manure—CPGM; cowpea-*sorghum* intercrop—CPSR; *sorghum* monocrops—SORG) on soil organic C (SOC) and soil total N at soil depth of 30 - 60 cm. Bars represent LSD values, and asterisks signify significant difference between treatments *P* = 0.05). CPGM did not receive the same N treatments as CPSR and SORG in 2010, and thus the LSD does not apply to those data points.

depth and was unaffected by any treatments (**Figure 5**). However, in the third year there was a trend for increased SOC for all treatments. Soil total N also decreased overall ($P = 0.02$) in 2011, but CPGM (0.21 g·kg^{-1}) was 16% lower ($P = 0.02$) than CPSR (0.25 g·kg^{-1}) following FALL. Cowpeas in the CPSR rotation produced taproots which likely extended further down into the soil profile than the *sorghum* roots in the CPGM rotation and may have resulted in the higher soil N. Soil total N and SOC may have continued to drop overall in 2011 if root production was not enough to offset soil respiration of SOC at that depth. Leaching of soluble SOC and mineralized N was likely not a problem due to the low rainfall in 2011.

4. Conclusions

Iron and Clay cowpea was very well adapted to the Pineywoods region of Texas and shows potential as a green manure crop. However, cowpeas proved to be too competitive for successful intercropping in high-biomass *sorghum* production systems; thus, intercropping cowpeas with *sorghum* was not a recommended cropping system in this vegetative region. Despite these results, warm-season annual legume species other than Iron and Clay cowpeas may better complement an intercropped system using high-biomass *sorghum*. The 2010 season of cowpea green manure (CPGM) increased ($P < 0.05$) *sorghum* yields in 2011 and 2012 by 5% and 18%, respectively. Under near-normal rainfall conditions, Dixie crimson clover was able to increase *sorghum* biomass yield as a cool-season green manure. However, there were no significant effects of N fertilizer treatments on *sorghum* yield, suggesting significant amounts of residual N remaining from the previously existing bermudagrass pasture. Declining levels of SOC and soil N over the course of the study indicated mineralization of organic matter and mining of N from the soil by *sorghum*. Dry weather and timing of N application may also explain why no N response was observed in biomass yield. As SOC reaches a new equilibrium with the current tillage system and N mineralization lessens, *sorghum* biomass would likely begin to respond to N fertilizer. Grasses such as high biomass *sorghum* require N from soil and/or inorganic fertilizer rates that may exceed 100 $kg \cdot ha^{-1}$ for desired production. Neither CPGM nor CLGM were able to increase SOC or soil total N after three years for the Lilbert soil in East Texas. Because plant residue contributions greatly impact SOC, the severe drought in 2011 which reduced overall biomass production by over half may have delayed any detectable effects on SOC and soil N. Because clover can be rotated annually, unlike cowpea green manure, it would contribute the most C and N to the soil over time and likely have the most beneficial effects on SOC and soil total N. High-biomass *sorghum* production was optimized at reduced rates of inorganic fertilizer N using legumes. Thus, a net increase in total soil N and SOC in the top 60 cm of the soil profile was generated during a 3 yr period. Longer term research would be helpful in revealing effects of various annual legumes on yield and soil management recommendations for high-biomass *sorghum* cropping systems in this region of Texas.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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