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Adaptation of C_4 Bioenergy Crop Species to Various Environments within the Southern Great Plains of USA

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Abstract: As highly productive perennial grasses are evaluated as bioenergy feedstocks, a major consideration is biomass yield stability. Two experiments were conducted to examine some aspects of yield stability for two biofuel species: switchgrass (*Panicum vigratum* L.) and *Miscanthus x giganteus* (*Mxg*). Biomass yields of these species were evaluated under various environmental conditions across the Southern Great Plains (SGP), including some sites with low soil fertility. In the first experiment, measured yields of four switchgrass ecotypes and *Mxg* varied among locations. Overall, plants showed optimal growth performance in study sites close to their geographical origins. Lowland switchgrass ecotypes and *Mxg* yields simulated by the ALMANAC model showed reasonable agreement with the measured yields across all study locations, while the simulated yields of upland switchgrass ecotypes were overestimated in northern locations. In the second experiment, examination of different N fertilizer rates revealed switchgrass yield increases over the range of 0, 80, or 160 kg N ha^{-1} $year^{-1}$, while *Mxg* only showed yield increases between the low and medium N rates. This provides useful insights to crop management of two biofuel species and to enhance the predictive accuracy of process-based models, which are critical for developing bioenergy market systems in the SGP.

Keywords: ALMANAC; switchgrass; *M. x giganteus*; bioenergy; climate; nitrogen

1. Introduction

Climate change or global warming, a gradual increase in average global temperature, is now well documented and widely accepted by scientists. To reduce the atmospheric CO_2 level, the Environmental Protection Agency (EPA) has recommended using more renewable energy from solar, wind, and bioenergy sources [1]. These renewable sources will play a role in providing energy services in a sustainable manner, in particular, in mitigating climate change [2,3]. Among renewable energy sources, bioenergy has the unique advantage of providing solid, liquid, and gaseous fuels that can be stored, transported, and utilized far from where they are produced [4]. However, current bioenergy production is associated with environmental challenges such as increases in net greenhouse gas emissions from direct/indirect land use changes, increased fertilizer use and use of fossil fuel powered equipment for crop management (e.g., harvest and tillage) [5,6]. To solve some of challenges, cellulosic biofuel species have gained attention for biofuel production [7]. Cellulosic biofuel crops can be produced on marginal lands not suitable for food crops and require relatively little fertilizer [7,8]. In addition, these biofuel crops can be pressed into dry pellets that can be used for heating and generation of electrical power [9,10].

Although cellulosic biofuel species have been considered a promising renewable energy source for decades, neither processing nor end-markets for cellulosic bioenergy feedstocks are fully developed. Uncertainty in regional adaptability and yield stability of biofuel plant species increases potential feedstock growers' concerns. Moreover, limited available land area can be a challenge to growers trying to find feasible dedicated feedstocks with consistently high yields in different environmental conditions, including soil nutrient limitations. For the success of the bioenergy industry, including potential growers, information on bioenergy feedstock productivity and stability in different environments, with particular emphasis on marginal lands, is needed to assess feasible and reduce investment risks. Switchgrass (*Panicum vigratum* L.) and *M. x giganteus* (*Miscanthus x giganteus*) are key potential cellulosic feedstocks for bioenergy production in the USA [11–13]. Evaluating the adaptability and production of these bioenergy crops across various geographic regions as well as in different environmental conditions, such as limited soil nutrient availability in soil, will provide important information for the development of the bioenergy industry.

Switchgrass and *M. x giganteus* are C_4 warm-season grasses capable of fast growth and steady high yield production in marginal locations not suited to food crop production [14]. These grasses have different yield potentials in different environments. Switchgrass is characterized by a wide degree of genetic variation, which results in broad geographic adaptations [15]. Switchgrass can be grouped into lowland and upland ecotypes, which are adapted to different edaphic conditions. Lowland ecotypes generally have high yields in the southern USA, whereas upland ecotypes generally have high yield potentials in the drier, colder Northern Great Plains [16,17]. *M. x giganteus* originated in East Asia and has been studied across Europe since 1983 [18,19]. Selected in the University of Illinois at Urbana-Champaign, USA in 1988 [20], *M. x giganteus* had promising initial data for biomass production in the USA [12,21]. Unlike switchgrass, *M. x giganteus* has limited genetic diversity with few genotypes available in the USA [22]. According to Glowacka et al. [22] who compared genetic similarities among a broad sample of *M. x giganteus* accessions from different locations, the accessions of *M. x giganteus* are genetically identical. It will be interesting to follow the adaptation of new *M. x giganteus* progeny such as "Freedom^{TM}" [23,24], "Amuri" [24], and "Nagara" [24,25] (which were released after our field study was initiated) in future studies.

This could restrict its geographic adaptability as suggested by the broad range of dry matter yield observed in the US Midwest. For instance, *M. x giganteus* can produce large amounts of biomass in central Illinois [11,20,26,27], while, in Kansas, it produces much lower biomass yield [11,28]. Biomass production of switchgrass and *M. x giganteus* vary significantly with N availability [12,19,29–33]. Both switchgrass and *M. x giganteus* are perennial rhizomatous grasses that efficiently translocate and store nutrients during leaf senescence, enabling them to efficiently use soil nutrients [11,34]. Both grasses need less than one-third of the amount of N required for maximizing maize (*Zea mays* L.) yield [12,26,35]. In the upper Midwestern USA, *M. x giganteus* is more productive and is likely to require less N than switchgrass [24,36]. Numerous studies have shown that switchgrass yields continuously increased with N addition between 0 and 160 kg N ha^{-1} $year^{-1}$ [12,30,32,33], while *M. x giganteus* yield increases were only shown with nitrogen fertilization of 50 to 70 kg N ha^{-1} $year^{-1}$ [19,29,31]. However, most of these studies on productivity of *M. x giganteus* and switchgrass were in the Central or Northern Great Plains. A contrasting result was found in central Texas. Compared to a high-fertility, irrigated part of a field, an adjacent area with no irrigation or added fertility had a 54% drop in "Alamo" switchgrass yield and a 72% drop in *M. x giganteus* yield [37]. Yields for switchgrass were 1.96 Mg ha^{-1} irrigated and 0.91 Mg ha^{-1} dryland. The values for *M. x giganteus* were 1.63 Mg ha^{-1} irrigated and 0.45 Mg ha^{-1} dryland. Thus the potential yield of *M. x giganteus* and switchgrass, even under various N rates, in the Southern Great Plains appears to differ drastically from the Midwestern US.

The Southern Great Plains (SGP) of the U.S. encompasses large areas of rangeland, dryland farms, and some irrigated areas [38], and will play an important role, as biofuel production is targeted for various "marginal" conditions. The SGP comprises an east-west precipitation gradient and north-south variations in soil type and topography. These differences lead to large variation in productivity of switchgrass and *M. x giganteus* across the regions. In addition, the SGP has experienced repeated and severe droughts, especially during summer, which limit crop production [39]. Therefore, the SGP is well-suited to quantify the productivity of these biofuel grasses in a broad range of environmental conditions.

In this study, two experiments were conducted. In the first experiment, yields of *M. x giganteus* and four switchgrass ecotypes were collected over multiple years at six locations in SGP regions in Texas, Louisiana, Oklahoma, and Missouri. This study expands upon previous research [40] which evaluated switchgrass productivity at multiple locations in the SGP by adding three additional years of yield data and a new site. The purpose of this study was to evaluate the most productive perennial plant variety in each location. This research is critical to identify the suitability of switchgrass ecotypes and *M. x giganteus* for SGP region and to test regional adaptability and stability of these biofuel crops. A process based model, ALMANAC (Agricultural Land Management Alternative with Numerical Assessment Criteria) [41–43], was used to simulate yields for different environmental effects including regional weather and soil characteristics. ALMANAC simulations of these perennial biofuel grasses will provide realistic predictions of biofuel production under various environmental conditions in the SGP region. The second experiment was designed to find the optimal amounts of N fertilizer to enhance switchgrass and *M. x gigenteus* yields at a single location in the SGP region. The purpose of this study is to investigate the effects of organic and inorganic fertilizer on yields of the two crops grown in multiple years in the SGP. This research could improve crop establishment and crop management, which are critical factors for promoting higher biofuel biomass production in the SGP.

2. Materials and Methods

2.1. Experiment 1: Evaluating Biomass Production in Multiple Locations

Six locations (Table 1) with different annual precipitation and different soil characteristics (type, electrical conductivity (EC), and sodium adsorption ratio (SAR)) across south-central USA were used in this study. Soil EC and SAR were used to evaluate the soil conditions, such as the level of salinity. EC is a measure of the amount of salt in soil (salinity), while SAR is an index for describing the proportion of sodium to calcium and magnesium in soil solution (sodicity). Seeds and fresh rhizomes of five perennial biofuel species including two switchgrass lowland ecotypes ("Alamo" and "Kanlow"), two switchgrass upland ecotypes ("Cave-In-Rock" and "Blackwell"), and *Miscanthus* (*M. x gigenteus*) were planted in 1 L volume pots filled with Houston black clay soil and grown under controlled greenhouse conditions (25 °C, 12 h day/12 h night) until transplanting. Young plants of these five entries were transplanted to the field nursery at the different study sites starting in either 2009 or 2010 (Table 2). In spring 2009, seedlings and rhizome were transplanted into all sites, except for the site in Calhoun. Except for Calhoun, the experiments were laid out as randomized complete block designs, with 5-m long single row plots consisting of five transplants (1 m apart) and four replicates. The distance between single-row plots was 1 m. In Calhoun, rhizomes of *M. x giganteus* were transplanted into the field in 2009, while seedlings of switchgrass were transplanted in 2010. The experiment was laid out as randomized completed block designs, with 5-m long single row plots consisting of four transplants (1 m apart) and three replicates. At each location, except for Calhoun, weeds were controlled by pre- and post-emergence herbicide applications [Prowl H20pendimethalin: (*N*-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine)) and 2,4-D(-2,-4-dichlorophenoxyacetic acid)], hoeing, and hand weeding. In Calhoun, weeds were controlled by hand weeding around plants and using a string-trimmer between rows. The plants were harvested every October after either 2009 or 2010.

Table 1. Soil type, latitude, average annual precipitation, and soil chemical characteristics such as EC (soil electrical conductivity) and SAR (sodium adsorption ratio) estimated in 100 cm soil depth for the six locations included in of experiment 1.

Location	Soil Type	Latitude	Precipitation [a] (mm)	EC [b]	SAR [b]
Columbia, MO	Mexico silt loam	38.89	1083	1.0	0
Mt.Vernon, MO	Gerald silt loam	37.07	1171	0	0
Stillwater, OK	Kirkland silt loam	36.12	932	1.4	5.9
Calhoun, LA	Ruston-Lucy Association [c]	32.5	1406	0	0
Nacogdoches, TX	Attoyac fine sandy loam	31.5	1251	0	0
Temple, TX	Houston black clay	31.04	910	1	0

[a] Obtained from US Climate Data [44]; [b] Obtained from Web Soil Survey [45]; [c] Ruston is a fine sandy loam and Lucy is a loamy sand.

Table 2. Plant type, county, state, and latitude of origin for all plant types used in experiment 1.

	Site of Origin		
	County	**State**	**Lattitude**
	Switchgrass		
Alamo	Live Oak	Texas	28
Blackwell	Kay	Oklahoma	37
Cave-In-Rock	Hardin	Illinois	38
Kanlow	Hughes	Oklahoma	35
	Miscanthus		
Miscanthus x giganteus [a]	-	Maryland	39

[a] *Miscanthus* rhizomes were developed in Maryland by Kurt Bluemal, Inc. [46].

The new data used in this study included dry weight from each plot collected in October 2012, 2014, and 2015 for all sites, except for Calhoun, LA. The yield data in Calhoun, LA were collected in October 2011, 2012, and 2014. In Calhoun, only the 2011 and 2012 samples had nutrients analyzed. A length of row of either 0.5 m or 1.0 m was harvested from each replicate for biomass determination. After harvest, the fresh samples were weighed for a total fresh weight, and a subsample of 200–500 g from every sample was saved for dry weight determination. The samples were dried at 66 °C in a forced-air oven until the dry weight had stabilized. The dry samples were weighed and ground for nutrient analysis. The dry ground samples were sent to Texas A&M AgriLife Extension Service Soil, Water and Forage Testing Laboratory (College Station, TX, USA) to determine the concentration of N, P, and K. Nutrient removal rates were determined by multiplying nutrient concentration by dry biomass yield. In addition to all the yield data obtained as described above, the 2011 yield data reported in Kiniry et al. [40] were also included. Using Statistical Analysis Software version 9.3 (SAS 9.3), Mixed-model ANOVA was conducted to test for significant differences among entries (switchgrass ecotypes and *M. x giganteus*) and study locations. The year was considered as a random effect, and variety and study location were considered as fixed effects.

Yields were simulated by ALMANAC using the batch run feature. Weather data used for each location were from the nearest and most complete NOAA station. Soils for each location were the same as shown in Table 1 and data were obtained via Web Soil Survey. Adjustments to the soil were made for Mt. Vernon, Stillwater, and Temple. The soils' field capacity and wilting point were adjusted to be more in line with mean values for each soil textural class, as described by Ratliff et al. [46]. Soil values in that study were derived from studies with plants drying down the soils, and so provide reasonable values for each texture class. Mt. Vernon's field capacity was adjusted for the second-to-lowest layer to 0.34, and the lowest layer to 0.248. The wilting point for the lowest layer was changed to 0.015. Stillwater's field capacity was changed for the second to lowest layer to 0.219, and the lowest layer to 0.17. Temple's field capacity was changed for every layer to 0.348 and the wilting points were adjusted to 0.219. The lowest soil layer was removed, and the new lowest soil layer depth was limited to 1.4 m. To account for plant growth in early years, simulations were started three years before field trials began so all simulated plants began at the same growth stage. Values for simulated averages were taken in years that corresponded to field harvests. Values used for Calhoun were from 2011, 2012, and 2014 (2014 did not have nutrient analysis values there), whereas values for all other sites were from 2011, 2012, 2014, and 2015.

Management in the first simulated year consisted of fertilizer application on 1 April, planting on 10 April, and harvesting on 31 December. Every year to follow had fertilizer applied on 1 April, and harvesting on 31 October until 9 years of management were reached. Fertilizer in the simulations was assumed to be non-limiting at all sites except Stillwater, where 100 N was applied in the simulations each year. Potential heat units (PHU), degree days to plant maturity, were adjusted based on location and somewhat by entry to match the actual growing seasons for each location. "Alamo" and *M. x giganteus* had 1700 PHUs for the two most northern sites, Columbia and Mt. Vernon, and 2000 PHUs for the remaining sites. For the other three switchgrass ecotypes, Stillwater was in the northern group, with 1700 PHUs for Columbia, Mt. Vernon, and Stillwater, and 2000 PHUs for Calhoun, Nacogdoches, and Temple. Switchgrass parameters were already included in the ALMANAC software, so minor adjustments were made to distinguish switchgrass varieties from one another.

Radiation use efficiency (RUE), the efficiency with which plants convert available sunlight to biomass, and leaf area index (LAI), the amount of leaf area per unit of ground area varied by site and variety. Population planting densities were adjusted to generate realistic potential LAI values. The parameters (RUE and LAI) used for the simulations are given in Table 3. The leaf area development curve also varied between northern and southern sites based on measured values [38]. For *M. x giganteus* in the north we assumed 36% of potential LAI was reached at 6% of the degree days to maturity and 84% of potential LAI at 13% of degree days. To compare between measured and simulated yields of all plant types across all study locations, the correction and linear regression were estimated using Proc REG in Statistical Analysis Software version 9.3 (SAS 9.3).

Table 3. Measured and ALMANAC-simulated biomass yields (dry matter basis) and simulation parameters radiation use efficiency (RUE) and potential LAI (DMLA) of all plant entries used in study averaged across years 2011–2015 for the five locations and for 2011, 2012, and 2014 for Calhoun.

	Switchgrass				
Location	**Alamo**	**Blackwell**	**Cave-In-Rock**	**Kanlow**	***Miscanthus x giganteus***
	Measured Yield (Simulated Yield) in Mg ha^{-1}				
Columbia, MO	24.8 (23.4)	12.9 (20.2)	13.6 (24.4)	24.7 (26.6)	33.2 (28.3)
Mt. Vernon, MO	19.3 (17.2)	13.7 (14.8)	15.4 (17.2)	22.6 (19.9)	25.0 (26.5)
Stillwater, OK	12.8 (7.7)	7.8 (2.6)	9.9 (2.5)	11.1 (7.5)	3.4 (2.8)
Calhoun, LA	27.3 (22.7)	-	-	-	16.5 (12.2)
Nacogdoches, TX	37.6 (23.1)	3.5 (5.7)	5.2 (5.5)	16.0 (24.4)	6.9 (11.2)
Temple, TX	27.5 (18.2)	5.0 (4.8)	5.6 (4.7)	12.6 (19.7)	4.5 (3.6)
	RUE in g per MJ Intercepted PAR (LAI)				
Columbia, MO	4.0 (12)	3.55 (5.5)	3.5 (5.5)	4.6 (12)	5.8 (12)
Mt. Vernon, MO	4.0 (12)	3.55 (5.5)	3.5 (5.5)	4.6 (12)	5.8 (12)
Stillwater, OK	4.0 (12)	3.55 (5.5)	3.5 (5.5)	4.6 (12)	5.8 (12)
Calhoun, LA	4.0 (12)	1.10 (2.6)	1.8 (2.6)	2.6 (5.5)	4.9 (2.6)
Nacogdoches, TX	4.0 (12)	1.10 (2.6)	1.8 (2.6)	2.6 (5.5)	4.9 (2.6)
Temple, TX	4.0 (12)	1.10 (2.6)	1.8 (2.6)	2.6 (5.5)	4.9 (2.6)

- Data is not available.

2.2. Experiment 2: Evaluating Effects of N Amount on Plant Productivity in Calhoun, LA

Seedlings of switchgrass "Alamo" and rhizomes of *M x giganteus* were transplanted in spring 2009 and spring 2010, respectively, in the Louisiana State University AgCenter at Calhoun, Louisiana. The experiments were laid out as randomized completed block designs, with 5-m long single row plots consisting of four transplants (1 m apart) and three replicates. Our field measurements were taken in 2011 and 2012. Treatments consisted of three nitrogen rates (0, 80, and 160 kg N ha^{-1} $year^{-1}$), two types of fertilizer sources (organic and inorganic) and two different species ("Alamo" switchgrass and *M. x giganteus*). Poultry litter was used as organic fertilizer, while inorganic fertilizer was prepared using tap water. Fertilizer applications were made annually in spring beginning in 2011 and continued through 2012. The treatments were laid out in split-split plots based on a randomized completed block design with three replicated blocks. The nitrogen application rate was considered as the main plot, and two types of fertilizer were treated as subplots. The species was considered as sub-sub plot.

In October of both 2011 and 2012, samples of either 0.5 m by 0.5 m or 1.0 m by 1.0 m were harvested from each treatment in each replication. After harvest, the fresh samples were weighed for a total fresh weight, and a subsample of 200–500 g from each sample was saved for dry weight measurement. The samples were dried at 66 °C in a forced-air oven until the dry weight had stabilized. The dry samples were weighed, ground, and sent to Texas A&M AgriLife Extension Service Soil, Water, and Forage Testing Laboratory (College Station, TX, USA) to determine the concentrations of N, P, and K. Nutrient removal rates were calculated by multiplying nutrient concentration by dry biomass yield. Using SAS 9.3, ANOVA was conducted using Proc Mixed to test the effects of species, N fertilizer rate, type of fertilizer, and their interaction effects on dry matter yields across years. Year was treated as a random effect. The N fertilizer rate, fertilizer type, and species were considered as fixed effects.

3. Results

3.1. Experiment 1: Biomass Yields at Six Locations across a Latitudinal Gradient

Measured dry biomass yields were not significantly different among entries ($p = 0.1465$), but they were significantly different among locations ($p = 0.0109$). Also, there was a significant interaction between plant entry and location ($p = 0.0002$), reflecting differential responses of entry to different location (Table 3). The highest biomass production was achieved by a lowland switchgrass ecotype, "Alamo", at Nacogdoches at 37.6 Mg ha^{-1}. The other lowland ecotype, "Kanlow", had the highest biomass yield in Columbia at 24.7 Mg ha^{-1}. The lowest biomass production of these two lowland ecotypes was at Stillwater at 12.8 Mg ha^{-1} and 11.1 Mg ha^{-1}. For upland switchgrasses, "Blackwell" and "Cave-In-Rock", had the lowest biomass yields at Nacogdoches at 3.5 Mg ha^{-1} and 5.2 Mg ha^{-1}, respectively. The highest biomass yields for these two were in Mt. Vernon at 13.7 Mg ha^{-1} and 15.4 Mg ha^{-1}, respectively. *M. x giganteus* yielded the highest biomass in Columbia at 33.2 Mg ha^{-1}, and its lowest biomass was in Stillwater at 3.4 Mg ha^{-1}. Overall, all plant types produced low yields in Stillwater. Higher values in EC and SAR were observed in Stillwater (Table 1), which reveals that soils in Stillwater have the highest salinity of all study sites. This high soil salinity may have resulted in stress and likely caused the low plant yields in Stillwater.

In general, the simulated yields of all plant entries agreed moderately well with the measured yields across locations while showing varying success for "Alamo" switchgrass, the other switchgrass ecotypes pooled, and *M. x giganteus* (Table 3 and Figures 1 and 2). Regression analysis for simulated and measured yields including data from all grasses revealed an R^2 of 0.67 and a slope of 0.76 (Figure 1). For "Alamo", measured yields in the northern regions (Columbia and Mt. Vernon) were in close agreement with simulated yields, but simulated yields in southern regions (Calhoun, Nacogdoches, and Temple) were underestimated (Figure 2a). The model simulations showed an R^2 of 0.65. Pooling the other three switchgrass ecotypes (Figure 2b), the model simulations had a correlation coefficient of only 0.16 when compared with measured yields. For upland switchgrass ecotypes, measured yields at the southern locations agreed closely with simulated yields. Simulated yields in northern locations for upland switchgrass ecotypes were overestimated. The yields of "Kanlow" were overestimated for the southern locations. Simulated yields for *M. x giganteus* showed the highest correlation with measured yields, with an R^2 of 0.92 and a regression line close to the 1:1 line (Figure 2c). The simulated yields in Stillwater were underestimated for all plant types.

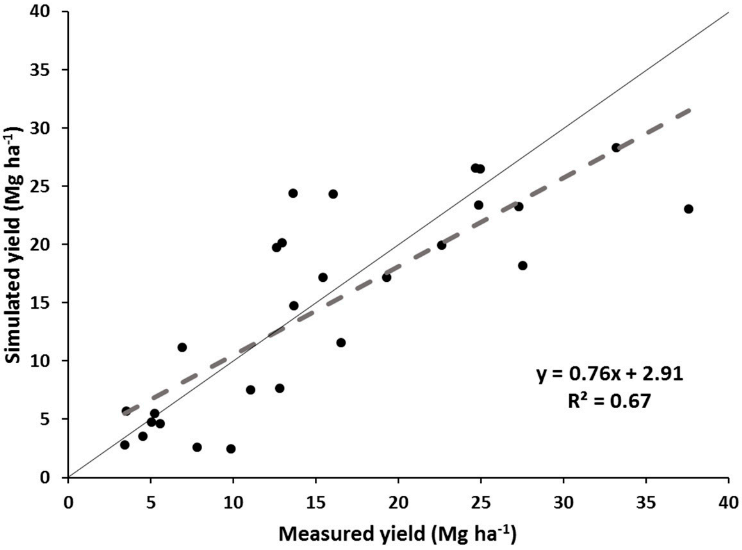

Figure 1. Relationship between measured and ALMANAC-simulated dry matter yields. Individual data points represent average yields across years for all plant types at each of the six study locations. The dashed line is the fitted regression line and solid line is the 1:1 line.

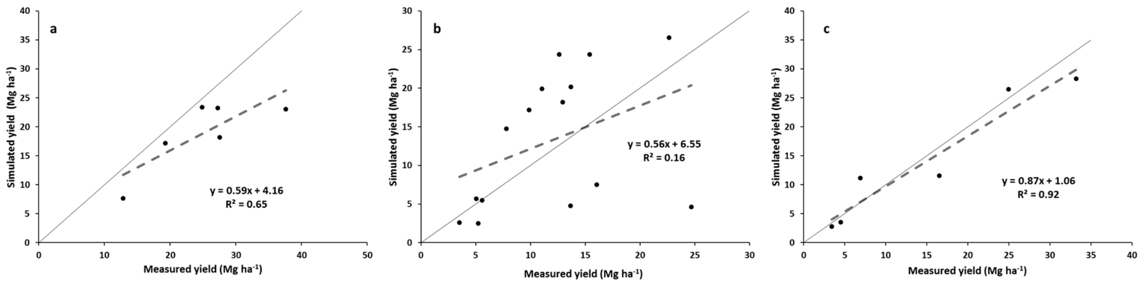

Figure 2. Relationship between measured and ALMANAC-simulated yields of (**a**) "Alamo" switchgrass; (**b**) all other switchgrass ecotypes; and (**c**) *Miscanthus x giganteus* averaged across years at six study locations. Dashed line is the fitted regression line and solid line is the 1:1 line.

To examine the relationship between yield and latitude, the average measured and simulated yields in two clusters that were formed based on the latitude of study location were compared to each other (Table 4). Cluster 1 includes Columbia (38.89°N) and Mt. Vernon (37.07°N), while Cluster 2 includes Calhoun (32.5°N), Nacogdoches (31.5°N), and Temple (31.04°N). Stillwater was excluded for this analysis because high soil salinity affected yields. In general, plant types have different yield patterns between the two clusters. For "Alamo", Cluster 2 had higher measured yield at 30.8 Mg ha^{-1}. In contrast, "Kanlow", upland switchgrass ecotype, and *M. x giganteus* showed that the measured plant yields of Cluster 1 were greater than Cluster 2. The simulated yields also followed measured yield patterns between the two clusters, except for lowland switchgrass ecotypes ("Alamo" and "Kanlow"). For these two ecotypes, the measured yield difference between the two clusters were 10 Mg ha^{-1}, while the simulated yields of the two clusters were only differed by 1 Mg ha^{-1}.

Table 4. Means of measured and simulated yield for each cluster. Within switchgrass ecotype and *Miscanthus x giganteus*, two clusters were defined based on the latitude of study location. Cluster 1 includes Columbia (38.95°N) and Mt. Vernon (37.07°N), whereas Cluster 2 includes Calhoun (32.5°N), Nacogdoches (31.60°N), and Temple (31.08°N). The Stillwater location was excluded because plant production at the location was limited by high soil salinity. Values in bold mark the cluster at which greater yields were observed within plant type.

	Switchgrass				*Miscanthus*
Cluster	**Alamo**	**Blackwell**	**Cave-In-Rock**	**Kanlow**	***Miscanthus x giganteus***
			Measured Yield Mg ha^{-1}		
1	22.1	13.3	14.5	23.7	29.1
2	30.8	4.3	5.4	14.3	9.3
			Simulated Yield Mg ha^{-1}		
1	20.3	17.5	20.8	23.3	27.4
2	21.3	5.2	5.1	22.1	9.0

Seasonal dynamics in simulated leaf area index (calculated using modified Beer's law) indicated that the greatest LAIs of all three plant types were observed on mid-June and early-June at Columbia (northern-most location) and Nacogdoches (southern-most location) locations, respectively (Figure 3). Seasonal LAI changes in northern locations had consistently higher LAI than in southern locations for upland switchgrass ecotypes and *M. x giganteus*. "Alamo" had similar values of LAI in both locations, but its LAI in the southern location was initiated earlier than "Alamo" grown in the northern locations. Growth at the northern and southern locations was affected by the prevailing photoperiod (changes in the length of day) and the annual extreme minimum temperature (Figure 4). Photoperiod duration in April and June in Columbia were 14.17 and 14.53 hours, respectively, while the lengths of photoperiods during April and June in Nacogdoches were 12.45 and 14.12 hours, respectively (Figure 4a). According to the USDA plant-hardiness zone map, the northern locations (Columbia and Mt. Vernon) were in Zone 6 where the range of minimum temperature is −23.3 to −17.8 °C (Figure 4b). The southern locations (Calhoun, Nacogdoches, and Temple) are in Zone 8 where the range in minimum temperature is −12.2 to −6.7 °C (Figure 4b). Stillwater is in Zone 7 where the range in minimum temperature is −17.8 to −12.2 °C (Figure 4b).

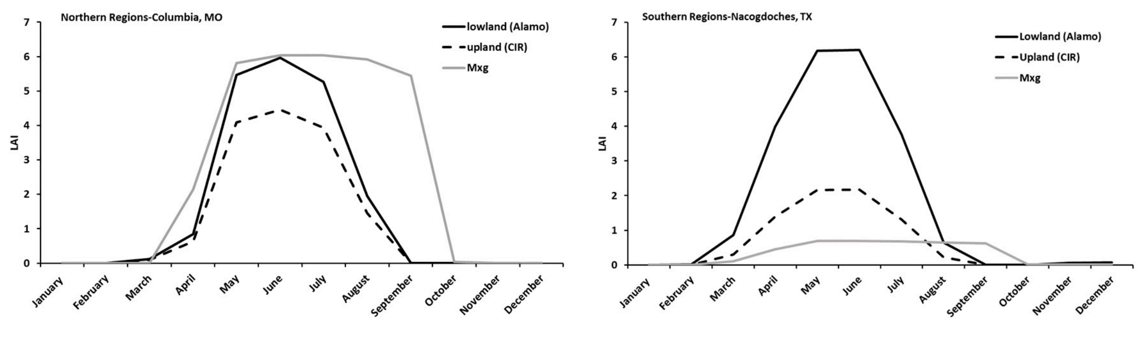

Figure 3. Seasonal changes in simulated leaf area index averaged across years (2011–2015) for lowland ("Alamo"), and upland ("Cave-In-Rock") ecotypes, and *Miscanthus x giganteus* grown in a northern location (Columbia, MO) and a southern location (Nacogdoches, TX) of the USA.

Biomass N, P, and K concentrations varied with different location (Table 5). In most of sites such as Temple, Stillwater, Mt. Vernon, and Columbia, upland switchgrass ecotypes had the largest N concentration, while in Nacogdoches and Calhoun, *M. x giganteus* had the largest N concentration. Similar result patterns were observed in P and K concentrations, except for the K concentration in Columbia. "Alamo" had the highest K concentration of harvest biomass in Columbia. The removal rates for N, P, and K by each plant variety also varied between different locations (Table 6). Unlike nutrient concentration, the nutrient removal rates were generally dependent on the harvested biomass yield. For example, in most study sites such as Temple, Nacogdoches, Calhoun, and Stillwater, lowland ecotypes such as "Alamo", which produced the highest biomass, had the largest N, P, and K removal rates. In both Mt. Vernon and Columbia, *M. x giganteus* had the highest removal rates of N, P, and K compared to switchgrass ecotypes.

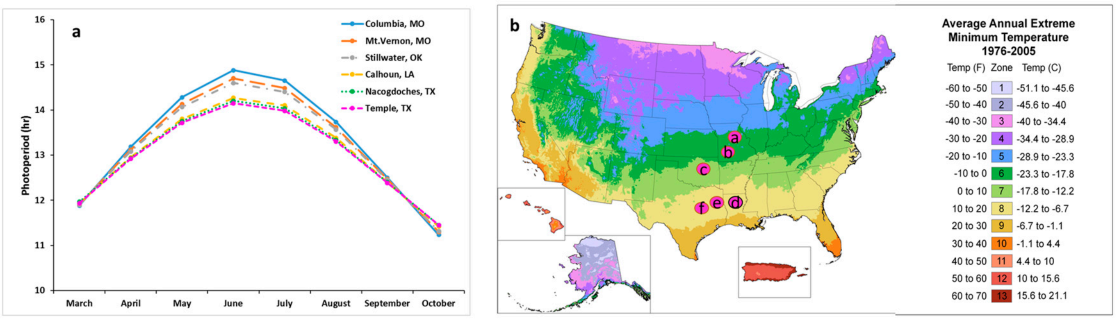

Figure 4. (**a**) Seasonal changes in the length of photoperiod measured on the 15th of each month at all six study locations and (**b**) USDA-Plant-hardiness-zone-map [47]. Pink circles in the map indicate study locations. a, Columbia, MO; b, Mt. Vernon, MO; c, Stillwater, OK; d, Calhoun, LA; e, Nacogdoches, TX; and f, Temple, TX.

Table 5. Average N, P, and K concentrations of biomass harvested in multiple years at the six study locations (Experiment 1). Values in bold mark the plant entry with the highest concentration within each location for each variable.

Plant Type	Temple, TX	Nacogdoches, TX	Calhoun, LA	Stillwater, OK	Mt. Vernon, MO	Columbia, MO
			Nutrient Concentration			
			N **(g kg^{-1})**			
Alamo	5.35	4.85	8.68	7.40	6.50	5.45
Blackwell	7.70	5.50	-	8.30	**9.10**	6.30
Cave-In-Rock	**12.10**	7.60	-	**10.70**	6.50	**8.10**
Kanlow	6.00	4.40	-	9.80	7.20	6.10
Miscanthus x giganteus	9.20	**8.45**	**11.51**	7.30	6.15	5.70
			P **(g kg^{-1})**			
Alamo	0.46	1.36	1.69	0.98	0.50	1.36
Blackwell	0.76	1.54	-	1.05	**0.67**	1.54
Cave-In-Rock	**1.52**	**1.64**	-	**1.35**	0.56	**1.64**
Kanlow	0.57	1.25	-	1.21	0.62	1.25
Miscanthus x giganteus	0.70	1.09	**1.85**	1.08	0.51	1.09
			K **(g kg^{-1})**			
Alamo	4.32	6.12	12.28	4.43	5.77	**6.96**
Blackwell	6.42	5.97	-	4.18	**7.56**	6.15
Cave-In-Rock	**9.35**	7.88	-	4.98	7.02	6.83
Kanlow	5.18	7.70	-	**6.32**	4.84	6.78
Miscanthus x giganteus	4.99	**9.94**	**12.48**	4.31	4.72	5.63

- Data is not available.

Table 6. Average yearly removal of N, P, and K in harvested biomass in multiple years at the six study locations (Experiment 1). Values in bold mark the plant entry with the highest concentration within each location for each variable.

	Nutrient Removal					
Plant Type	**Temple, TX**	**Nacogdoches, TX**	**Calhoun, LA**	**Stillwater, OK**	**Mt. Vernon, MO**	**Columbia, MO**
			***N* (kg ha^{-1})**			
Alamo	**104.01**	**169.88**	**215.05**	61.62	129.81	132.30
Blackwell	33.75	14.73	-	44.78	120.94	105.95
Cave-In-Rock	77.37	40.48	-	58.43	86.03	128.83
Kanlow	55.03	62.83	-	**75.49**	143.73	144.49
Miscanthus x giganteus	37.17	82.69	173.16	27.06	**213.33**	**188.48**
			***P* (kg ha^{-1})**			
Alamo	8.92	**47.50**	**41.77**	8.14	9.95	32.92
Blackwell	3.31	4.11	-	5.66	8.94	25.82
Cave-In-Rock	**9.72**	8.74	-	7.37	7.37	26.10
Kanlow	5.25	17.82	-	**9.29**	12.42	29.56
Miscanthus x giganteus	2.82	10.64	27.80	4.01	**17.62**	**35.94**
			***K* (kg ha^{-1})**			
Alamo	**83.95**	**214.37**	**304.40**	36.86	115.16	169.05
Blackwell	28.13	15.97	-	22.57	100.46	103.43
Cave-In-Rock	59.76	41.96	-	27.18	92.89	108.58
Kanlow	47.54	109.98	-	**48.68**	96.58	160.50
Miscanthus x giganteus	20.15	97.31	187.66	15.96	**163.85**	**186.20**

- Data is not available.

3.2. Experiment 2: N Amount Effect on Biomass Yield

Based on the statistical analysis to test significant main effects and interactions of nitrogen, fertilizer resources (organic and inorganic), species, and interaction (Figure 5), nitrogen rate and species significantly affected biomass yield. No significant effects on yield were observed for fertilizer types (organic and inorganic, $p = 0.443$) and treatment interactions. Measured biomass yields significantly differed by species ($p < 0.0001$, Figure 5). Switchgrass had significantly higher biomass yield than *M. x giganteus* across all nitrogen fertilizer rates (Figure 5). Also, there were significant effects of nitrogen rate ($p = 0.016$) on biomass yield. The measured biomass yield of switchgrass significantly increased as nitrogen fertilizer increased from 0 to 160 kg N ha^{-1} year^{-1}. In contrast, higher *M. x giganteus* yield was observed at the 80 kg N ha^{-1} year^{-1} than at the 160 kg N ha^{-1} year^{-1}.

Nutrient concentrations for N, P, and K for *M. x giganteus* were greater than nutrient concentrations for harvested switchgrass biomass (Table 7). Moreover, the nutrient concentrations in the control for *M. x giganteus* were higher than switchgrass. *M. x giganteus* generally had the highest nutrient concentration at 160 kg N ha^{-1} year^{-1} in poultry litter. The nutrient concentrations for switchgrass were generally high at either 80 or 160 kg N ha^{-1} year^{-1}. Overall, the nutrient removal rates for N, P, and K for switchgrass were consistently higher than *M. x giganteus*. The highest nutrient removal rates for N, P, and K by switchgrass were observed at 160 kg N ha^{-1} year^{-1} in inorganic N fertilizer, while the highest nutrient removal rates for *M. x giganteus* were observed at 160 kg N ha^{-1} year^{-1} in organic fertilizer.

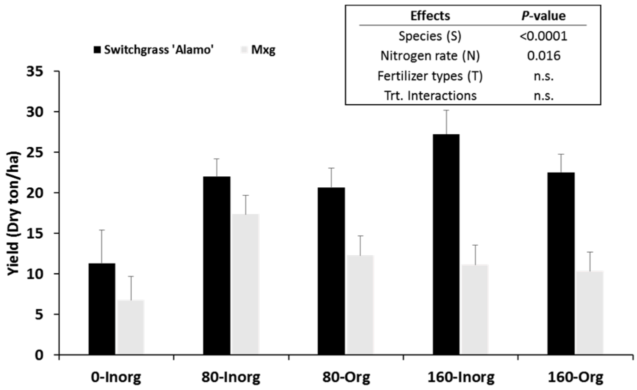

Figure 5. Measured biomass yields (dry matter basis) for switchgrass ("Alamo") and *Miscanthus x giganteus* treated by three N rates (0, 80, and 160 kg N ha^{-1} $year^{-1}$) and two fertilizer types (organic and inorganic) averaged across years (2011–2012) at Calhoun, LA. ANOVA significant tests for main effects and interaction of species, nitrogen fertilizer rate, and fertilizer type on yields ($p < 0.05$). n.s. indicates no significant difference. The error bars are the SE.

Table 7. Means of nutrient concentration and removal rates of N, P, and K within each year under different N fertilizer and water resources for switchgrass and *Miscanthus x giganteus* used in experiment 2. The bold values were selected as the largest value within each species for each variable.

	N Rates	Fertilizer Resources	Nutrient Concentration (g kg^{-1})			Nutrient Removal (kg ha^{-1})		
Species			**N**	**P**	**K**	**N**	**P**	**K**
Swichgrass (Alamo)	0	Inorganic	4.52	0.76	5.99	71.55	11.68	91.54
	80	Inorganic	5.33	1.11	**7.41**	90.63	18.90	125.53
		Poultry	**5.40**	**1.29**	7.29	109.83	26.22	143.67
	160	Inorganic	5.17	1.20	6.34	**139.83**	**32.64**	**163.65**
		Poultry	5.31	1.06	7.28	113.10	24.61	150.87
Miscanthus x giganteus	0	Inorganic	7.98	1.10	7.74	74.80	10.06	74.16
	80	Inorganic	8.64	0.79	7.80	77.80	7.12	73.56
		Poultry	8.07	1.40	8.02	99.96	17.09	108.81
	160	Inorganic	8.50	0.63	6.50	92.47	6.68	71.89
		Poultry	**9.90**	**2.01**	**12.27**	**101.46**	**20.77**	**130.78**

4. Discussion

In the first experiment, yields of two switchgrass ecotypes (upland and lowland) and *M. x giganteus* were estimated for six sites distributed across the Southern Great Plains (SGP) with different climate characteristics and soil types. According to the results of measured yield patterns for all five entries, greater measured yields were observed in study sites that are closest to where they were originated. For example, "Alamo" had higher biomass yield in southern locations (Calhoun, Nacogdoches, and Temple), closest to its origin in Live Oak county, Texas. In contrast, the other three switchgrass ecotypes and *M. x giganteus* showed the different yield patterns than "Alamo". Their yields increased in northern locations that were close to their geographic origins. This result reveals that plants tend to show optimal growth performance near where they have been established and persisted. Similar results have been reported by Jefferson and McCaughey [48] who reported that latitude of origin of a switchgrass ecotype was positively correlated to biomass production.

The optimal growth performance near their geographic origins may be reasonable because plants thrive in such environments due to factors including rainfall, temperature, and length of the photoperiod [49–51]. Among the environmental factors, photoperiod (length of day) can significantly influence plant development, including plant dormancy, formation of storage organ, asexual reproduction, leaf development, stem elongation, germination, and flowering initiation [50,52].

Kiniry et al. [40] reported significant correlation between photoperiod and yields for two switchgrass ecotypes and *M. x giganteus*, but the values of correlation coefficients varied among entries. "Alamo" had a negative value of correlation coefficient between photoperiod and its yield, whereas yields of "Kanlow", upland switchgrass ecotypes, and *M. x giganteus* yields were positively correlated with photoperiod [40]. The results of this study show that the yields of "Alamo" in lower latitudes were 25% greater than in higher latitudes, while "Kanlow", upland switchgrass, and *M. x giganteus* had three-times greater biomass yield in higher latitude study locations. Moreover, leaf area development showed the same pattern as yield for all entries, except for "Alamo", across all study locations. In the seasonal changes in simulated leaf area index, much higher maximum values of leaf area index were observed for upland switchgrass ecotypes and *M. x giganteus* in higher latitudes. In contrast, "Alamo" had similar maximum leaf area index during the growing seasons in both northern and southern locations, but its growing period in southern locations was longer than in northern locations. The "Alamo" leaf area index increased rapidly in mid-February in southern locations, while the leaf area index increased rapidly in March in northern locations.

In addition to photoperiod, temperature also plays an important role in controlling plant development, both during the dormant period and during the growth phase [50,53]. In perennial plants, temperature is a critical factor for inducing and controlling dormancy in their rhizomes. This is a mechanism for rhizomatous perennial plants to survive adverse conditions by pulsing growth. Many plants require sufficient days with chilling temperatures during winter to completely release dormancy for the normal processes of plant growth, reproductive development and subsequent yield [54–56]. In this study, the six locations belong to different cold hardiness zones which differ in their extreme minimum temperatures. The minimum temperature in lower latitude study locations was 11 °C higher than northern study locations. The higher winter temperatures in the southern region may not satisfy the chilling requirements for "Kanlow", upland switchgrasses, and *M. x giganteus*, which may result in prolonged dormancy leading to their lower yields in this region [57,58]. These results can be supported by Kiniry et al. [40] who reported positive correlation between cold stress and yields for "Kanlow", upland switchgrass ecotypes, and *M. x giganteus*, indicating that colder winter temperature favored their growth and development.

The simulated yields showed reasonable trends when compared with the measured yields for all switchgrass ecotypes and *M. x giganteus* pooled, but showed variable results when looking at the individual switchgrass ecotypes and *M. x giganteus*. The model appears quite reasonable for simulating "Alamo" switchgrass and *M. x giganteus* across this range of latitudes but may need some improvement before it can capture the yield variability of the other three switchgrass ecotypes. The model accounted for two-thirds of the variability in all the pooled data and showed a realistic regression line. "Alamo" simulated yields also accounted for nearly two thirds of the variability in measured yields, but tended to underpredict yields at the higher-yielding, more southern sites. For the other pooled three switchgrass ecotypes, the model had a regression line for simulated yields:measured yields that was reasonably close to the 1:1 line, but the model only accounted for 16% of the variability in measured yields. Thus the model did only a fair job in predicting these yields. Finally, for *M. x giganteus*, the model did an excellent job simulating yields across sites, with the regression line close to the 1:1 line and the correlation coefficient being greater than 0.90.

Based on the results of measured and simulated yields, unlike upland switchgrass ecotypes and *M. x giganteus*, "Alamo" could consistently produce high yields across all study locations, which may reflect that "Alamo" growth was less affected by photoperiod and temperature changes. A similar result has been observed by Li et al. [59], who reported that the southern ecotypes are usually less sensitive to the inductive signals than northern ecotypes. Based on these yield results, "Alamo" can be selected as the optimal biofuel species growing in both southern and northern location in the SGP, while upland switchgrass ecotypes and *M. x giganteus* can be great bioenergy crop candidates only in northern locations of the SGP.

The nutrient concentration in harvested biomass is greater when biomass yield is lower, which may be due to the relatively high leaf to stem biomass ratio for smaller plants [60,61]. Mattos et al. [60] reported that nutrient concentrations were higher in leaves compared to other plant parts (e.g., root and stem) because nutrients taken up by roots are primarily transported to the leaves, where most important biochemical reactions occur. The nutrient removal rates for N, P, and K followed the biomass trends. This is shown by nutrient removal rates by plants for N, P, and K that increased as biomass yield increased. Similar results have been observed in switchgrass [60]. According to Kering et al. [60], nutrients accumulate in plant tissue as they grow, so increased plant size may reflect increased nutrient removal in harvested biomass.

In Experiment 2, the effects of nitrogen fertilization on biomass yield of "Alamo" switchgrass and *M. x giganteus* were investigated at a single location in the SGP. Most previous yield evaluations under various nitrogen fertilizer application rates for *M. x giganteus* were conducted in Central and Northern USA or Europe, where *M. x giganteus* is well-adapted [19,29,31,62]. It is still unclear about the effect of nitrogen fertilizer application rate on biomass yield of *M. x giganteus* in southern locations in the USA, where, based on the results from our first experiment, *M. x giganteus* is not well adapted. The second experiment, therefore, provides useful information about relationships between geographic adaptation and nitrogen response in *M. x giganteus*.

Switchgrass and *M. x giganteus* significantly responded to nitrogen fertilizer application rates. As nitrogen fertilizer application rates increased, yields of switchgrass significantly increased. Unlike switchgrass, *M. x giganteus* yields increased only from 0 to 80 kg N ha^{-1} $year^{-1}$ application rates. Similar results have been reported in elsewhere [60,63–65]. In the southern US, switchgrass yield increased as nitrogen fertilizer application rates increased up to 224 kg N ha^{-1} [63], and "Alamo" switchgrass produced the maximum yield at 168 kg N ha^{-1} [64]. Although no significant effects on yield were observed for fertilizer types (organic and inorganic), compared with inorganic fertilizer, smaller yield differences between 80 and 160 kg N ha^{-1} $year^{-1}$ of organic (poultry litter) fertilizer were observed in both switchgrass and *M. x giganteus*. This may be because poultry litter is a slow-release fertilizer, which can delay nutrient uptake of the plant [66]. In switchgrass, N removal difference between 80 and 160 kg N ha^{-1} $year^{-1}$ was 49.2 N kg ha^{-1} for inorganic fertilization, while only 3.27 kg N ha^{-1} was removed by plants from the organic fertilizer. The removal difference between 80 and 160 kg N ha^{-1} $year^{-1}$ for *M. x giganteus* tended to show similar pattern with switchgrass, but the N removal amount was much lower. In *M. x giganteus* 14.7 and 1.5 N kg ha^{-1} were removed by plant for inorganic and organic fertilization, respectively. Although the nutrient removal increased from 80 and 160 kg N ha^{-1} $year^{-1}$ of inorganic fertilizer, *M. x giganteus* yields decreased. This may have been due to environmental limitations at the study site. This result is supported by other studies [67,68], where *M. x giganteus* yields increased with increased nitrogen fertilizer in Illinois, but not across the eastern USA, where *M. x giganteus* was not as well adapted as in Illinois. In addition, Vergeer et al. [69] reported that plant yields are more influenced by regional adaptation (e.g., flowering time and growth rate), rather than nitrogen rate. This may be why *M. x giganteus* yield was much less than switchgrass at 0 kg N ha^{-1} $year^{-1}$.

The nutrient concentration and removal rates by plants varied by either nitrogen rates or species. The highest nutrient concentrations for switchgrass and *M. x giganteus* harvested were observed at either 80 or 160 kg N ha^{-1}. The nutrient removal rates by harvested switchgrass followed the biomass trend, shown as the nutrient removal rates increased as nitrogen rate increased. Unlike switchgrass, the nutrient removal by harvested *M. x giganteus* was not associated with its yield, but increased as nitrogen fertilizer application increased. This result indicates that *M. x giganteus* applied with greater nitrogen application rate may increase biomass ratio of leaves over stem because higher nutrient concentrations are observed in leaves compared to other plant parts (e.g., root and stem) [61].

5. Conclusions

In conclusion, two experiments were conducted to evaluate the stability of two of the most promising bioenergy crops—switchgrass and *M. x giganteus*—under various environmental conditions across the Southern Great Plains (SGP). The first experiment examined the productivity of upland and lowland switchgrass ecotypes and *M. x giganteus* at six locations distributed in the SGP. Productivity of biofuel species was highly related to the localities where they originated or have persisted. One of the lowland switchgrass ecotypes, "Alamo", showed the highest yield in southern locations and also consistently produced the highest biomass yields among other entries across all study locations. Unlike "Alamo", yields for the upland switchgrass ecotypes, "Kanlow" switchgrass, and *M. x giganteus* increased as latitude of study locations increased. The simulated yields of lowland switchgrass ecotypes and *M. x giganteus* agree relatively well with their measured yields across all study locations, whereas the simulated yields of upland switchgrass ecotypes were overestimated in northern locations. In the second experiment, the effects of organic and inorganic nitrogen fertilizers on crop yields were evaluated in switchgrass and *M. x giganteus*. Switchgrass yield increased as N rate increased, while yields of *M. x giganteus* increased only from 0 to 80 kg N ha^{-1}. The two experiments provide valuable inputs for process-based models to realistically simulate the performance of these important perennial grasses at SGP locations, and to estimate nutrient needs for extending their biomass production yield. Moreover, they provide useful information about the most productive perennial grasses and their appropriate nitrogen application rates to farmers and the bioenergy industry, which is critical for developing the bioenergy market system in the SGP.

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References

1. EPA (Environmental Protection Agency). Clean Power Plan for Existing Power Plants. Available online: http://www2.epa.gov/cleanpowerplant/clean-power-plan-exiting-power-plants (accessed on 14 December 2016).
2. Moomaw, W.; Yamba, F.; Kamimoto, M.; Maurice, L.; Nyboer, J.; Urama, K.; Weier, T. Introduction. In *IPCC Special Report of Renewable Energy Sources and Climate Change Mitigation*; Edenhofer, O., Pichs-Madruga, R., Sokona, Y., Seyboth, K., Matschoss, P., Kadner, S., Zwickel, T., Eickemeier, P., Hansen, G., Schlömer, S., et al., Eds.; Cambridge University Press: Cambridge, UK; New York, NY, USA, 2012.
3. Olivia, R.; Mihăilă, S. Rural economy and bioethanol production. *Sustainability* **2016**, *8*, 1148. [CrossRef]
4. Demirbas, A. Biofuels sources, biofuel policy, biofuel economy and global biofuel projections. *Energy Convers. Manag.* **2008**, *49*, 2106–2116. [CrossRef]
5. Van Alfen, N.K. *Encyclopedia of Agriculture and Food System*; Elsevier: Davis, CA, USA, 2014; Volume 1.
6. Kung, C.C.; Xie, H.; Wu, T.; Chen, S.C. Biofuel for energy security: An examination on pyrolysis systems with emissions from fertilizer and land-use change. *Sustainability* **2014**, *6*, 571–588. [CrossRef]
7. Buchanan, G. *Increasing Feedstock Production for Biofuels: Economic Drivers, Environmental Implications, and the Role of Research*; DIANE Publishing: Collingdale, PA, USA, 2010.
8. Mehmood, M.A.; Ibrahim, M.; Rashid, U.; Nawaz, M.; Ali, S.; Hussain, A.; Gull, M. Biomass production for bioenergy using marginal lands. *Sustain. Prod. Consum.* **2016**. [CrossRef]
9. Timmons, D.; Allen, G.; Damery, D. *Biomass Energy Crops: Massachusetts Potential*; Massachusetts Division of Energy Resources and Massachusetts, Department of Conversion and Recreation: Boston, MA, USA, 2008.

10. Porter, P.A.; Barry, J.; Samson, R.; Doudlah, M. *Growing Wisconsin Energy: A Native Grass Pallet Bio-Heat Roadmap for Wisconsin*; Agrecol Corp: Madison, WI, USA, 2008; Available online: http://www.agrecol.com/AgrecolADDReport.pdf (accessed on 14 December 2016).
11. Anderson, E.; Arundale, R.; Maughan, M.; Oladeinde, A.; Wycislo, A.; Voigt, T. Growth and agronomy of *Miscanthus x giganteus* for biomass production. *Biofuels* **2011**, *2*, 71–87. [CrossRef]
12. Heaton, E.; Voigt, T.; Long, S.P. A quantitative review comparing the yields of two candidate C-4 perennial biomass crops in relation to nitrogen, temperature and water. *Biomass Bioenergy* **2004**, *27*, 21–30. [CrossRef]
13. Sanderson, M.A.; Reed, R.L.; Mclaughlin, S.B.; Wullschleger, S.D.; Conger, B.V.; Parrish, D.J.; Wolf, D.D.; Taliaferro, C.; Hopkins, A.A.; Ocumpaugh, W.R.; et al. Switchgrass as a sustainable bioenergy crop. *Bioresour. Technol.* **1996**, *56*, 83–93. [CrossRef]
14. Quinn, L.D.; Straker, K.C.; Guo, J.; Kim, S.; Thapa, S.; Kling, G.; Lee, D.K.; Voigt, T.B. Stress-tolerant feedstocks for sustainable bioenergy production on marginal land. *Bioenergy Res.* **2015**, *8*, 1081–1100. [CrossRef]
15. Luo, H.; Wu, Y.; Kole, C. *Compendium of Bioenergy Plants: Switchgrass*; CRC Press: New York, NY, USA, 2014; pp. 17–183.
16. Zalapa, J.E.; Price, D.L.; Kaeppler, S.M.; Tobias, C.M.; Okada, M.; Casler, M.D. Hierarchical classification of switchgrass genotypes using SSR and chloroplast sequences: Ecotypes, ploidies, gene pools, and ecotypes. *Theor. Appl. Genet.* **2011**, *122*, 805–817. [CrossRef] [PubMed]
17. Zhang, Y.; Zalapa, J.; Jakubowski, A.R.; Price, D.L.; Acharya, A.; Wei, Y.; Brummer, E.C.; Kaeppler, S.M.; Casler, M.D. Natural hybrids and gene flow between upland and lowland switchgrass. *Crop Sci.* **2011**, *51*, 2626–2641. [CrossRef]
18. Jones, M.B.; Walsh, M. *Miscanthus for Energy and Fibre*; James and James Ltd.: London, UK, 2001.
19. Lewandowski, I.; Clifton-Brown, J.C.; Scurlock, J.M.O.; Huisman, W. Miscanthus: European experience with a novel energy crop. *Biomass Bioenergy* **2000**, *19*, 209–227. [CrossRef]
20. Heaton, E.A.; Dohleman, F.G.; Long, S.P. Meeting US biofuel goals with less land: The potential of Miscanthus. *Glob. Chang. Biol.* **2008**, *14*, 2000–2014. [CrossRef]
21. Heaton, E.A.; Clifton-Brown, J.; Voigt, T.B.; Jones, M.B.; Long, S.P. Miscanthus for renewable energy generation: European Union experience and projections for Illinois. *Mitig. Adapt. Strateg. Glob. Chang.* **2004**, *9*, 433–451. [CrossRef]
22. Glowacka, K.; Clark, L.V.; Adhikari, S.; Peng, J.; Stewart, R.; Nishiwaki, A.; Yamada, T.; Jøgensen, U.; Hodkinson, T.R.; Gifford, J.; et al. Genetic variation in *Miscanthus x gigantesu* and the importance of estimating genetic distance thresholds for differentiating clones. *GCB Bioenergy* **2015**, *7*, 386–404. [CrossRef]
23. Drapala, P. *MSU Lets "Freedom" Ring as Viable Biofuel Feedstock*; Mississippi State University: Starkville, MS, USA, 2010.
24. Heaton, E.A.; Dohleman, F.G.; Miguez, A.F.; Juvik, J.A.; Lozovaya, V.; Widholm, J.; Zabotina, O.A.; Mcisaac, G.F.; David, M.B.; Voigt, T.B.; et al. Miscanthus: A promising biomass crop. *Adv. Bot. Res.* **2010**, *56*, 75–137.
25. New Energy Farms. Miscanthus. Available online: http://www.newenergyfarms.com/crops/miscanthus/ (accessed on 5 December 2016).
26. Dohleman, F.; Heaton, E.; Leakey, A.; Long, S. Does greater leaf-level photosynthesis explain the larger solar energy conversion efficiency of Miscanthus relative to switchgrass? *Plant Cell Environ.* **2009**, *32*, 1525–1537. [CrossRef] [PubMed]
27. Mclsaac, G.F.; David, M.B.; Mitchell, C.A. Miscanthus and switchgrass production in central Illinois: Impacts on hydrology and inorganic nitrogen leaching. *J. Environ. Qual.* **2010**, *39*, 1790–1799. [CrossRef]
28. Clifton-Brown, J.C.; Lewandowski, I.; Bangerth, F.; Jones, M.B. Comparative responses to water stress in stay-green, rapid- and slow senescing genotypes of the biomass crop, Miscanthus. *New Phytol.* **2002**, *154*, 335–345. [CrossRef]
29. Beale, C.V.; Long, S.P. Seasonal dynamics of nutrient accumulation and partition-ing in the perennial C-4-grasses *Miscanthus x giganteus* and Spartina cynosuroides. *Biomass Bioenergy* **1997**, *12*, 419–428. [CrossRef]
30. Bransby, D.I.; Mclaughlin, S.B.; Parrish, D.J. A review of carbon and nitrogen balances in switchgrass grown for energy. *Biomass Bioenergy* **1998**, *14*, 379–384. [CrossRef]

31. Christian, D.G.; Poulton, P.R.; Riche, A.B.; Yates, N.E.; Todd, A.D. The recovery over several seasons of *N*-15-labelled fertilizer applied to *Miscanthus x giganteus* rangng from 1 to 3 years old. *Biomass Bioenergy* **2006**, *30*, 125–133. [CrossRef]
32. Parrish, D.J.; Fike, J.H. The biology and agronomy of switchgrass for biofuels. *Crit. Rev. Plant Sci.* **2005**, *24*, 423–459. [CrossRef]
33. Vogel, K.P.; Brejda, J.J.; Walters, D.T.; Buxton, D.R. Switchgrass biomass productionin the Midwest USA: Harvest and nitrogen management. *Agron. J.* **2002**, *94*, 413–420. [CrossRef]
34. Coyne, P.I.; Trlica, M.J.; Owensby, C.E. Carbon and Nitrogen Dynamics in Range Plants. In *Wildland Plants. Physiological Ecology and Developmental Morphology*, 1st ed.; Bedunah, D.J., Sosebee, R.E., Eds.; Society for Range Management: Denver, CO, USA, 1995; pp. 59–167.
35. Camberato, J.; Nielsen, R.L. Nitrogen Management Guideline for Corn in Indiana. Purdue Nitrogen Management Update. Available online: https://www.agry.purdue.edu/ext/corn/news/timeless/nitrogenmgmt.pdf (accessed on 14 December 2016).
36. Ng, T.L.; Eheart, J.W.; Cai, X.; Miguez, F. Modeling miscanthus in SWAT to simulate its water quality effects as a bioenergy crop. *Environ. Sci. Technol.* **2010**, *44*, 7138–7144. [CrossRef] [PubMed]
37. Kiniry, J.R.; Johnson, M.V.; Bruckerhoff, S.B.; Kaiser, J.U.; Cordsiemon, R.L.; Harmel, R.D. Clash of the titans: Comparing productivity via radiation use efficiency for two grass giants of the biofuel field. *Bioenergy Res.* **2012**, *5*, 41–48. [CrossRef]
38. Savage, D.A.; Costello, D.F. *The Southern Great Plains: The Region and Its Needs*; U.S. Department Yearbook of Agriculture: Washington, DC, USA, 1948; pp. 503–506.
39. Krishna, K.R. *Agricultural Prairies: Natural Resources and Crop Productivity*; CRC Press: Boca Raton, FL, USA, 2015; pp. 42–43.
40. Kiniry, J.R.; Anderason, L.C.; Johnson, M.V.V.; Behrman, K.D.; Brakie, M.; Burner, D.; Cordsiemon, R.L.; Fay, P.A.; Fritschi, F.B.; Houx, J.H., III; et al. Perennial biomass grasses and the Mason-Dixon line: Comparative productivity across latitudes in the Southern Great Plains. *Bioenergy Res.* **2013**, *6*, 276–291. [CrossRef]
41. Kiniry, J.R.; Williams, J.R.; Gassman, P.W.; Debaeke, P. A general, process-oriented model for two competing plant species. *Trans. ASAE* **1992**, *35*, 801–810. [CrossRef]
42. Kiniry, J.R.; Sanderson, M.; Williams, J.R.; Tischler, C.R.; Hussey, M.A.; Ocumpaugh, W.R.; Read, J.C.; Van Esbroeck, G.; Reed, R.L. Switchgrass with the ALMANAC model. *Agron. J.* **1996**, *88*, 602–606. [CrossRef]
43. Kiniry, J.R.; Cassida, K.A.; Hussey, M.A.; Muir, J.P.; Ocumpaugh, W.R.; Read, J.C.; Reed, R.L.; Sanderson, M.A.; Venuto, B.C.; Williams, J.R. Switchgrass simulation by the ALMANAC model at diverse sites in the southern US. *Biomass Bioenergy* **2005**, *29*, 419–425. [CrossRef]
44. National Dept. of Commerce, National Oceanic and Atmospheric Administration. Climate Data Online Search. Available online: http://www.ncdc.noaa.gov/cdo-web/search (accessed on 20 May 2016).
45. Natural Resources Conservation Service, United States Dept. of Agric. Web Soil Survey. Available online: http://websoilsurvey.sc.egov.usda.gov/App/WebSoilSurvey.aspx (accessed on 20 May 2016).
46. Ratliff, L.F.; Ritchie, J.T.; Cassel, D.K. Field measured limited of soil water availability as related to laboratory measured properties. *Soil Sci. Soc. Am. J.* **1983**, *47*, 770–775. [CrossRef]
47. PRISM Climate group. USDA-Plant-Hardiness-Zone Map. Available online: http://planthardiness.ars.usda.gov/phzmweb/interactivemap.aspx (accessed on 20 May 2016).
48. Jefferson, P.G.; McCaughey, W.P. Switchgrass (*Panicum virgatum* L.) cultivar adaptation, biomass production, and cellulose concertation as affected by latitude of origin. *Int. Sch. Res. Not. Agron.* **2012**. [CrossRef]
49. Badr, A.; El-Shazly, H. Molecular approaches to origin, ancestry and domestication history of crop plants: Barley and clover as examples. *J. Gen. Eng. Biotechnol.* **2012**, *10*, 1–12. [CrossRef]
50. Craufurd, P.Q.; Wheeler, T.R. Climate change and the flowering time of annual crops. *J. Exp. Bot.* **2009**, *60*, 2529–2539. [CrossRef] [PubMed]
51. Kang, Y.; Khan, S.; Ma, X. Climate change impacts on crop yield, crop water productivity and food security-A review. *Prog. Nat. Sci.* **2009**, *19*, 1665–1674. [CrossRef]
52. Thomas, B.; Vince Prue, D. Juvenility, photoperiodism, and vernalization. In *Advanced Plant Physiology*; Wilkins, M.B., Ed.; Longman Scientific &Technical: Essex, UK, 1984; pp. 408–439.
53. Rees, A.R. *Ornamental Bulbs, Corms and Tubers*; CAB International: Wallingford, UK, 1992.

54. Crabbé, J. Dormancy. In *Encyclopedia of Agricultural Science*; Arntzen, C., Ed.; Academic Press: New York, NY, USA, 1994; Volume 1, pp. 597–611.
55. Melke, A. The physiology of chilling temperature requirements for dormancy release and bud-break in temperate fruit trees grown at mild winter tropical climate. *J. Plant Stud.* **2015**, *4*, 110–156. [CrossRef]
56. Sarath, G.; Baird, L.M.; Mitchell, R.B. Senescence, dormancy and tillering in perennial C4 grasses. *Plant Sci.* **2013**, *217–218*, 140–151. [CrossRef] [PubMed]
57. Cook, N.C.; Jacobs, G. Suboptimal winter chilling impedes development of acrotony in apple shoots. *HortScience* **1999**, *34*, 1213–1216.
58. Oukabli, A.; Mahhou, A. Dormancy in sweet cherry (*Prunus avium* L.) under Mediterranean climatic conditions. *Biotechnol. Agron. Soc. Environ.* **2007**, *11*, 133–139.
59. Li, C.; Junttila, O.; Ernstsen, A.; Heino, P.; Palva, E.T. Photoperiodic control of growth, cold acclimation and dormancy development in silver birch (Betula pendula) ecotypes. *Physiol. Plant.* **2003**, *117*, 206–212. [CrossRef]
60. Kering, M.K.; Biermacher, J.T.; Butler, T.J.; Mosali, J.; Guretzky, J.A. Biomass yield and nutrient responses of switchgrass to phosphorus application. *Bioenergy Res.* **2012**, *5*, 71–78. [CrossRef]
61. Mattos, D., Jr.; Quanggio, J.A.; Cantarella, H.; Alva, A.K. Nutrient content of biomass components of Hamlin sweet orange trees. *Sci. Agricola* **2003**, *60*, 155–160. [CrossRef]
62. Ercoli, L.; Mariotti, M.; Masoni, A.; Bonari, E. Effect of irrigation and nitrogen fertilization on biomass yield and efficiency of energy use in crop production of Miscanthus. *Field Crops Res.* **1999**, *63*, 3–11. [CrossRef]
63. Ma, Z.; Wood, C.W.; Bransby, B.I. Impact of row spacing, nitrogen rat, and time on carbon partitioning of switchgrass. *Biomass Bioenergy* **2001**, *20*, 413–419. [CrossRef]
64. Muir, J.P.; Sanderson, M.A.; Ocumpaugh, W.R.; Jones, R.M.; Reed, R.L. Biomass production of "Alamo" switchgrass in response to nitrogen, phosphorus, and row spacing. *Agron. J.* **2001**, *93*, 896–901. [CrossRef]
65. Nikiéma, P.; Rothstein, D.E.; Min, D.H.; Kapp, C.J. Nitrogen fertilization of switchgrass increases biomass yield and improves net greenhouse gas balance in northern Michigan, USA. *Biomass Bioenergy* **2011**, *35*, 4356–4367. [CrossRef]
66. Ma, B.L.; Dwyer, L.M.; Gregorich, E.G. Soil nitrogen amendment effects on nitrogen uptake and grain yield of maize. *Agron. J.* **1999**, *91*, 650–656. [CrossRef]
67. Davis, M.P.; David, M.B.; Voigt, T.B.; Mitchell, C.A. Effect of nitrogen addition on *Miscanthus x giganteus* yield, nitrogen losses, and soil organic matter across five sites. *GCB Bioenergy* **2015**, *7*, 1222–1231. [CrossRef]
68. Teat, A.L.; Neufeld, H.S.; Gehl, R.J.; Gonzales, E. Growth and Yield of *Miscanthus x giganteus* Grown in Fertilized and Biochar-Amended Soils in the Western North Carolina Mountains. *Castanea* **2015**, *80*, 45–58. [CrossRef]
69. Vergeer, P.; Van den Berg, L.L.J.; Bulling, M.T.; Ashmore, M.R.; Kunin, W.E. Geographical variation in the response to nitrogen deposition in Arabidopsis lyrata petraea. *New Phytol.* **2008**, *179*, 129–141. [CrossRef] [PubMed]