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Switchgrass yield and stand dynamics from legume intercropping based on seeding rate and harvest management

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Abstract: Intercropping legumes may reduce inputs and enhance sustainability of forage and feedstock production, especially on marginal soils. This approach is largely untested for switchgrass (*Panicum virgatum* L.) production, yet producer acceptance should be high given the traditional use of legumes in forage/agricultural systems. Our objectives were to evaluate three cool-season and two warm-season legumes and their required densities to influence yield and supply nitrogen (N) compared to three inorganic N levels (0, 33, and 66 kg N ha^{-1} [0, 30, and 60 lb N ac^{-1}]) at three locations in Tennessee (Knoxville [Sequatchie Silt Loam], Crossville [Lilly Loam], and Milan [Loring B2 Series]). Fall of 2010 seeded, cool-season legumes (red clover [*Trifolium pratense* L.], hairy vetch [*Vicia villosa* L.], ladino clover [*Trifolium repens* L.]), arrowleaf clover (*Trifolium vesiculosum* L.), and a spring of 2011 seeded, warm-season legume (partridge pea [*Chamaecrista fasciculate* L.]) were interseeded into switchgrass at three (high, medium, and low) seeding rates each in two experiments. Harvest treatments were annual single, postdormancy biofuel (Experiment One) or integrated forage-biofuel (preanthesis and postdormancy; Experiment Two). Year one yield impacts were minimal. During the second harvest year, legumes increased yield versus Year 1; in general, yields for 33 and 67 kg N ha^{-1} did not differ from those for red clover, hairy vetch, ladino clover, or partridge pea ($p < 0.05$). Arrowleaf clover yields were not different from 0 kg N ha^{-1}. Forage biomass yields were generally more responsive to legumes ($p < 0.05$) than the biomass regime. Legume persistence after three years was generally greatest for ladino clover and partridge pea. Forage quality (switchgrass only) in some cases was positively influenced by legume treatments, notably hairy vetch and partridge pea ($p < 0.05$). Intercropping selected legumes in switchgrass may enhance forage quality and yield while reducing nonrenewable inputs, fertilizer costs, and emissions/runoff to air and groundwater.

Key words: biological nitrogen fixation—biomass—forage—legume integration—switchgrass—sustainability

Nitrogen (N) is the principal nutrient required in cropping systems and is applied at a rate of nearly 11 million t (10.8 million tn) of commercial or synthetic N per year in the United States (GAO 2003; USDA 2004). Among all macronutrients, N is the fourth most abundant found in plant tissue and has the greatest impact on soil fertility and subsequent crop productivity (Taiz and Zeiger 2006). Petroleum and natural gas are the primary fuels required for synthetic-N production and therefore exert a strong influence on inorganic-N prices (Pimentel et al. 2008). Thus, increased fossil fuel costs may place pressure on farmers to seek alternative sources of fertilizers, or accept reduced profit margins and compromised economic viability. These realities create challenges to sustainable feedstock production, and reduction of external inputs has important implications for producer profitability (Boyer et al. 2012), carbon (C) balances (Franzluebbers et al. 2000), and environmental sustainability/conservation (McLaughlin and Walsh 1998; Sanderson et al. 2004b).

The main nutrient requirement for switchgrass production is N (Vogel et al. 2002), which is affected by the frequency and timing of harvests, amount of biomass removed, and soil N-mineralization rates (McLaughlin and Kszos 2005; Parrish and Fike 2005). Even under optimal cropping management, plants usually take-up less than 60% of applied fertilizer and often only 40% or less (Sinclair 2006). Nutrient uptake and removal in crops is highly variable within a single year, among years, and among sites, even when N supplies from both the soil and additional fertilizer inputs are adequate (Gastal and Lemaire 2002). The variability and rate of N uptake during development and its effects on yield have many impacts on the quality of biomass and the environment, as well as the overall feasibility of integrated biomass and forage production (Adler et al. 2007). Standard practices for switchgrass have called for at least 50 kg N ha^{-1} (44.6 lb N ac^{-1}) during the year after switchgrass establishment (Year 2), followed by 67 to 100 kg N ha^{-1} (59.6 to 89.6 lb N ac^{-1}) thereafter (Wolf and Fiske 1995; McLaughlin and Walsh. 1998; Mooney et al. 2009).

Because legumes biologically fix N, they have been used as intercrops for centuries and can be grown in tandem with agricultural crops in lieu of synthetic N (Peoples 2009; Graham 2005). Therefore, integration of legumes into switchgrass forage and feedstock production may provide a viable alternative to inorganic N, but data on appropriate species and seeding rates are lacking. If a viable model can be developed for switchgrass systems, producer acceptance should be high given the traditional use of legumes in forage-agricultural systems. Soil fertility and yield can be increased by intercrops because they increase soil organic C (SOC), N, and phosphorus (P) compared with weedy fallows (Tonitto et al. 2006);

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however, the extent of this in switchgrass systems is unknown. An additional benefit of intercrops is that they suppress weed growth and development by competing for soil-water and nutrients. In addition to direct competition for growth resources, intercrops enhance weed seed decay and increase plant residue (Adler and Chase 2007; Conklin et al. 2002). However, a data-gap exists on legume species as well as proper seeding rates that can be successfully integrated into forage-biofuel systems in the humid, southeastern United States, and contribute to yield, thereby reducing synthetic N inputs and subsequent greenhouse gas emissions.

Switchgrass can offset lost forage production by cool-season forages during hot, dry months, and interseeding legumes may increase forage quality by taking advantage of legume growth patterns and improving seasonal distribution of forage production. Other benefits from increasing diversity in pasture systems include greater yield stability under stress or disturbance, ecological facilitation, reduction of weed abundance, and niche-differentiation (Sanderson et al. 2004a; Sanderson et al. 2005; Finn et al. 2013). In grass-legume swards, legume competiveness is affected by companion species growth habit, photosynthetic pathway, management, and hard seed level (Posler et al. 1993). Because of these potential incompatibilities, botanically stable pasture/feedstock-legume mixtures are often not sustained, and either the legume or grass component declines long term (Blanchet et al. 1995; George et al. 1995; Warwick 2011). A study by Blanchet et al. (1995) found that hairy vetch interseeded into switchgrass stands persisted into their second year; however, they were not tracked beyond two years. In addition, establishment and persistence of legumes may adversely affect switchgrass growth early in the growing season if legume growth habits are not considered (George et al. 1995), as Taylor and Jones (1983) found that the red clover (*Trifolium pratense*) component of a switchgrass/red clover mixture overwhelmed switchgrass stands after Year 2. However, research is limited on the persistence of successful legume intercrop species in established switchgrass stands, particularly under various seeding rates.

Harvest management is another factor likely affecting legume persistence and establishment success, as some species may respond to canopy removal whereas others may be unaffected, due to contrasting windows for maximizing legume species photosynthesis and companion crop canopy removal. Such differences have not been investigated in detail, and when to remove grass canopy for effective legume management is still an unresolved issue (Wang et al. 2010). A study by George et al. (1995) tested only forage harvest regimes (June and July), and determined that adequate defoliation in early June is important for minimizing switchgrass competition with legumes (crownvetch [*Coronilla varia* L.], birdsfoot trefoil [*Lotus corniculatus* L.], and red clover). Other studies have looked at the establishment, yield, and persistence of legumes interseeded into switchgrass, but did not include harvest treatments (Blanchet et al. 1995; Butler et al. 2013), which is necessary for assessing legume intercropping success under various production systems.

To address these data gaps, we conducted research examining switchgrass compatibility with cool- and warm-season legume intercrops compared to synthetic-N fertilization under two harvest systems (two-cut, simulating dual-purpose forage-biomass [preanthesis and postsenescence] and one-cut, biofuel harvest [postsenescence]). We evaluated each legume species at three seeding rates to determine densities required to impact switchgrass yield, thereby promoting labile-N in the soil sphere. Specific objectives were to evaluate five (four cool-season and one warm-season) legume species, each seeded at three rates and managed under two harvest systems, and determine their (1) influence on switchgrass yield and forage quality compared to three inorganic-N levels; (2) determine persistence of legumes over three years; and (3) identify legume density thresholds and their associated impact on switchgrass yields.

Materials and Methods

Site Description. Locations included the East Tennessee Research and Education Center, Knoxville, Tennessee (ETREC; 35.53° N, –83.57° W), on a soil mapped as Huntington silt loam (fine-silty, mixed, active, mesic Fluventic Hapludolls); Plateau Research and Education Center, Crossville (PREC, Northern Cumberland Plateau; 36.0° N, –85.1° W) on a Lilly Loam (Fine-loamy, siliceous, semiactive, mesic Typic Hapludults); and, Research and Education Center at Milan (RECM, East Gulf Coastal Plain, 35.5° N, –88.4° W) on a soil mapped Collins silt loam (coarse-silty, mixed, active, acid, thermic Aquic Udifluvents). Mean annual precipitation during the duration of the study (2010 to 2012) at ETREC was 127 m (50 in), with mean annual temperature at 15.3°C (60°F; NOAA 2013). This site had been under orchardgrass (*Dactylis glomerata* L.) hay production for four years prior to initiation of this experiment. At PREC, precipitation was 153 cm (60 in), mean annual temperature was 13.3°C (56°F; NOAA 2013), and previous management had been tall fescue (*Schedonorous arundinaceus* [Schreb.]) pasture. Mean annual precipitation at MREC was 128 cm (50 in), mean annual temperature was 15.7°C (60°F; NOAA 2013), and the site had been under row crop production for four years prior to experiment initiation.

Experimental Design. This experiment tested two factors arranged factorially under a randomized complete block design. The first factor was harvest system, and included two levels, a single harvest (postdormancy) and a two-cut system (preanthesis stage and postdormancy). These harvests were chosen to represent a biomass and an integrated forage-biomass production scenario, respectively. The second factor, N treatments, included five legume species drilled at low, medium, and high seeding rates, and inorganic-N applied at 0 (control), 33, and 67 kg N ha^{-1} (60, 30, and 0 lb ac^{-1}) in the form of ammonium nitrate (NH_4NO_3) into established Alamo switchgrass. Inorganic-N was applied in a single application when switchgrass was about 30 cm (12 in) tall (approximately April 15) in 2011 and 2012. Legumes and respective seeding rates were the following: red clover at 9.0, 13.4, and 17.9 kg pure live seed (PLS) ha^{-1} (8, 12, 16 lb PLS ac^{-1}); hairy vetch (*Vicia villosa*) at 6.7, 10.1, and 13.4 kg PLS ha^{-1} (6, 9, 12 lb PLS ac^{-1}); ladino clover (*Trifolium repens*) at 3.4, 5.0, and 6.7 kg PLS ha^{-1} (3, 4.5, 6 lb PLS ac^{-1}); partridge pea (*Chamaecrista fasciculata*) at 13.4, 20.2, and 26.9 kg PLS ha^{-1} (12, 18, 24 lb PLS ac^{-1}); and, arrowleaf clover (*Trifolium vesiculosum*) at 11.2, 16.8, and 22.4 kg PLS ha^{-1} (10, 15, 20 lb PLS ac^{-1}). Species and seeding rates were selected based on previous work by Warwick (2011) in that species tested herein were deemed most successful in terms of establishment into switchgrass, and medium seeding rates were considered adequate, with high and low levels adjusted accordingly. Therefore, there were 18 N treatment levels ([3 cool-season + 2 warm-season legumes] ×

3 seeding rates + 3 inorganic N levels) and 2 harvest levels, installed in three blocks, creating 108 plots (experimental units) per locale.

Switchgrass was planted at 9 kg PLS ha^{-1} (8 lb PLS ac^{-1}) in spring of 2007 at ETREC and PREC and in spring of 2004 at RECM. Plots at ETREC and PREC were 7.6 by 1.5 m and 7.6 by 1.8 m (6 by 25 ft and 6 by 30 ft), respectively, with 18 cm (7 in) row-spacing. Plots at RECM were 7.6 by 3.8 m (25 by 12 ft) with 25.4 cm (10 in) row-spacing. Weeds were controlled at ETREC with nicosulfuron (2- [{4,6-dimethoxypy rimidin-2-yl}aminosulfonyl]-N,N-dimethyl-3-pyridinecarboxamide) at 0.98 L ha^{-1} (0.10 gal ac^{-1}) in 2009. Weeds at PREC and RECM were controlled by 2, 4-dichlorophenoxyacetic acid at 0.9 L ha^{-1} (0.09 gal ac^{-1}) in 2009.

Legumes were no-till drilled into switchgrass stubble at ETREC and PREC using a 5-row Hege (Colwich, Kansas) plot drill and an 8-row ALMACO (Nevada, Iowa) plot drill at RECM without subsequent reseeding. Cool-season legumes (red clover, hairy vetch, arrowleaf clover, and ladino clover) were planted on October 20, September 9, and September 28, 2010, and the warm-season legume (partridge pea) was seeded on March 24, April 12, and April 13, 2011, at ETREC, PREC, and MREC, respectively. Planting depth ranged from 0.6 to 1.3 cm (0.2 to 0.5 in), depending on seed size. All legume seeds were inoculated prior to seeding: partridge pea and hairy vetch with cow pea group inoculum (*Bradyrhizobium* spp.), and clover species with clover group inoculum (*Sinorhizobium meliloti*).

Data Collection. Legume stand densities were estimated late-spring annually following green-up using a 1 m^2 (3.3 ft^2) frequency grid (Vogel and Masters 2001). Four, 1 m^2 density counts were taken on each experimental unit and averaged. In 2010 (pretreatment), soil tests were conducted at 15 cm (6 in) depths to determine preliminary levels of pH and soil N, P, potassium (K), magnesium (Mg), and calcium (Ca). Samples were ground to pass through a 1 mm (0.04 in) sieve on a Wiley mill (Thomas Scientific, Swedesboro, New Jersey) and Mehlich-1 extractable nutrients were measured by inductively coupled plasma (ICP) using a 7300 ICP-OES DV (Perkin-Elmer, Waltham, Massachusetts). The pH was determined on a 1:1 soil to water ratio using a AS3010D Dual pH Analyzer (Labfit, Burswood, Australia).

Harvest regimes included (1) a single, end-of-season harvest in November (one-cut system), (2) an integrated forage and biofuel production paradigm in June and November (two-cut system) with each harvest treatment analyzed separately, and (3) the sum of the two-cut system under an integrated approach. Plots were harvested at ETREC and PREC using a Carter forage harvester (Brookston, Indiana) with a 91 cm (36 in) cutting width, and at RECM with a New Holland Crop Cruiser 850 forage chopper with a 2.1 m (6.9 ft) cutting width. For both harvest regimes, switchgrass was cut to a 20.3 cm (8 in) stubble height in 2011 and 2012. Grab samples of switchgrass (1 to 2 kg [2.2 to 4.4 lb]) were collected from all plots at harvest, and then weighed, dried at 49°C (120°F) in a batch oven (Wisconsin Oven Corporation, East Troy, Wisconsin) for 48 to 72 hours, and weighed again to determine moisture content. Samples were then ground to a 2 mm (0.08 in) particle size on a Wiley mill.

Forage quality was analyzed on the first (forage) cut of the two-cut harvest system. The analysis included acid detergent fiber (ADF), neutral detergent fiber (NDF), crude protein (CP), hemicellulose, and ash content. Ground (2 mm [0.08 in]) switchgrass tissue (separated from legumes) was analyzed with near-infrared spectroscopy (NIRS) using a LabSpec Pro Spectrometer (Analytical Spectral Devices, Boulder, Colorado). Five scans were taken per sample and the scan range was 1,003 to 2,500 nm. Samples were compiled across replications per legume seeding level such that only one sample was analyzed per species and N-level per harvest (n = 8 per site). In a separate run (n = 3), partridge pea and switchgrass tissue composition was analyzed postsenescence (mid-November) to determine biofuel digestibility affects from intercropping this species with switchgrass. Equations for the forage nutritive analysis and biomass quality were standardized and checked for accuracy using the Grass Hay equation developed by the NIRS Forage and Feed Consortium (NIRSC [Hillsboro, Wisconsin]).

Statistical Methods. Three separate models were analyzed to elucidate the relationship between selected legume intercrops and switchgrass. For all models, analysis of variance (ANOVA) assumptions of normally distributed residuals (Shapiro-Wilk test) and homogeneity of variances (Levene's F-test) were confirmed. When significant differences were found, pairwise post-hoc comparisons of the least squares means were conducted using Least Significant Difference (LSD) at $p \leq 0.05$. Mean separation was performed by the SAS macro pdmix800 (Saxton 1998) with Fisher's with a Type-I error rate of 5%.

Legume Intercropping Impacts on Switchgrass Yield and Forage Quality versus Inorganic Nitrogen Model. Dependent variables, switchgrass yield and forage quality (for forage harvest only; i.e., ADF, NDF, CP, hemicellulose, and ash) were analyzed separately by harvest regime (i.e., one-cut biomass; forage, biomass in an integrated system, and their summation), and included appropriate year and location interactions using ANOVA. In all models, N-treatment was the fixed effect and year, location, and block were entered as random effects using PROC MIXED (SAS V9.3; SAS Institute, Cary, North Carolina).

Intercropped Legume Persistence in Switchgrass Sward Model. Legume persistence (dependent variable; i.e., legume density over years) by species, seeding rate, and harvest treatment (main effects) were analyzed under a repeated measures ANOVA using PROC MIXED over the three year sampling period with block entered as a random effect. Legume species, seeding rate, and harvest treatment were all entered as fixed effects and location as a random effect, with year being a repeated measure. For the repeated measure, an autoregressive covariance was used and the denominator degrees of freedom for the Type III F-test were adjusted with the Kenward-Roger method (Gomez et al. 2005). However, the –2 log-likelihood did not change under the repeated-measure analysis (did not drop by at least 5 per covariance parameter) and the autoregressive correlation value (0.24) indicated a weak correlation among observations, so autoregressive covariance was dropped. Thereafter, an additional model was analyzed using legume density by species and seeding rate within a year to assess persistence based on seeding rate impacts by species.

Switchgrass Yield and Legume Density Relationship Model. A multiple-regression analysis was performed to examine the relationship between legume density (independent variable) and biomass yield (dependent variable). One requirement was that adequate legumes existed; therefore, due to poor persistence at PREC in Year 2, this location-year combination was

Location	pH	P (kg ha^{-1})	K (kg ha^{-1})	Ca (kg ha^{-1})	Mg (kg ha^{-1})
RECM	7.1	30.2 M	149.0 M	2,222 S	191.5 S
ETREC	6.7	29.1 M	50.4 L	1,676 S	293.4 S
PREC	6.8	35.8 H	40.3 L	2,177 S	26.9 D

Notes: L = low. M = medium. H = high. S = sufficient. D = deficient.

Table 1
Mehlich 1 soil extractable nutrients (phosphorus [P], potassium [K], calcium [Ca], and magnesium [Mg]; measured with inductively coupled plasma [ICP]) and pH (based on 1:1 [soil to water ratio]) baseline (2010) results at Research and Education Centers in Tennessee (East Tennessee Research and Education Center [ETREC], Plateau Research and Education Center [PREC], and Milan Research and Education [RECM]).

dropped from this model. Initially, a mixed model analysis of variance (MMAOV) was run separately for legume density per harvest treatment (one-cut biomass; forage, biomass in an integrated system, and their total) with replication, location, and year entered as random effects; legume species, seeding rate, and seeding rate × legume species were fixed effects, with $\alpha = 0.05$. Because location and year were not important ($p \geq 0.05$) predictive variables for legume density in the MMAOV, these effects were removed in the multiple regression model. Therefore, the simplified regression model (pooled across years and location) accessed legume density per species with the interaction of density and legume species for each of the four harvest treatments. All residuals in the aforementioned models were normally distributed ($p \geq 0.05$; Shapiro-Wilk > 0.90).

Results and Discussion

Mean soil test results per location indicate moderate-high P levels in the upper 15 cm (5.9 in), with moderate-low K, sufficient Ca levels, and sufficient-deficient Mg levels (table 1). In general, legume persistence and establishment was lowest at PREC, which also corresponds to low-deficient soil-nutrient conditions compared to other experimental sites, suggesting soil fertility was important for successful legume establishment and persistence.

Legume Intercropping Impacts on Switchgrass Yield and Forage Quality versus Inorganic Nitrogen. When combined across all locations and years (2011 and 2012), there were no differences in forage quality results of switchgrass, except for CP ($p = 0.013$) with hairy vetch being greatest (7.9%), ladino clover lowest (6.6%), and the remaining treatments not differing ($p < 0.05$). This was not found to be the case by Posler et al. (1993), who reported increased CP levels of legume-grass mixtures compared to sole grass crops. However, Posler et al. (1993) as well as others (George et al. 1995) did not separate legume and grass tissue before analysis; consequently, in situ forage quality of grass-legume mixtures is likely more positively impacted than what was observed in this study.

For the first study year (2011), RECM was the only location to have forage quality components (CP, NDF, and hemicellulose) impacted by legumes ($p < 0.05$; table 2). For this location and year combination, CP for switchgrass tissue intercropped with hairy vetch and red clover exceeded all other N-treatments ($p < 0.05$). For this location × year combination, hairy vetch treatments also resulted in the lowest NDF levels in the switchgrass among all treatments, indicating greater digestibility. Similarly, hairy vetch resulted in the lowest hemicellulose levels, with partridge pea and inorganic-N levels being the greatest ($p < 0.05$), indicating greater 5 and 6 C sugars in switchgrass plant tissue when intercropped with partridge pea and under inorganic-N inputs.

During 2012 forage harvests, forage quality results were impacted by legume species at ETREC. Specifically, CP, ADF, NDF, and ash were impacted by legume intercrops and inorganic-N treatments ($p < 0.05$; table 2). For CP, legumes did not differ from inorganic-N rates; however, partridge pea treatments had higher levels than that of ladino clover intercrops. Acid detergent fiber and NDF were lowest in switchgrass grown with partridge pea, and was greatest for ladino and red clovers ($p < 0.05$; table. 2). Therefore, results suggest greater digestibility and intake by ruminants of switchgrass-forage when intercropped with partridge pea during June. Conversely, greater ash levels were observed in partridge pea intercropped switchgrass tissue compared to inorganic-N treatments ($p < 0.05$), suggesting more adverse feedstock characteristics due to slagging in combustion chambers.

Based on postsenescence partridge pea–only tissue composition, if harvested in a switchgrass-biomass mixture, biofuel digestibility may decrease substantially compared to switchgrass-only biomass (average ADF increases of 43% and total digestible nutrient losses of 44%), due to partridge peas' stemmy and fibrous composition. Such declines from switchgrass-only biomass will likely reduce ethanol conversion efficiency and affect enzymatic requirements if harvested in a mixture where partridge pea is a significant component.

When combined across locations and years, the forage and integrated (forage + biomass harvests) harvest treatment yields were impacted by N and legume treatments, whereas the biomass and biomass-only harvest treatments were not ($p < 0.05$). This suggests that the annual removal of the switchgrass canopy may affect yield impacts from intercropping. Under each harvest treatment models, there were no location or year effects ($p > 0.05$), but a slight interaction was observed for location × year impacts for the forage harvest ($p = 0.05$). Under the forage harvest, the 67 kg N ha^{-1} (60 lb N ac^{-1}) rate resulted in the greatest yield, followed by 33 kg N ha^{-1} (30 lb N ac^{-1}), and the 0 kg N ha^{-1} rate being the lowest ($p < 0.05$); and, the latter two not differing from any legume species (except for that of arrowleaf clover, which was the lowest). For integrated harvests, a similar pattern was observed; however, none of the tested legumes were different than the 0 and 33 kg N ha^{-1} (30 lb N ac^{-1}) rates ($p > 0.05$).

For the first year of legume establishment, minimal yield impacts were observed as a result of intercropping. During 2011, the only legume treatment-imposed variation in yield occurred at PREC during the forage ($p = 0.029$) and biomass-only harvests ($p = 0.005$); all other yields were not impacted by intercrops or inorganic inputs ($p < 0.05$; table 3). Forage yields at PREC during the first year were greatest for 67 kg N ha^{-1} (60 lb N ac^{-1}) and did not differ from the 33 kg N ha^{-1} rate (30 lb N ac^{-1}; $p < 0.05$). In addition, the 33 kg N ha^{-1} rate was not different than any legume treatment except red clover, which was not different than the 0 kg N ha^{-1} rate (table 3). Similarly, for the biomass-only harvest, the highest N rate resulted in the greatest yields, but was not different than the moderate rate (table 3), nor arrowleaf clover, partridge pea, or red clover ($p > 0.05$).

Table 2

Switchgrass forage (early June) quality results (switchgrass tissue only) by legume and nitrogen (N) treatments by location (East Tennessee Research and Education Center [ETREC], Plateau Research and Education Center [PREC], and Milan Research and Education Center [RECM]) and by year (2011 and 2012).

Year	Location	Treatments*	Crude protein (%)	ADF (%)	NDF (%)	Ash (%)	Hemi-cellulose (%)
2011	ETREC	AC	10.6a	38.6a	66.5a	6.2a	27.9a
		RC	10.0a	41.0a	66.5a	6.3a	25.5a
		LC	10.0a	39.2a	67.5a	5.9a	28.3a
		PP	10.8a	37.3a	69.2a	5.9a	31.8a
		HV	10.1a	43.4a	68.1a	6.5a	24.7a
		IF	11.2a	41.8a	64.3a	6.1a	22.6a
	PREC	AC	8.8a	41.1a	67.0a	5.5a	25.9a
		RC	9.3a	37.3a	66.5a	5.1a	29.2a
		LC	9.2a	40.2a	66.7a	5.3a	26.5a
		PP	9.2a	39.6a	65.8a	5.4a	26.2a
		HV	10.9a	36.7a	64.2a	5.1a	27.5a
		IF	10.6a	35.0a	64.3a	4.8a	29.2a
	RECM	AC	8.4b	44.0a	79.0a	7.2a	34.9ab
		RC	9.6ab	44.3a	78.2a	7.4a	33.9ab
		LC	7.5b	46.7a	78.3a	6.7a	31.7b
		PP	8.9b	45.5a	79.3a	7.2a	35.7a
		HV	11.9a	46.2a	70.6b	6.8a	24.4c
		IF	8.6b	43.6a	79.3a	7.0a	35.7a
2012	ETREC	AC	5.6ab	46.2c	83.1ab	4.5ab	36.9a
		RC	5.2ab	48.3a	85.4a	3.8b	37.2a
		LC	4.8b	48.6a	85.3a	3.8b	36.7a
		PP	6.0a	45.8c	82.0b	5.0a	36.2a
		HV	5.5ab	46.4bc	83.6ab	4.6ab	37.2a
		IF	5.1ab	48.1ab	84.1ab	3.8b	36.1a
	PREC	AC	7.7a	40.2a	79.6a	2.8a	39.4a
		RC	7.9a	40.0a	79.7a	2.9a	39.7a
		LC	7.8a	39.8a	79.1a	3.2a	39.3a
		PP	7.7a	39.6a	78.7a	3.1a	39.1a
		HV	7.7a	39.6a	79.0a	3.0a	39.3a
		IF	7.8a	39.9a	79.0a	2.9a	39.1a
	RECM	AC	1.3a	52.5a	91.1a	6.7a	38.6a
		RC	1.8a	52.7a	89.9a	7.4a	37.2a
		LC	0.4a	58.9a	87.5a	7.2a	28.6a
		PP	1.6a	53.0a	90.0a	7.0a	37.0a
		HV	1.4a	52.9a	90.7a	6.8a	37.8a
		IF	1.2a	53.7a	91.8a	7.2a	38.1a

Notes: Different letters indicate differences within a given location and year combination at the $p < 0.05$ level. ADF = Acid detergent fiber. NDF = neutral detergent fiber.

*Legume intercrop treatments: arrowleaf clover (AC), red clover (RC), ladino clover (LC), partridge pea (PP), hairy vetch (HV), and inorganic fertilizer (IF; combined across inorganic-N rates: 67 and 33 kg ha^{-1}).

During the second harvest year, legumes had more beneficial impacts on yield compared to that of the first legume establishment year. For 2012, the forage yield ($p = 0.028$) and biomass-only ($p < 0.0001$) at PREC was impacted by treatments. Similarly, at RECM, forage ($p = 0.005$), biomass ($p = 0.012$), and integrated ($p = 0.003$) yields were affected by legume intercrop and N inputs (table 3). Both forage yields at PREC and RECM were greatest at 67 and 33 kg N ha^{-1} (60 and 30 lb N ac^{-1}). At PREC, the 67 and 33 kg N ha^{-1} rate produced equivalent forage yields to that of ladino clover and hairy vetch (table 3). For this location-year combination, the lowest yielding treatments were red clover, then partridge pea and arrowleaf clover. The biomass-only harvest regime at PREC also had no differences between high and medium synthetic N rates, which were greater than all other treatments ($p < 0.05$); however, partridge pea yields exceeded that of intercropped arrowleaf clover (table 3). At RECM, partridge pea mixtures yielded comparable biomass to the inorganic treatments for forage, biomass, and integrated harvests ($p < 0.05$). Biomass harvests at RECM revealed that in addition to partridge pea, hairy vetch, and ladino clover, intercropping can supply equivalent N as 33 kg N ha^{-1} for switchgrass yields. On the other end of the spectrum, for these harvest regimes, arrowleaf clover consistently produced the poorest yields, not different than 0 kg N ha^{-1}.

Intercropped Legume Persistence in Switchgrass Swards. Harvest treatment did not impact legume persistence ($p = 0.99$) and consequently this fixed effect was dropped from the model. In turn, legume species, year, and legume species × year were all important factors ($p < 0.0001$). However, neither seeding rate within legume species ($p = 0.38$), nor seeding rate × year within legume species was significant ($p = 0.78$). The combined model for legume persistence (across locations and seeding rates) illustrated declining legume density trends over years, with the exclusion of arrowleaf clover as it was close to initial low legume levels (4.19 and 4.15 plants m^{-2} [1.28 and 1.26 plants ft^{-2}] in Years 1 and 3, respectively [figure 1]). Legume species' persistence slightly increased from Year 2 to Year 3 for arrowleaf clover (1.6 plants m^{-2} [0.49 plants ft^{-2}]), ladino clover (2.1 plants m^{-2} [0.64 plants ft^{-2}]), and partridge pea (0.3 plants m^{-2} [0.09 plants ft^{-2}]). This suggests that legume density either leveled off, or could potentially increase over time due to self-reseeding (partridge pea) or asexual reproduction via lateral stolons (i.e., arrowleaf and ladino clovers).

Among all legume treatments (across all seeding rates and years), red clover resulted in the highest density (12.6 plants m^{-2} [3.8 plants ft^{-2}]), with partridge pea, ladino clover, and hairy vetch densities not differing from one another (10.3, 9.8, and 9.6 plants m^{-2} [3.1, 3.0, and 2.9 plants ft^{-2}], respectively), and arrowleaf clover being the lowest (3.6

Table 3
Switchgrass forage (early June), biomass only (mid-November), biomass, and integrated (forage + biomass) yield by treatment at the East Tennessee Research and Education Center (ETREC), Plateau Research and Education Center (PREC), and Milan Research and Education Center (RECM) for 2011 and 2012.

		2011				2012			
Location	Treatment*	Biomass only (Mg ha^{-1})	Forage (Mg ha^{-1})	Biomass (Mg ha^{-1})	Integrated (forage biomass) (Mg ha^{-1})	Biomass only (Mg ha^{-1})	Forage (Mg ha^{-1})	Biomass (Mg ha^{-1})	Integrated (forage + biomass) (Mg ha^{-1})
ETREC	AC	11.2a	5.7a	8.5a	14.3a	11.8a	4.7a	5.6ab	10.3a
	RC	11.1a	5.5a	9.1a	14.6a	11.2a	5.3a	5.9a	11.2a
	LC	9.91a	5.3a	7.9a	13.1a	11.2a	4.2a	5.9a	10.1a
	PP	10.9a	6.1a	7.8a	13.9a	10.9a	4.2a	5.6ab	9.8a
	HV	10.3a	5.4a	9.3a	14.7a	12.1a	4.6a	5.8a	10.4a
	N-0	9.3a	6.1a	8.7a	14.8a	12.0a	4.6a	5.1ab	9.7a
	N-33	9.4a	6.0a	10.4a	16.5a	12.1a	4.2a	4.2b	8.4a
	N-67	12.9a	6.8a	8.1a	15.0a	10.6a	5.2a	5.1ab	10.3a
PREC	AC	9.0bc	3.5bc	2.8a	6.3a	6.0d	1.8cd	3.3a	5.1a
	RC	8.7bc	3.1c	2.6a	5.8a	6.9bcd	1.7d	3.3a	5.0a
	LC	8.4bc	4.0bc	2.8a	6.6a	6.1cd	2.3abc	3.4a	5.8a
	PP	9.7b	3.3bc	2.6a	5.9a	7.4b	1.9cd	3.3a	5.2a
	HV	8.4bc	3.2bc	2.9a	6.1a	7.1bc	2.0bcd	3.5a	5.5a
	N-0	8.2bc	3.4bc	2.4a	5.8a	5.9bcd	1.5d	3.0a	4.5a
	N-33	9.9ab	4.2ab	3.1a	7.4a	8.8a	2.7ab	3.5a	6.2a
	N-67	11.6a	5.1a	3.2a	8.3a	9.5a	2.8a	2.9a	5.7a
RECM	AC	10.0a	4.2a	4.0a	8.1a	8.4a	3.5cd	3.8c	7.2d
	RC	10.0a	5.3a	4.5a	9.9a	8.1a	4.3bc	3.8c	8.1bcd
	LC	9.7a	5.4a	4.0a	10.2a	7.9a	3.6cd	4.4abc	8.0cd
	PP	8.7a	5.4a	3.9a	9.4a	7.6a	5.0ab	4.6ab	9.6ab
	HV	9.9a	6.3a	4.1a	10.5a	7.6a	4.1bcd	4.7ab	8.9bc
	N-0	8.2a	5.5a	2.7a	8.1a	7.0a	2.7d	4.0bc	6.7d
	N-33	9.9a	5.7a	4.3a	10.0a	7.1a	6.1a	5.3a	11.4a
	N-67	8.0a	9.0a	3.9a	13.0a	7.5a	6.2a	5.0ab	11.2a

Note: Different letters indicate a significant difference within a given harvest, location, and experimental year at the $p < 0.05$ level.

*Legume intercrop treatments: arrowleaf clover (AC), red clover (RC), ladino clover (LC), partridge pea (PP), hairy vetch (HV), and inorganic fertilizer ([IF], combined across inorganic-N rates: 67 and 33 kg ha^{-1}).

plants m^{-2} [1.1 plants ft^{-2} ; $p < 0.05$]). When combined across all years, red clover at the high and medium seeding rates (17.9 and 13.4 kg PLS ha^{-1} [16 and 12 lb PLS ac^{-1}]) had the greatest densities across all seeding rate legume combinations (27.3 and 25.2 plants m^{-2} [8.3 and 7.7 plants ft^{-2}], respectively). On the opposite end of the legume density spectrum, arrowleaf clover was the lowest, with seeding rates not affecting density over time ($p < 0.05$; figure 1).

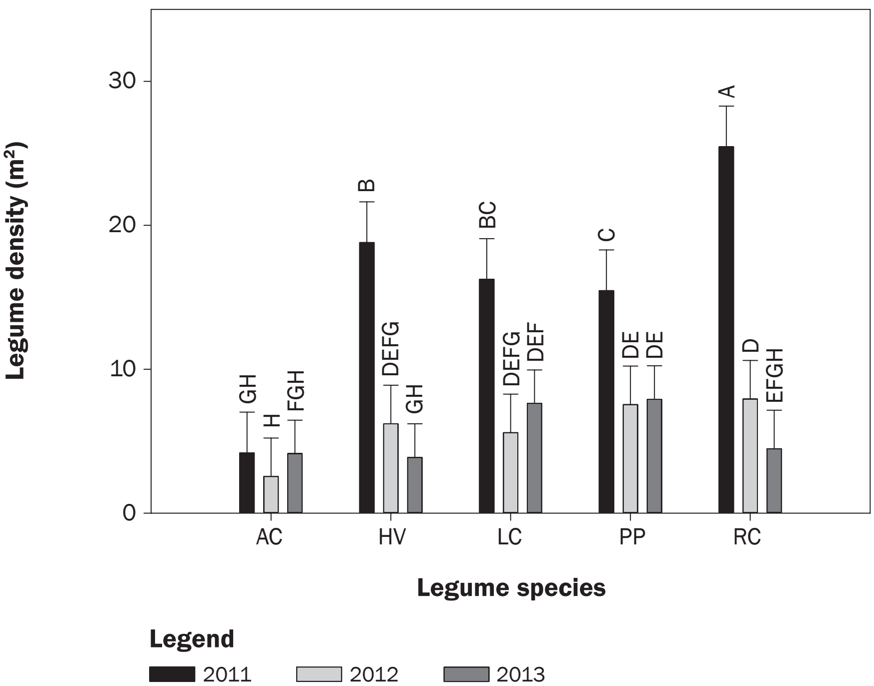
Within years and across all seeding rates by legume species, persistence differed the greatest during the establishment year (4.2 to 25.5 plants m^{-2} [1.2 to 7.7 plants ft^{-2}]) with red clover being the highest, followed by hairy vetch = partridge pea = ladino clover, > arrowleaf (figure 1). During Year 2 (across all legume species) densities dropped approximately 63%, with declines leveling off during Year 3 (only 6% decline from Year 2). Consequently, arrowleaf clover density during Year 2 was the only species that differed among all legume treatments during 2013 ($p < 0.05$).

Year 1 density (16.0 plants m^{-2} [4.9 plants ft^{-2}]) was greater than Year 2 (6.0 plants m^{-2} [1.8 plants ft^{-2}]), which was not different than that of Year 3 (5.6 plants m^{-2} [1.7 plants ft^{-2}; $p < 0.05$]), when compared across all seeding rates, legume treatments, and locations. ETREC and RECM during Year 1 had the greatest densities (18.6 and 18.8 plants m^{-2} [5.6 and 5.7 plants ft^{-2}], respectively) compared across all locations and years, followed by PREC in Year 1 (10.6 plants m^{-2} [3.2 plants ft^{-2}]), which was different than the other locations for that year ($p < 0.05$), but not different than ETREC and RECM in Year 2 (8.7 and 8.2 plants m^{-2} [2.7 and 2.5 plants ft^{-2}], respectively). However, the latter two were not different than RECM in Year 3 (7.49 plants m^{-2} [2.3 plants ft^{-2}]). Finally, ETREC densities in Year 3 were different than RECM in Year 3 (3.8 plants m^{-2} [1.2 plants ft^{-2}]); albeit still greater than PREC in Year 2 (1.0 plants m^{-2} [0.3 plants ft^{-2}; $p < 0.05$]).

Seeding rates × legume species did not impact plant density by year ($p < 0.05$). Although red clover, partridge pea, and hairy vetch were among the only species that higher seeding rate resulted in greater numeric legume densities for Year 1 (figure 2), this trend did not persist in Year 2, with ladino clover and red clover being the only species where greater seeding rates were enumerating in Year 3. Conversely, low and medium

Figure 1

Legume density by species (arrowleaf clover [AC], hairy vetch [HV], ladino clover [LC], partridge pea [PP], and red clover [RC]) combined across locations (East Tennessee, Plateau, and Milan Research and Education Centers) and seeding rates (low, medium, and high) per species during 2011 to 2013. Different letters indicate significant differences at $p \leq 0.05$, compared across years and species. Vertical bars are +/– one standard error.



seeding rates resulted in numerically greater legume densities for hairy vetch, arrowleaf clover, and partridge pea during Year 3.

Based on legume persistence for all tested species, reseeding would be recommended during Year 4, due to companion crop densities likely dropping below an acceptable persistence threshold for target N fixation (≤67 kg ha^{-1} [60 lb N ac^{-1}; Warwick 2011; Peoples et al. 1995]). Reseeding during Year 4 may not be necessary for red clover, ladino clover, and partridge pea, due to exceptionally high persistence and in some cases, out competing switchgrass (in field observations). Consequently, reseeding would be predicated on site management objectives, soil texture, and physiographic location, and selected legume intercrop.

Among all legume intercrops, partridge pea demonstrated the greatest potential for legume persistence, self-reseeding in both harvest systems, and for all seeding rates across a diversity of soil textures. However, given the difficulty in seed procurement, potential toxicity for cattle (Liener 1962), need for inoculating seed by-hand, unknown diazotrophic species for partridge pea host, and great potential for dormant and hard seed, this species may not be an ideal candidate. Consequently, ladino and red clover may be more appropriate as a legume intercrop given that persistence was also among the highest after three years (9.8 and 8.8 plants m^{-2} [3.0 and 2.7 plants ft^{-2}], respectively), seed come preinoculated and with rock phosphate protectant to combat unfavorable conditions, have low dormant-seed level, have high N fixation rates (Peoples et al. 1995), and are widely distributed. Further, based on the theoretical fixation-transfer of these cool-season species (Peoples and Baldock 2001), and their complementary growth habit from fall through early-spring, these species are likely good intercrop candidates in switchgrass swards.

Switchgrass Yield and Legume Density Relationship. In the regression model (combined across locations and years) for the integrated harvest (forage + biomass) system, seeding rate and seeding rate × legume species interaction were marginally important (p = 0.05) descriptors of yield, whereas legume species alone was not important for describing combined yields (p = 0.08; figure 3a). Under the integrated model, relationships with legume density were all generally positive (ranging from β = 1.63 to 2.56 ± 1.22 se) with increasing legume density until approximately 10 plants m^{-2} (3 plants ft^{-2}). This trend excluded hairy vetch, suggesting the growth habit of this species (climbing with tendrils) may negatively affect integrated switchgrass yield (p = 0.04). Overall, across all seeding rates, for all species, there were some marginal trends suggesting positive benefits from legume integration.

Varying yield and legume density relationships were observed for the forage-only and biomass-only harvest regimes. Similar to integrated yield results, forage yields were not impacted by legume species (p = 0.38), neither by seeding rate (p = 0.13), nor legume species × seeding rate interactions (p = 0.16). Conversely, switchgrass biomass-only yields were impacted by legume density (p < 0.0001), but only marginally by legume seeding rate (p = 0.07; figure 3b), but not by legume species (p = 0.16), nor legume species × seeding rate interactions (p = 0.98). This indicates biomass yields may potentially benefit at proper legume seeding rate, or be adversely affected at too high of densities (seeding rate). For this harvest regime, cool-season legumes (red clover, ladino clover, and arrowleaf clover) tended to occur with less frequency at higher legume densities, as biomass was not removed during peak production of these legumes (i.e., forage harvest period).

Summary and Conclusions

There are a multitude of potential benefits from introducing legumes into pasture and monoculture biofuel systems in the humid east, including reduced fertilizer inputs, increased soil C additions from green manure, reduced weed pressure due to niche differentiation, and reduced leaching of soil nitrate (NO_3) to groundwater. Switchgrass pastures and biofuel swards can be interseeded successfully with cool-season legumes (ladino and red clovers) and the warm-season legume, partridge pea, without annual reseeding (≥3 years; depending on soil texture, soil fertility, and rainfall). Although annual reseeding may not be required, substantial legume density loss did occur during Year 2 for all species (approximately 63% decreases), likely due to competition from the dense stands of switchgrass used in this study and the canopy closure of this spe-

Figure 2

Legume density by seeding rate (low, medium, and high) and species ([a] hairy vetch [HV], [b] arrowleaf clover [AC], [c] ladino clover [LC], [d] partridge pea [PP], and [e] red clover [RC]) combined across locations (East Tennessee, Plateau, and Milan Research and Education Centers) during 2011 to 2013, and analyzed within years. Vertical bars are +/– one standard error.

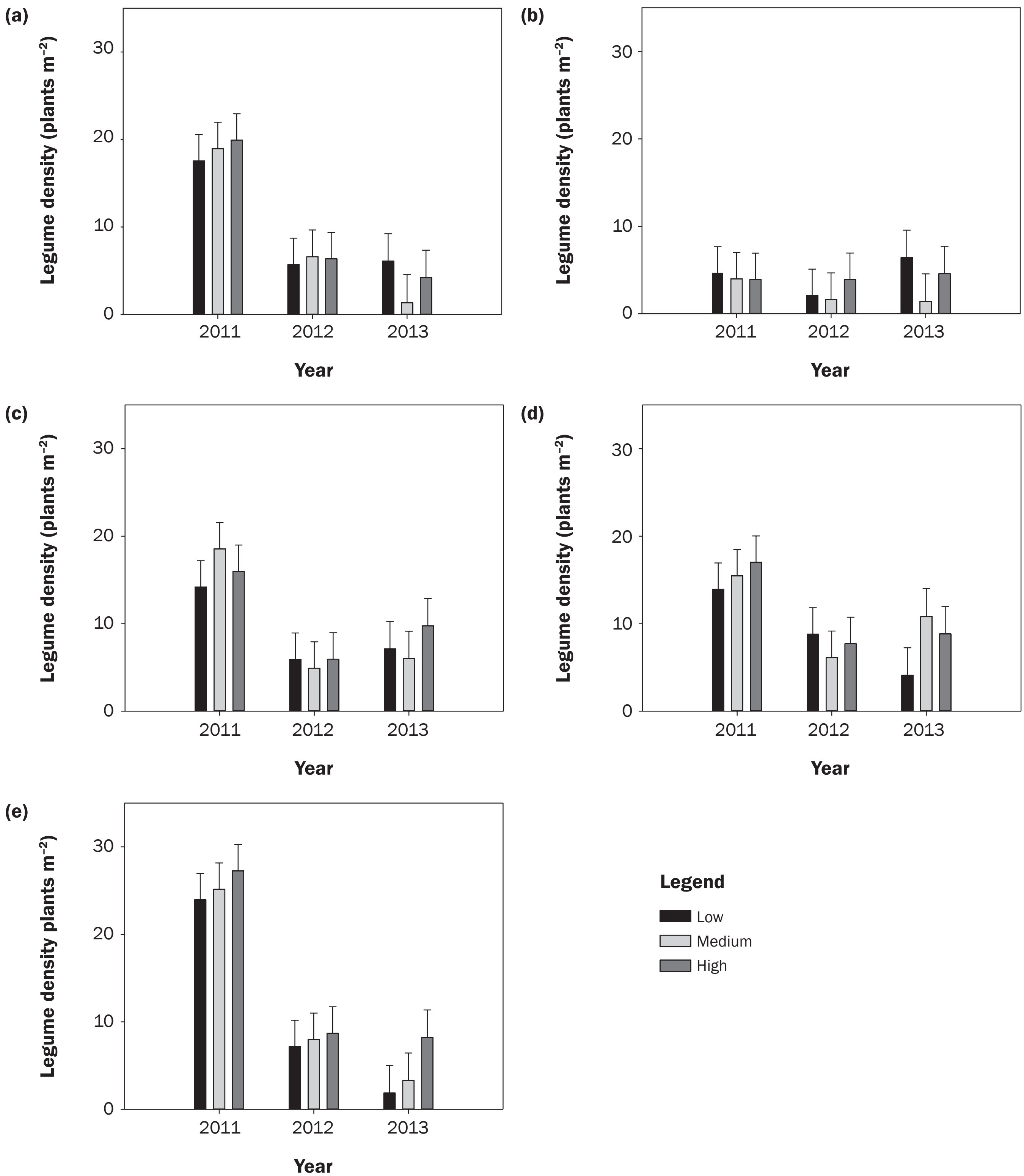
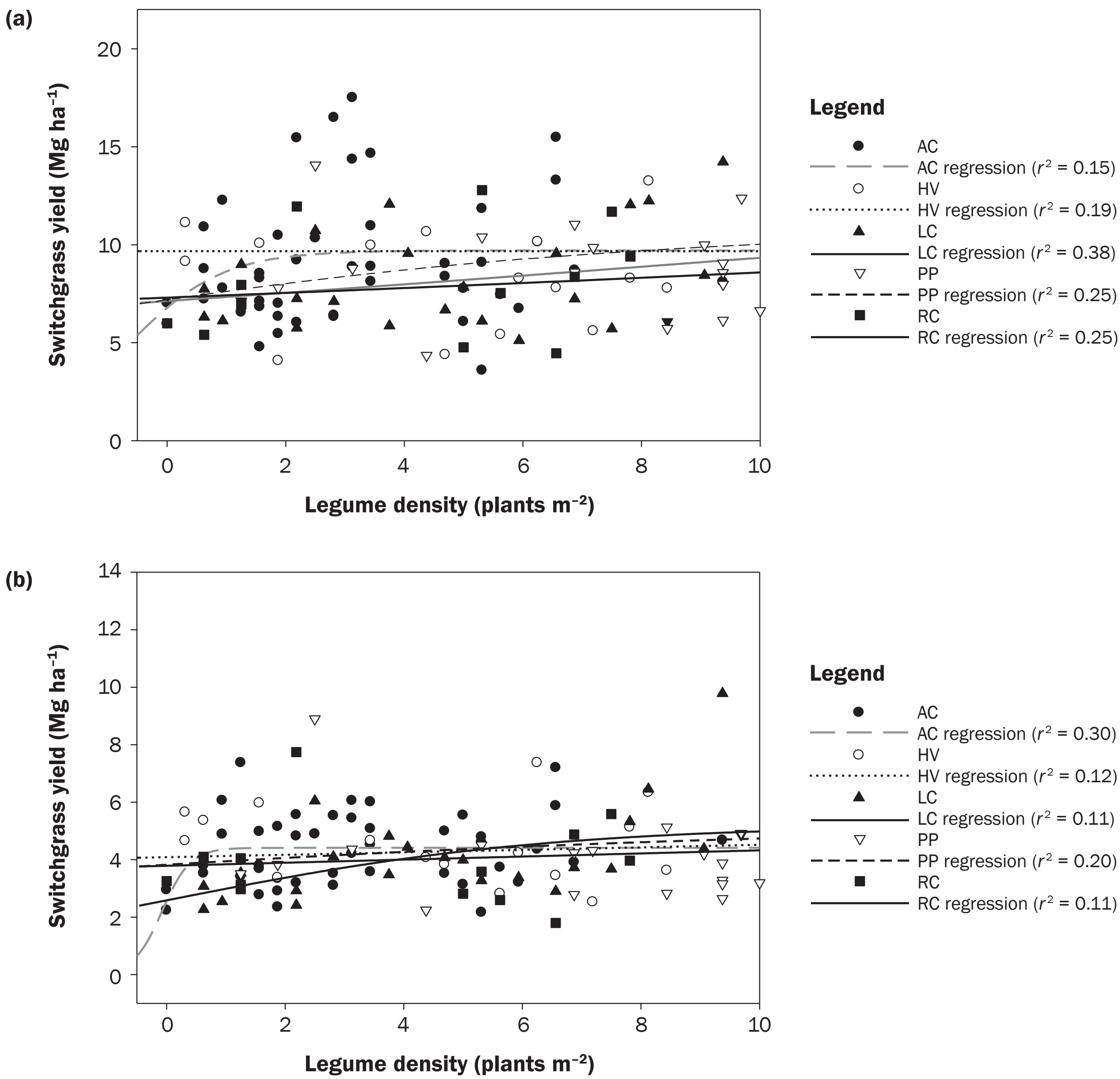


Figure 3

Switchgrass integrated ([a] early June + biomass mid-November) and biomass-only ([b] one-cut; mid-November) yields regressed against legume density by species (arrowleaf clover [AC: $Y = 9.7/ (1+ e^{-(x+0.67)/0.81})$; $Y = 4.4/ (1+ e^{-(x+0.08)/0.24})$, respectively], hairy vetch [HV: $Y = 9.7/ (1+ e^{-(x-32.2)/-2.16})$; $Y = 797.7/ (1+ e^{-(x-524.6)/99.6})$, respectively], ladino clover [LC: $Y = 22.5/ (1+ e^{-(x-18.1)/23.53})$; $Y = 5.2/ (1+ e^{-(x-0.05)/3.21})$, respectively], partridge pea [PP: $Y = 10.9/ (1+ e^{-(x+3.7)/5.5})$; $Y = 5.3/ (1+ e^{-(x+8.1)/8.5})$, respectively], red clover [RC: $Y = 494.7/ (1+ e^{-(x-254.9)/60.9})$; $Y = 8.4/ (1+ e^{-(x-8.1)/39.2})$, respectively]). Data were collected from the East Tennessee, Plateau, and Milan Research and Education Centers from 2011 to 2012, and regressed with nonlinear regression (equation: Sigmoidal, Sigmoid 3 Parameter).



cies early in the growing-season. Substantial legume density losses occurred for one of the three locations (PREC), with the lowest persistence occurring by Year 2 (1.0 plants m^{-2}), corresponding to low soil fertility at this site.

Despite differences in legume establishment success, all tested legumes could be recommended for interseeding into switchgrass (with the exclusion of arrowleaf clover, as it never established well in our study). The abundance of legumes fluctuated from establishment year to the third study year and consequently, producers would need to reestablish every 3 to 5 years (depending on intercrop species) to maintain the legume component, with the possible exceptions of ladino clover, red clover, and partridge pea. More research is needed to determine persistence long term of legumes, particularly of annual reseeding species whose densities may increase over time.

Considering neither legume density nor persistence was impacted by seeding rate, the lower seeding rates could therefore be used for proper legume establishment; albeit, red clover densities may benefit from a higher seeding rate. However, the higher seeding rate is not recommended due to the observed competition from red clover and partridge pea (particularly in bottomland areas). In addition, economic assessments of legume intercropping at lower seeding rates to determine breakeven points are needed for switchgrass biofuel and forage cropping systems to ascertain economic feasibility of intercropping systems.

There were some indications that legume intercropping may improve switchgrass forage quality results (reduced ADF, NDF, and increased CP levels), even with legume tissue removed before quality analysis. More beneficial legume intercropping results were observed during the second year, suggesting more cumulative beneficial forage quality impacts from legume integration. Specifically, there are indications that greater digestibility and intake of switchgrass-forage may occur when intercropped with partridge pea, hairy vetch, and red clover after the establishment year.

Similarly, yield during the second harvest year was more positively impacted compared to that of the first legume establishment year. In addition, of the 42% significant legume intercropping harvest regime location combinations during Year 2, the majority occurred during forage harvests. Consequently, legume intercropping might be considered more remunerating for switchgrass-forage systems. Hairy vetch, ladino clover, and partridge pea in some cases, had the greatest efficacy for improving yields when compared to medium and low inorganic-N levels (67 and 33 kg N ha^{-1} [60 and 30 lb N ac^{-1}]), and in other instances did not differ from 0 kg N ha^{-1}. However, arrowleaf clover consistently induced the least yields, generally not differing than the 0 kg ha^{-1} rate.

Overall, across all seeding rates and all species, in some cases there were marginal trends suggesting a positive relationship between switchgrass yield and legume frequency for selected legumes. For integrated and biomass yields, relationships with legume density were generally positive with increasing legume density until reaching approximately 10 plants m^{-2} (3 plants ft^{-2}). Consequently, red clover, partridge pea, and ladino clover intercrops may enhance forage quality and yield (equivalent to 33 kg N ha^{-1} [30 lb N ac^{-1}]) while reducing fertilizer costs and C-positive inputs in the mid-South.

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