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Evaluation of intercropped switchgrass establishment under a range of experimental site preparation treatments in a forested setting on the Lower Coastal Plain of North Carolina, U.S.A.

Janine M. Albaugh [a,*], *Eric B. Sucre* [b], *Zakiya H. Leggett* [b], *Jean-Christophe Domec* [a], *John S. King* [a,c]

[a] *Department of Forestry and Environmental Resources, Box 8008, North Carolina State University, Raleigh, NC 27695-8008, USA*
[b] *Weyerhaeuser NR Company, 1785 Weyerhaeuser Rd, Vanceboro, NC 28586, USA*
[c] *Department of Biology, University of Antwerp, B-2610, Wilrijk, Belgium*

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ABSTRACT

There is growing interest in using switchgrass (*Panicum virgatum* L.) as a biofuel crop and for its potential to sequester carbon. However, there are limited data on the establishment success of this species when grown as a forest intercrop in coastal plain settings of the U.S. Southeast. Therefore, we studied establishment success of switchgrass within experimental intercropped plots and in pure switchgrass plots in an intensively managed loblolly pine (*Pinus taeda*) plantation in eastern North Carolina. Pine trees were planted in the winter of 2008, and switchgrass was planted in the summer of 2009. Establishment success of switchgrass was measured over the growing season from May to October 2010, and quantified in terms of percent cover, height (cm), tiller density (number of tillers m^{-2}), leaf area index and biomass (Mg ha^{-1}). At the end of the growing season, pure switchgrass plots were taller than the intercropped treatments (114 $\pm$ 2 cm versus 98 $\pm$ 1 cm, respectively), but no significant treatment effects were evident in the other variables measured. Switchgrass biomass across all treatments increased from 2.65 $\pm$ 0.81 Mg ha^{-1} in 2009 to 4.14 $\pm$ 0.45 Mg ha^{-1} in 2010. There was no significant effect of distance from the pine row on any switchgrass growth parameters. However, we anticipate a shading effect over time that may limit switchgrass growth as the pines approach stand closure.

1. Introduction

Despite energy prices in the United States (U.S.) being among the cheapest worldwide, the economic, environmental and national security concerns associated with using fossil fuels have led to the search for renewable energy sources and development of the U.S. biofuel market. In 1985, the U.S. Department of Energy (DOE) began funding research on herbaceous species with the potential to serve as cellulosic biofuel feedstock [1]. Bioenergy and bio-based products have important environmental benefits including near-zero net emissions of greenhouse gases and improved soil and water quality [2]. Further, herbaceous energy crops can contribute to crop diversity and economic viability of growers [3]. Switchgrass (*Panicum virgatum* L.), a perennial warm-season grass, was chosen by the DOE as the model herbaceous species for

* *Corresponding author*. Tel.: +1 919 2590676; fax: +1 919 5156193.
E-mail address: Janine_Albaugh@ncsu.edu (J.M. Albaugh).
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development as a biomass energy feedstock [4,5]. This species is indigenous to the Central and North American tall-grass prairie, found in regions from the Atlantic coast to the eastern Rocky Mountains, and north into Canada [6].

Favorable characteristics of switchgrass for use as a candidate feedstock for energy production are well documented. These characteristics include its perennial nature, diverse geographic range [7] and high yield potential attained in screening trials compared with other herbaceous species [3,7–9]. In addition, switchgrass has a wide array of positive environmental attributes [1,3] which include a low nutrient demand [7], soil and water conservation benefits [7,9,10], ability to provide wildlife habitat [11], and increased below-ground carbon (C) sequestration [4,11,12] compared to annual and other perennial species. Importantly, switchgrass can be harvested with conventional agricultural equipment [12].

Using native warm-season perennial grasses as herbaceous energy crops may offer producers an alternative cash source and an opportunity to diversify their land [4]. However, there is economic risk associated with growing a crop dedicated largely to the relatively new biofuel market [13]. An alternative approach is an intercropped forestry-biofuel management system where switchgrass could be grown in rows between the crop trees. The switchgrass and any tree components not traditionally used as forest products (e.g. residues remaining after a thinning or harvesting operation) could be used as feedstock for biofuel production. The product diversity offered by such a system has important potential environmental and economic benefits which include below-ground C sequestration, potentially improved wildlife habitat for some species, lower economic risks associated with entering a new market, and increased yields with optimum use of available growing space [13].

Successful establishment of herbaceous biomass crops is essential for economically meeting land management objectives [14,15]. Failure to rapidly (within one year of seeding) establish a productive switchgrass stand reduces the economic viability of this species as a biomass feedstock [4]. Unfortunately, many warm-season perennial grasses like switchgrass can be difficult to establish [8,14,16,17]. Switchgrass stand failure may be attributed to a combination of factors which most often includes seed dormancy [6,18], incorrect seed placement [1], and competition from weeds [6,8,12]. Growth of switchgrass in the establishment year is determined by soil moisture and fertility, competition from other species [12], and soil pH, with seedling survival being significantly reduced by soil pH ≤ 4 or >8 [11,19]. Once established, well-managed switchgrass stands can successfully out-compete weeds, and require minimal maintenance [1,10]. Important elements of grass stand establishment include setting management objectives, site selection, plant material selection, site preparation, factors associated with planting (i.e. planting date and rate and depth of planting) management during establishment, and plant stand assessment, including growth and development [15].

Given the difficulty in establishing switchgrass stands, and the limited data on switchgrass establishment and growth in an intercropped system on a low pH forest soil, our objective was to determine the establishment success and quantify the growth rate of switchgrass during its second growing season when intercropped with loblolly pine (*Pinus taeda* L.), compared to pure switchgrass stands on the Lower Coastal Plain of North Carolina. Different levels of site preparation were employed in this study: the pine-switchgrass intercropped plots were V-sheared prior to planting, whereas the pure switchgrass plots were V-sheared and root raked, thereby creating a more uniform seed bed, but also a higher level of disturbance. Typically, there is a flush of soil available nitrogen (N) (Assart effect) following harvesting and site preparation, as these disturbances provide conditions suitable for rapid decomposition and nutrient release from the forest floor and harvest residue from the previous rotation [20–23]. These increases in available N are usually transitory, and only last for the first few years following harvesting [21,23]. Therefore, we hypothesized that the differences in site preparation between the pure and intercropped switchgrass stands would create a temporal gradient in soil N availability, resulting in productivity differences over the switchgrass growth rotation. Capturing the abundant mineralized soil N in switchgrass crop biomass and retaining it in root systems and subsequent soil organic C at the beginning of a rotation before the trees are large enough for significant uptake [24] could provide a mechanism for greater site N retention over the course of a tree rotation. Evidence of this has been demonstrated by Minick et al. [25], who showed that mineralized N was effectively used by switchgrass when grown as a pure stand, and when intercropped with pine.

2. Materials and methods

A long-term field study (Lenoir 1 Intercropping Sustainability Study) was established on the Lower Coastal Plain of North Carolina, U.S.A., to determine effects of intercropping and/or biomass management on site productivity and sustainability within the context of intensive forest management for production of solid wood products and biofuel feedstock. This study was established and is being maintained by Catchlight Energy, LLC (a Chevron|Weyerhaeuser Joint Venture) on forest land owned and managed by Weyerhaeuser Company. As this multifunctional intercropped production system has potential to be broadly applicable throughout the southeast U.S., we evaluated a range of potential operational treatments that could be used by forest landowners. However, it is important to note that our study is within an experimental and not an operational context.

2.1. Site description and experimental design

The field site was located in Lenoir County, on the Lower Coastal Plain of North Carolina, U.S.A. (35° N, 77° W). The soils were classified as Pantego (fine, loamy, siliceous, semiactive, thermic Umbric Paleaquults) and/or Rains (fine, loamy, siliceous, semiactive, thermic Typic Paleaquults) soil series which are deep, poorly drained, moderately permeable soils. The previous stand was a 109 ha loblolly pine plantation planted in 1974, with a site index of 21.3 m at age 25.

The study was installed as a randomized complete block design with four blocks. Treatment plots were 0.8 ha in size, with 0.4 ha measurement plots. Treatment plots had a minimum outer buffer of 15 m. As the objective of our study was to measure the establishment success of the switchgrass, the following three treatments were selected from three out of the four blocks: switchgrass-only, and two treatments where pine was intercropped with switchgrass. The two treatments where pine was intercropped with switchgrass were laid out in combination with different biomass management options, either: 1) Biomass retained (designated as 'intercropped + biomass'), where all non-merchantable logging material from harvesting the previous rotation remained on site, or 2) Biomass removed (designated as 'intercropped − biomass'), where any non-merchantable material that could potentially be used for biofuel production (i.e. coarse woody debris (CWD) >5 cm in diameter was removed by a grapple-claw excavator and piled along the sides of each treatment plot; the remaining woody material was left as groundcover within the plot boundary. Coarse woody debris mass and corresponding C and N contents removed during site preparation were quantified by Beauvais [26].

The biomass retained treatment represented standard operating procedures under normal harvesting scenarios. The pine-switchgrass intercropped treatments were V-sheared and bedded and pine trees were planted by hand during winter 2008 at 1100 stems ha^{-1} on bedded rows spaced 6 m apart. Weyerhaeuser's liquid suspension-based fertilizer with 3% N, 6.2% phosphorus (P), 2.5% potassium (K), 4.5% magnesium (Mg) and 2% calcium (Ca) was incorporated into beds to promote seedling root development and establishment.

Prior to planting the switchgrass, the inter-bed areas between the pine tree rows were V-sheared. In the switchgrass-only treatment, the entire plots were V-sheared and root raked (thereby removing most harvesting residuals). The switchgrass-only treatment had the highest level of disturbance during site preparation followed by the intercropped − biomass treatment, and the intercropped + biomass treatment. We anticipated that these varying levels of disturbance would produce a temporal gradient in soil N availability: on a short-term basis, the highest level of disturbance in the pure switchgrass stand would produce a large pulse of N compared to the lowest level of disturbance in the intercropped + biomass treatment, with the intercropped − biomass treatment intermediate between these two treatments. However, over the long-term, it is possible that without additional fertilizer, the pure switchgrass stands may become N deficient in comparison to the intercropped treatments, where there will likely be a delay in the peak N availability (especially in the intercropped + biomass treatment; see [23]). This would allow the intercropped treatments to catch up to the pure stand, unless the switchgrass in the intercropped treatments becomes shaded by the pine trees, or there are negative below-ground interactions between the pines and switchgrass at the root level.

2.2. *Extractable inorganic soil N and potentially mineralizable N*

Extractable inorganic soil N and potentially mineralizable N were determined in October 2008 after the previous rotation had been harvested and the biomass removed treatments had been installed. Soil samples were collected over a depth of 0–15 cm and were pooled from two locations per plot. Extractable inorganic N (exchangeable ammonium (NH_4^+) and nitrate (NO_3^-)) were determined by adding 50 ml of 2 mol l^{-1} KCl to 5.0 g of air-dried soil and shaken for 1 h [27]. A seven-day anaerobic incubation was used to determine potentially mineralizable soil N. This method has been used to provide an index of potentially available N released from the soil in the form of ammonium (NH_4^+) and has been frequently correlated to N uptake by plants [28,29]. For each soil sample, 5.0 g of air-dried soil was added to a glass vial and filled with deionized water to ensure that the sample was devoid of oxygen. Following the seven-day incubation, samples were quantitatively transferred using 3 mol l^{-1} KCl and shaken for 1 h. Final sample extractions contained a 10:1 KCl:soil ratio with a final normality of 2 [30]. Extracts from the inorganic N and potentially mineralizable N procedures were analyzed using a Bran and Leubbe TRACCS 2000 Auto-Analyzer (SPX Corporation); these were expressed in mg kg^{-1} and converted to a kg ha^{-1} basis.

2.3. *Switchgrass establishment*

Switchgrass (cultivar Alamo) seed was machine-planted in June 2009 at 9 kg pure live seed ha^{-1} in rows spaced 40 cm apart. Seed was planted at a depth of 0.6 cm and covered with soil. These seeding rates and planting depths were consistent with recommended values [10,31]. Simultaneous with planting, the aforementioned liquid suspension fertilizer was applied between each row of switchgrass. The combination of high phosphorus and base cations in this fertilizer was intended to increase soil pH, which was inherently very acidic (pH 3.9), and promote root growth. In the switchgrass-only plots, the entire 0.8 ha plot was planted to switchgrass in rows spaced 40 cm apart. In the intercropped plots, switchgrass seed was planted between each pine row in a swath approximately 2 m-wide; there were six 2 m-wide rows of intercropped switchgrass in each 0.4 ha measurement plot. Switchgrass plots were sprayed with 2,4–D (4.68 ℓ ha^{-1}) and a post-emergent herbicide (Basagran; 0.88 ℓ ha^{-1}) in May 2010. Weyerhaeuser's coated Arborite® fertilizer, supplying 65.6 kg N ha^{-1}, 6.6 kg P ha^{-1} and 0.2 kg boron ha^{-1}, was applied in June 2010. Fertilizer levels were determined based on published literature, where typical recommendations of N applications for switchgrass range from 50 to 112 kg ha^{-1} [32–35].

2.4. *Switchgrass measurements*

Establishment success was determined by assessing percent cover, tiller density (measured as number of tillers m^{-2}; a tiller is an individual grass shoot comprised of a meristem, leaves, stem, roots and latent buds), height growth (cm), leaf area index (m^2 m^{-2}), and quantifying the biomass (Mg ha^{-1}) of switchgrass and other competing vegetation (competing vegetation refers to all non-crop vegetation); see methods for each measurement described below. Measurements commenced in May 2010 and switchgrass growth was assessed on a monthly basis until October 2010 (measurement dates were 5 May (day of year (DOY) 125), 9 June (DOY 160), 14 July (DOY 195), 18 August (DOY 230), and 6 October (DOY 279)). By October 2010, more than 55% of the most recent fullyformed leaves of the measured switchgrass tillers were undergoing senescence, or had senesced. No measurements were collected during September, when the site was flooded (389 mm of rain fell during September; Fig. 1). The switchgrass was harvested after complete senescence (end of November 2010) and the biomass produced was compared to that harvested at the end of the 2009 growing season.

2.4.1. *Sampling design*

Switchgrass percent cover, tiller height (cm) and tiller density (number of tillers m^{-2}) measurements in the pine-switchgrass intercrop treatments were based on a stratified random sampling approach: the 2 m-wide planted switchgrass area between each pine row was divided into three zones and an equal number of measurements ($N = 30$) were randomly collected from each zone. At each measurement point, the distance (m) to the nearest pine row was recorded to determine whether there was a significant effect of distance from a pine row on any of the switchgrass growth parameters measured. The same sampling design was used in the switchgrass-only plots for comparison across all treatments, i.e. in each switchgrass-only plot, six 2 m-wide permanent sampling areas were established; these areas were divided into three zones, and an equal number of measurements ($N = 30$) were randomly collected from each zone.

2.4.2. *Percent cover*

Percent cover was quantified monthly from May to July using the point intercept method [36], in which a sampling pole is placed at systematic intervals to sample within plot variation and quantify statistical changes in plant species cover and height over time. We measured percent cover with a vertically-oriented 2 cm diameter sampling pole which was carefully lowered to the ground at each sample point. The starting point was randomly assigned and the distance between points was approximately 6 m. Contact of the sampling pole with any part of a switchgrass plant was scored as a “hit”. Only one hit was recorded for each contact with the switchgrass, regardless of the pole intercepting a plant more than once. The number of hits from 90 points per plot (15 evenly spaced points per switchgrass row) was tallied, and cover was calculated as the percentage of hits relative to the total number of points sampled [37]. Percent cover estimates were discontinued after July, due to the increase in height of the switchgrass, where the sampling pole would have to be lowered from a position above the observer’s head, making it difficult to accurately read the sampling pin.

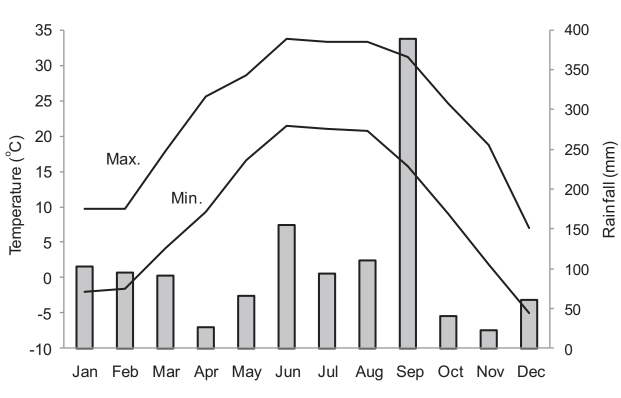

Fig. 1 – Average monthly minimum (Min.) and maximum (Max.) temperature and rainfall data measured at the field site on the Lower Coastal Plain of North Carolina during 2010. The line graphs represent the temperature, and bars represent the rainfall data.

2.4.3. *Height growth*

Switchgrass stem height (cm) was measured from May to July as the mean height from the soil to the collar of the most recent fully expanded leaf blade at each of the locations where a “hit” was scored for the percent cover estimate. Sanderson [17] refers to the most recent fully expanded leaf blade as one where the collar is fully visible. As the percent cover estimates were discontinued from July, height growth in August and October was measured at 90 points per plot based on the stratified random sampling approach.

2.4.4. *Tiller density*

Tiller density was determined from May to August by counting the number of live tillers rooted within three 0.25 m^2 quadrats per plot. Quadrat location was based on the stratified random sampling design; one quadrat was centered in each of the three zones. Tiller counts were scaled to a m^2 basis.

2.4.5. *Leaf area index*

Leaf area index (LAI) was measured from June to October using the Li-Cor LAI2000 plant canopy analyzer [38]. Eighteen LAI measurements (three measurements per switchgrass row) were collected per plot within 3 h of sunrise or sunset. Three measurements were collected at random intervals in the middle of each switchgrass row using a 15° view cap to ensure the sensor was restricted to sampling within the planted switchgrass area. One above-canopy light measurement was collected above the height of the switchgrass followed immediately by a below-canopy light measurement at ground level. Leaf area index estimates were not adjusted for other vegetation and are therefore more representative of total cover than pure switchgrass LAI.

2.4.6. *Aboveground biomass*

Aboveground switchgrass biomass samples, cut at a height of 10 cm above the soil surface, were collected at the end of the growing season in November 2009 and 2010, once senescence was complete. The 2009 samples were collected from five randomly located 1×1 m^2 quadrats per plot; the 2010 samples were collected from five randomly located 1×3 m^2 quadrats per plot. In the intercropped treatments, the quadrats were positioned in the center of the planted switchgrass area. Samples were transported to the laboratory, dried at 70 °C to a constant mass and weighed. Biomass values were scaled to a hectare value to estimate switchgrass biomass on a land area basis.

2.5. *Statistical analyses*

We used repeated measures analysis of variance (ANOVA) to test the hypothesis that stand establishment was not different among treatments using percent cover, tiller height and density, and leaf area index as response variables, treatment, day of year and treatment × day of year interactions as fixed effects, block as a random effect, and day of year as the repeated measure. We used a mixed model analysis of variance (Proc. Mixed [39]) to examine each growth metric over the growing season using a heterogeneous autoregressive model of the variance/covariance matrix structure. An ANOVA (Proc. GLM [39]) was performed to test the null hypothesis that removal of CWD during site preparation did not affect extractable inorganic soil N or potentially mineralizable N. Similarly, an ANOVA (Proc. GLM [39]) was performed to test the null hypothesis that treatment did not differ with respect to aboveground switchgrass or competing vegetation biomass harvested at the end of the growing season in 2009 and 2010. As there was no significant treatment difference within a year, values from the three treatments were pooled and comparisons made between biomass values in 2009 and 2010. We used regression analyses (Proc. REG [39]) to test the null hypothesis that there was no effect of distance from the nearest pine row on switchgrass growth parameters. When a significant difference was observed using the mixed model ANOVA, least square means were compared using the Tukey–Kramer adjustment method for multiple comparisons between treatments. Dependent variables were checked for normality and homoscedasticity and transformed as necessary. Biomass data were Log_e transformed; all means and standard errors are presented as untransformed values. In all cases, an $\alpha = 0.05$ significance level was used.

3. Results

3.1. Weather data

Historical average daily temperature at this site was 16.5 °C and annual precipitation was 1262 mm. The average temperature measured during the growing season (April to September, 2010) was 24.2 °C (Fig. 1). Minimum and maximum temperatures of 0.7 °C and 39.5 °C were recorded in April and July, respectively. Precipitation over the growing season measured 840 mm, with a minimum of 26 mm recorded in April. Maximum rainfall occurred in September (389 mm compared to the long-term September average of 127 mm). Annual precipitation for 2010 was 1252 mm.

3.2. Biomass removal during site preparation

As expected, root raking the switchgrass-only treatment removed more CWD and C and N contained in this material compared to the intercrop – biomass treatment. In the pure switchgrass treatment, 9.6 Mg CWD ha^{-1} containing 4.6 Mg C ha^{-1} and 14.9 kg N ha^{-1} were removed, compared to 6.1 Mg CWD ha^{-1} containing 2.9 Mg C ha^{-1} and 9.5 kg N ha^{-1} in the intercrop – biomass treatment (Table 1 [26]).

Assessment of CWD and C and N contents contained within this material at the beginning of the trial indicated there was approximately six times the amount of CWD and C and N distributed on the soil surface in the intercropped + biomass compared to the intercropped – biomass treatment. There was 10.6 Mg CWD ha^{-1} containing 5.1 Mg C ha^{-1} and 15.5 kg N ha^{-1} in the intercrop + biomass treatment versus 1.7 Mg CWD ha^{-1} containing 0.8 Mg C ha^{-1} and 2.7 kg N ha^{-1} in the intercrop – biomass treatment (Table 1).

3.3. Extractable inorganic soil N and potentially mineralizable N

Removal of CWD did not affect extractable inorganic soil NH_4^+ or NO_3^- ($p = 0.98$ and $p = 0.50$, respectively; data not shown) measured in October 2008. Therefore, data were pooled across the three treatments to yield extractable NH_4^+ and NO_3^- concentrations of 26.0 ± 2.2 kg ha^{-1} and 5.0 ± 1.9 kg ha^{-1}, respectively. Similarly, potentially mineralizable N, which measured 66.8 ± 11.8 kg ha^{-1}, did not vary with treatment ($p = 0.37$, data not shown).

3.4. Percent cover

Switchgrass percent cover increased linearly in all treatments over the period May (day of year (DOY) 125) to July (DOY 195), from 36 ± 4% to 60 ± 7%, respectively (Fig. 2). Treatment differences were not significant (Table 2).

3.5. Height growth

Switchgrass height increased from 18.7 ± 0.9 cm in May (DOY 125) to 114.3 ± 1.5 cm in October (DOY 279) in the pure switchgrass treatment (Fig. 3), which was significantly taller than the intercropped treatments from July (DOY 195) until measurements ceased in October (Tables 2 and 3). The pure switchgrass treatment was 23% taller than the intercropped treatments over the course of the season. There was no difference between the intercropped treatments; final tiller height in these treatments measured 97.5 ± 1.1 cm in October. Switchgrass height growth followed a sigmoidal curve, with a rapid increase in height early in the growing season, followed by slower height growth from July (DOY 195) until measurements ceased in October.

Table 1 – Mass of coarse woody debris (CWD) and carbon (C) (Mg ha^{-1}) and nitrogen (N) (kg ha^{-1}) contents contained in the CWD removed during site preparation in a pine-switchgrass intercropped study on the Lower Coastal Plain of North Carolina during October 2008. Values presented are the average amounts removed or retained in place at the beginning of the trial for the switchgrass-only treatment, and for loblolly pine intercropped with switchgrass where material from harvesting the previous rotation was retained on site (intercrop + biomass) or removed (intercrop – biomass) [26]. Values presented are means and (standard errors).

Treatment	Removed			Retained		
	CWD	C	N	CWD	C	N
Switchgrass-only	9.6 (2.0)	4.6 (1.0)	14.9 (3.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Intercrop + biomass	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	10.6 (2.1)	5.1 (1.0)	15.5 (2.4)
Intercrop – biomass	6.1 (0.6)	2.9 (0.3)	9.5 (1.0)	1.7 (0.6)	0.8 (0.3)	2.7 (0.9)

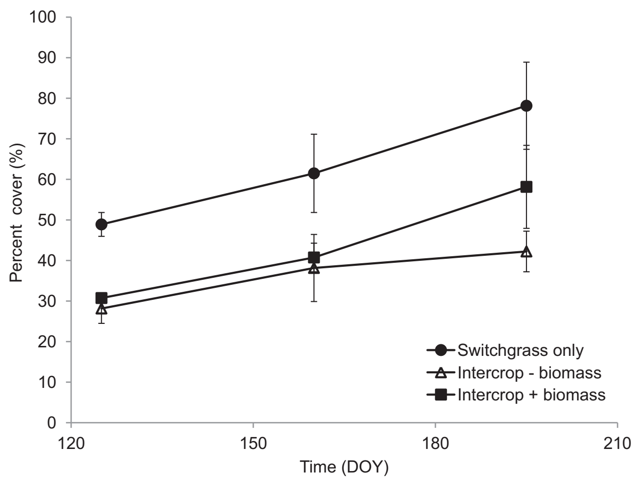

Fig. 2 – Increase in switchgrass percent cover during the 2010 growing season expressed as day of year (DOY) in the switchgrass-only treatments, and in the pine-switchgrass intercropped treatments where material from harvesting the previous rotation was retained (intercrop + biomass) or removed (intercrop − biomass). Values presented are means; error bars indicate one standard error of the mean.

3.6. *Tiller density*

There were no significant treatment effects on tiller density (Fig. 4, Table 2). Tiller density increased from an average of 141 ± 19 tillers m^{-2} in May (DOY 125) to reach a seasonal maximum of 463 ± 52 tillers m^{-2} in July (DOY 195), which then decreased to 342 ± 32 tillers m^{-2} in August (DOY 230) (Fig. 4).

3.7. *Effect of distance from the nearest pine row on switchgrass growth*

Switchgrass measurements collected across the width of the planted switchgrass area in the intercropped treatments and regressed against the distance from the nearest pine row indicated there was no significant effect of position from the nearest pine row on switchgrass percent cover, height or tiller density (data not shown).

Table 2 – Mixed model analysis of variance results ($P > F$) for switchgrass percent cover, height (cm), tiller density (number of tillers m^{-2}) and leaf area index (LAI) measured over the 2010 growing season in a pine-switchgrass intercropped study on the Lower Coastal Plain of North Carolina. The day of year (DOY) × DOY interaction was not significant (n.s.) for percent cover and was removed from the model.

	Percent cover	Height	Tiller density	LAI
Treatment	0.613	0.308	0.917	0.617
Day of year (DOY)	<0.001	<0.001	<0.001	<0.001
Treatment × DOY	0.397	0.049	0.372	0.094
DOY x × DOY	n.s.	<0.001	<0.001	<0.001

3.8. *Leaf area index*

There was no significant treatment effect on switchgrass leaf area index which increased from 1.6 ± 0.2 m^2 m^{-2} in June (DOY 160), reached a peak of 2.7 ± 0.2 in August (DOY 230), and declined to 2.4 ± 0.3 m^2 m^{-2} in October (DOY 279; Fig. 5).

3.9. *Switchgrass aboveground biomass and competing vegetation*

As there was no treatment effect on the amount of switchgrass or competing vegetation produced in either 2009 or 2010 ($p = 0.32$ and $p = 0.70$ in 2009 and 2010, respectively; data not shown), biomass data from the three treatments were pooled and comparisons made between 2009 and 2010; these were not significant for switchgrass ($p = 0.19$) or competing vegetation biomass ($p = 0.26$). Switchgrass biomass across all treatments increased from 2.65 ± 0.81 Mg ha^{-1} in 2009 to 4.14 ± 0.45 Mg ha^{-1} in 2010, which was accompanied by a decrease in the amount of competing vegetation, which declined from 0.81 ± 0.17 Mg ha^{-1} in 2009 to 0.50 ± 0.11 Mg ha^{-1} in 2010.

4. Discussion and conclusions

Our objective was to determine the establishment success and quantify the growth rate of switchgrass during its second growing season when intercropped with loblolly pine, compared to pure switchgrass stands. In this study, switchgrass grown in the pure treatments was significantly taller than where it was intercropped with loblolly pine, but there was no significant treatment effect on any of the other measured variables (percent cover, tiller density, leaf area index or aboveground biomass).

The seasonal development patterns observed for switchgrass height, tiller density and leaf area growth (see Figs. 3–5) were consistent with other studies, e.g. [40–44]. The pattern of switchgrass tiller density development (Fig. 4), which reached a seasonal maximum and then declined, has been described by Hernández Garay et al. [45] as a size-density compensation, where the tiller number decreases, but biomass increases. Similar results have been reported in other studies (e.g. [46].).

There is considerable information on switchgrass establishment, growth and biomass yield (see for example [5,8,11,14,44]); however, most of these data are from small test plots, some of which were established only for screening purposes, and while there are some data from field trials, none exist from an intercropped system with forest trees established at an operational scale on the Lower Coastal Plain of North Carolina.

Switchgrass tiller heights measured in this study are comparable to those reported from other trials across the U.S. [40,44] and Canada [41]. Whilst switchgrass tiller density and leaf area index development followed a similar pattern of development to other studies, maximum values for leaf area index measured in this study were lower than those reported in the literature. Maximum tiller density (568 tillers m^{-2}) was measured in the pure switchgrass planting in July (Fig. 4), compared to values of 580 tillers m^{-2} (southeast U.S. region

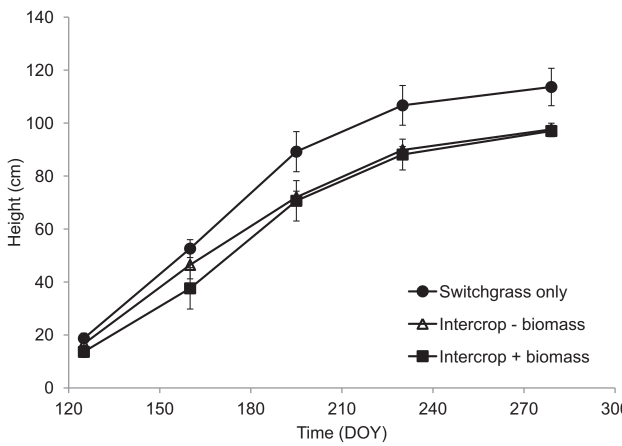

Fig. 3 – Switchgrass height growth during 2010, expressed as day of year (DOY) in the switchgrass-only treatments, and in the pine-switchgrass intercropped treatments where material from harvesting the previous rotation was retained (intercrop + biomass) or removed (intercrop − biomass). Values presented are means; error bars indicate one standard error of the mean.

[47]), 128–772 tillers m^{-2} (southeast U.S. [48]), 797 tillers m^{-2} (Midwest region [44]), and 356–947 tillers m^{-2} (Canada [41]). The peak leaf area index (3.3 m^2 m^{-2}) was recorded in the pure switchgrass planting in October (Fig. 5), which is lower than the range of 4.4–8.0 reported in the literature [42–44,49]. There are several possible reasons for the lower leaf area index values observed in the current study; these include the small scale of the test plots in studies from the literature (ranging in size from 3.7 × 0.9 m to 10 × 10 m), the use of different switchgrass cultivars (compared to Alamo used in this study) grown in different regions across the U.S. and Canada, some experiments were hand-weeded, others were seeded with a higher rate of pure live seed, or were re-seeded to ensure adequate stand populations, and some of the studies were well-established (>three years old). Importantly, our study was on a forested site with a lower soil pH, less uniform seed bed conditions, and history of minimal management and inorganic inputs compared to an agricultural setting. While mature switchgrass is relatively tolerant of diverse soil and climatic conditions, seedling survival can be significantly reduced by soil pH ≤ 4.0 or >8.0 [11]. The optimal soil pH for successful switchgrass seed germination is 6.0 [19]. Although the pre-treatment soil pH at this site was 3.9, use of the pre-plant fertilizer with adequate magnesium and calcium, increased the soil pH in switchgrass plots to 4.5–4.8.

Research on switchgrass as a biomass energy crop has shown yields of well-established stands from several sites across the southeastern, south central and mid Atlantic U.S. range from 12.2 to 26.0 Mg ha^{-1} [4], from 6.7 to 21.3 Mg ha^{-1}, from 13.0 to 26.6 Mg ha^{-1} [5], and from 9.8 to 16.6 Mg ha^{-1}, over several years growth [11]. According to George et al. [10], switchgrass biomass production of 13 Mg ha^{-1} across the state of North Carolina is expected to be a good yield average with existing cultivars in well-managed stands. As an agricultural crop, switchgrass produces high yields over much of the U.S., from 2.2 to 9.0 Mg ha^{-1} in North Dakota, from 11.2 to 13.4 Mg ha^{-1} in the western Corn Belt and 15.7 to 35.8 Mg ha^{-1} in the southeast [50].

This study was a large and variable field experiment; there were patches of bare soil where either the switchgrass seed was not sown, or it was sown but did not germinate. Further, this was a dynamic system with cool-season grasses and sedges being replaced by warm-season grasses and broadleaf species in mid- to late summer (visual assessment). In well-established switchgrass stands, competing vegetation is less problematic as weeds do not readily encroach into well-managed switchgrass [1]. Once established, switchgrass plantations are robust, with an ability to grow over and shade out low growing plants [10]. Switchgrass is a small-seeded species that initially allocates a large amount of energy to developing a strong root system. As a result, switchgrass

Table 3 – Differences of the least square means using the Tukey–Kramer adjustment method to compute P-values (Adj. *P*) for pairwise comparisons between pure switchgrass, loblolly pine intercropped with switchgrass where the material from harvesting the previous rotation was retained on site (intercrop + biomass), and where this harvesting material was removed (intercrop − biomass) for tiller height (cm) measured over the 2010 growing season in a pine-switchgrass intercropped study on the Lower Coastal Plain of North Carolina from May to October (day of year (DOY) 125–279, respectively).

Treatment comparison		DOY	t-value	P > |t|	Adj. P
Switchgrass	Intercrop + biomass	125	1.50	0.142	0.302
Switchgrass	Intercrop − biomass	125	0.93	0.359	0.626
Intercrop + biomass	Intercrop − biomass	125	0.57	0.570	0.835
Switchgrass	Intercrop + biomass	160	2.42	0.021	0.052
Switchgrass	Intercrop − biomass	160	2.09	0.044	0.107
Intercrop + biomass	Intercrop − biomass	160	0.34	0.739	0.940
Switchgrass	Intercrop + biomass	195	2.72	0.010	0.026
Switchgrass	Intercrop − biomass	195	2.63	0.013	0.033
Intercrop + biomass	Intercrop − biomass	195	0.10	0.925	0.995
Switchgrass	Intercrop + biomass	230	2.71	0.010	0.027
Switchgrass	Intercrop − biomass	230	2.77	0.009	0.023
Intercrop + biomass	Intercrop − biomass	230	−0.06	0.952	0.998
Switchgrass	Intercrop + biomass	279	2.59	0.014	0.036
Switchgrass	Intercrop − biomass	279	2.78	0.009	0.023
Intercrop + biomass	Intercrop − biomass	279	−0.18	0.856	0.982

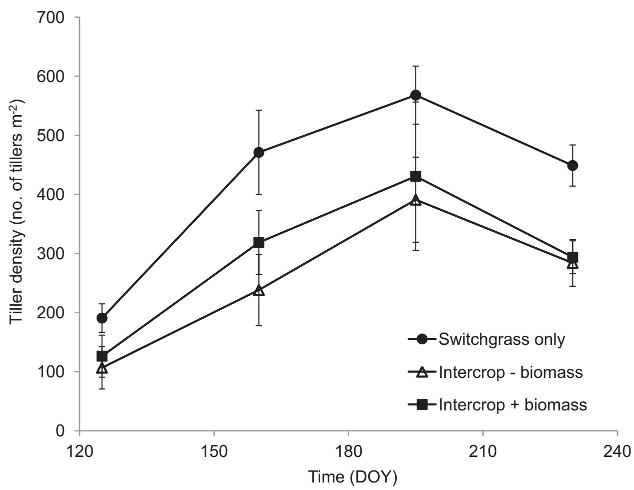

Fig. 4 – Switchgrass tiller density during 2010, expressed as day of year (DOY) in the switchgrass-only treatments, and in the pine-switchgrass intercropped treatments where material from harvesting the previous rotation was retained (intercrop + biomass) or removed (intercrop − biomass). Values presented are means; error bars indicate one standard error of the mean.

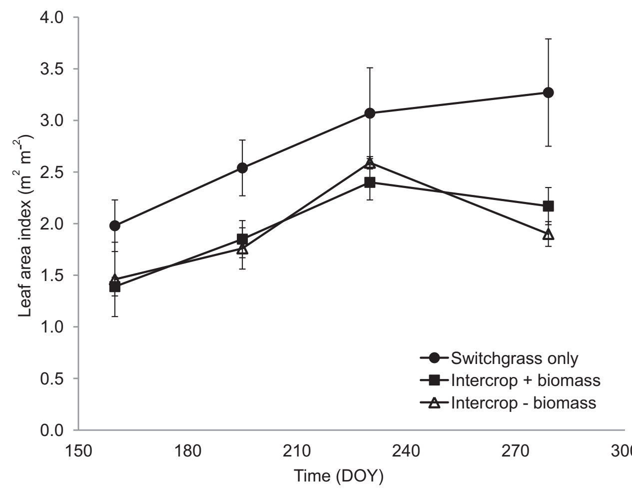

Fig. 5 – Seasonal development of switchgrass leaf area index, expressed as day of year (DOY) in the switchgrass-only treatments, and in the pine-switchgrass intercropped treatments where material from harvesting the previous rotation was retained (intercrop + biomass) or removed (intercrop − biomass). Values presented are means; error bars indicate one standard error of the mean.

typically only attains between 33 and 66% of its maximum production capacity during the first and second years before reaching its full capacity during the third year after planting [11]. However, results observed by Heaton et al. [44] led these authors to suggest that switchgrass had not reached ceiling yields in three years. We anticipate that as the switchgrass continues to grow and spread in our study, it will capture the site and out-compete other vegetation. Evidence supporting this is the 56% increase in switchgrass biomass across all treatments from the first (2009) to the second year (2010) of growth, and a decrease in competing vegetation: aboveground switchgrass biomass increased from an average of 2.7 Mg ha^{-1} across all treatments in 2009 to 4.1 Mg ha^{-1} in 2010; the amount of competing vegetation in 2009 was 0.8 Mg ha^{-1} compared to 0.5 Mg ha^{-1} in 2010. We anticipate the switchgrass biomass yield will continue to increase, possibly reaching a maximum at the end of the 2012 growing season.

A greater amount of N was removed with the CWD in the switchgrass-only and intercropped – biomass treatments (14.9 and 9.5 kg N ha^{-1}, respectively), compared to the intercropped + biomass treatment (Table 1). Despite this, removal of CWD did not affect extractable inorganic soil NH_4^+ or NO_3^-, or potentially mineralizable N measured in October 2008. In addition, there was no treatment effect on aboveground switchgrass biomass produced at the end of 2009 or 2010. This implies that the inherent soil fertility of the site was sufficient for switchgrass establishment and growth. However, there was much variability associated with the switchgrass growth measurements (Figs. 2–5). A field experiment of this size limits sampling replication for practical reasons. Whilst we hypothesized that the differences in site preparation between the treatments would create a temporal gradient in soil N availability, resulting in productivity differences, limited replication combined with the variation associated with the large physical area covered by the plots did not allow us to detect treatment differences as statistically significant, either in terms of variables (percent cover, tiller density, leaf area index, biomass) measured over the 2010 growing season, or in the increase in biomass yield from 2009 to 2010. According to Hanson and Johnson [19], there are large variations in the establishment success of switchgrass when new stands are initiated, and switchgrass yields are variable in the early (<3) years after planting [51]. We expect variability to decrease over time as the switchgrass captures the site; however, variability will likely increase again in the intercropped plots as the switchgrass becomes shaded as the pine trees approach canopy closure.

The intercropped system examined in our study allows for optimum use of the available growing space by planting switchgrass between the pine rows [13]. Regression analyses based on the stratified random sampling approach adopted in the intercropped treatments revealed that at this stage, proximity to a pine row did not affect any switchgrass growth parameter measured, nor was there any significant biomass difference between the pure switchgrass planting and the intercropped treatments. However, as we are uncertain how this will change as this intercropped system develops over time, a future focus for this project is to determine potential interactions between the pines and the switchgrass, that is, whether they compete or complement each other in terms of resource utilization. It is possible that these potential interactions can occur below-ground at the root level, and aboveground as light is intercepted by the pines, and subsequent shading may affect the switchgrass growth.

This study is the first to demonstrate that switchgrass can successfully be established as an intercrop in forested settings on the Lower Coastal Plain of North Carolina. However, the economic and C cycling consequences are yet to be determined. Even though we focused only on quantifying the biomass production, other factors such as switchgrass establishment, harvesting and transport costs [10], farm-gate feedstock cost, conversion efficiency of switchgrass to ethanol [52], and other factors such as government tax incentives, will play a large role in determining the economic viability of this type of system.

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