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BIOMASS AND MORPHOLOGICAL DEVELOPMENT OF MISCANTHUS
COMPARED WITH NATIVE GRASS IN SOUTH DAKOTA

BY
EHSAN J. AL AINIZI

A thesis submitted in partial fulfillment of the requirements for the
Master of Science
Major in Plant Science
South Dakota State University
2020
This thesis is approved as a creditable and independent investigation by a candidate for the master’s degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

David Wright  
Advisor  
Date

David Wright  
Department Head  
Date

Nicole Lounsbery, PhD  
Director, Graduate School  
Date
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ABSTRACT

BIOMASS AND MORPHOLOGICAL DEVELOPMENT OF MISCANTHUS COMPARED WITH NATIVE GRASS IN SOUTH DAKOTA

EHSAN AL AINIZI

2020

Little is known about Miscanthus for bioenergy in the northern Great Plains. This study compared 10-year-old stands of Miscanthus x giganteus to switchgrass (Panicum virgatum L.) and prairie cordgrass (Spartina pectinata Link) for biomass on marginal cropland in eastern South Dakota.

This study was conducted during 2017 and 2018 at Brookings, SD. Day of year (DOY), species, and N fertility were evaluated for effects on biomass yield and related components (tillers m⁻², plant height, and weed biomass). All three variables had significant effects on biomass yield, tiller m⁻², and tiller height in both years. N rate had no effect on weed biomass. Effect of N was most notable for M. x giganteus. Biomass yields of fertilized M. x giganteus, prairie cordgrass and switchgrass were 1.7 times that of unfertilized. Biomass distribution among phytomers was determined for Miscanthus in June, July, August, and September. At end of growing season about 40% of the total biomass yield was in the five most proximal phytomers. The most distal four phytomers remained undeveloped due to killing frost.

Biomass was estimated in April 2018 and 2019 from stockpiled over-wintered growth at Brookings, SD. The design was a split-plot with species whole plots and N fertilizer (0 or 112 kg N per ha) sub-plots. Species and N rate were significant in both years. In 2017, biomass yield was from 15.7 Mg per ha for Miscanthus to 9.4 Mg per ha
for switchgrass. Fertilized produced 80% more biomass than unfertilized plots. The species x N rate mean square was significant in 2017, with Miscanthus having a much greater response to N than the other grasses. No difference was found among species at 0 kg N ha\(^{-1}\), whereas species means were significant at 112 kg N ha\(^{-1}\). In 2018, Miscanthus (12.9 Mg ha\(^{-1}\)) and prairie cordgrass (12.3 Mg ha\(^{-1}\)) produced more than switchgrass (5.5 Mg ha\(^{-1}\)). Miscanthus produced large amounts of cellulosic biomass, compared to native grasses in the NGP; however, its superiority was only at a high level of N, a costly input for sustainable biofuel production systems.
CHAPTER 1

ABSTRACT

BIOMASS PRODUCTION OF *MISCANTHUS X GIGANTEUS* COMPARED TO TWO NATIVE SPECIES SWITCHGRASS AND PRAIRIE CORDGRASS IN THE NORTHERN GREAT PLAINS

EHSAN AL AINIZI

2020

Alternative renewable herbaceous sources of energy to fossil fuel should be environmentally friendly, require less inputs and have high biomass yield. In the northern Great Plains, there are three candidate species for high potential biomass production. These cellulosic herbaceous grasses are exotic *Miscanthus* (*Miscanthus x giganteus*) and natives switchgrass (*Panicum virgatum*) and prairie cordgrass (*Spartina pectinata*). All have the capability to grow in diverse conditions over a wide range of soil types, including marginal cropland unsuitable for conventional grain crop production.

My objective was to compare biomass production of *M. x giganteus* to the two native species under two nitrogen fertilizer (N) rates on poorly drained and saline cropland in eastern SD.

This study was conducted during 2017 and 2018 at Brookings SD. Three fixed effects variables [day of year (DOY), species, and N fertility] were evaluated for their effects on crop biomass yield and related components (tiller m$^{-2}$, plant height, and weed competition measured as biomass). All three variables had significant effects on biomass yield, tiller m$^{-2}$, and tiller height in both years. N rate had no effect on weed biomass in
both years. N rate had a significant effect on biomass yield most notably for *M. x giganteus*. Mean biomass yield of fertilized *M. x giganteus*, prairie cordgrass and switchgrass were about 1.7 times that of unfertilized in both years. Stockpiled overwinter biomass differed among species. *M. x giganteus* produced 1.5 times as much as the natives in 2017 and 2.3 times more than switchgrass in 2018. The species x N rate interaction was significant in 2017, but not in 2018.

*Miscanthus x giganteus* is a new candidate species for biofuel feedstock in the northern Great Plains with high yield expectation and unknown ecosystem effect. Biomass production of *Miscanthus* was twice that of switchgrass and 20% higher than prairie cordgrass. *Miscanthus* required N fertilizer to achieve those yields in both years of the study.

INTRODUCTION

Fossil fuels are a primary source of energy worldwide. However, due to increasing prices and their negative effects on ecological systems (e.g., pollution of our atmosphere by carbon dioxide and other greenhouse gases), more sustainable energy sources, such as biofuels, are needed to mitigate global climate change.

Perennial grasses are considered the best source of bioenergy because they have a high water-use efficiency, minimize soil erosion, sequester carbon in the soil, and require limited fertilizer application (Heaton et al., 2004b). Also, unlike annual crops, perennial grasses mobilize nutrients from leaves and stems and translocate them to their root system (Heaton et al., 2009).
First generation biofuels are derived from annual crops typically grown for food and feed; therefore, the production of first generation biofuels displaces food and feed crops (Davis et al., 2012). In addition, they need significant inputs to maximize production (Hülsbergen et al., 2001). Furthermore, the continuous planting of annual crops (e.g., maize, soybean, and wheat) in fertile soil in the United States (US) has led to decreased soil fertility and increased soil erosion, both of which negatively impact ecological systems. These problems could be reduced by cultivating tall prairie grasses as alternative biofuel crops on marginal cropland (Gonzalez-Hernandez et al., 2011).

Herbaceous perennial grasses are second generation bioenergy crops that are also useful for forage. They provide many advantages over first generation biofuel feedstock, including higher biomass production and a lower need for fertilizer application, herbicides, and other chemical inputs. In addition, they enhance and improve the ecosystem by decreasing soil erosion, provide habitat for wildlife, and sequester carbon into the soil (Mitchell et al., 2008).

Switchgrass

Switchgrass (*Panicum virgatum*) was selected as a model species for cellulosic biofuel from perennial grasses in the United States based on its high water use efficiency, wide distribution, and high productivity (McLaughlin, 1992). It also can grow in various soil types (Bouton, 2007; Parrish and Fike, 2005). Moser and Vogel (1995) described two ecotypes of switchgrass (upland and lowland) with different morphological traits. The interaction of switchgrass ecotype and environment has a major effect on biofuel feedstock production (Cassida et al., 2005). Switchgrass has been extensively studied for
its high potential biomass in the Great Plains and Midwest e.g., (Boe and Lee, 2007; Casler and Boe, 2003; Sanderson et al., 1999)

Prairie cordgrass

Prairie cordgrass (*Spartina pectinata* Link.) is a $C_4$ warm-season perennial grass that is widespread in the US. The species is strongly rhizomatous, and it is adapted to growth in marginal lands with high salinity and low drainage or wet soil that is not suitable for food crops. It provides shelter for animals and wildlife and traps snow which provides more moisture for the soil. The evaluation of prairie cordgrass in the northern Great Plains and Europe revealed its potential for higher biomass yield than corn or switchgrass in poor soils (Boe et al., 2009).

Miscanthus

Another perennial grass candidate cellulosic biofuel species Miscanthus (*Miscanthus x giganteus*) is a hybrid and sterile species produced naturally by crossing *M. sacchariflorus* and *M. sinensis*. In 1935, Olsen in Japan collected rhizomes of *M. x giganteus* due to its high productivity and transferred them to Denmark for planting. It was then taken to other countries in Europe then later to the US (Jones and Walsh, 2001; Lewandowski et al., 2000). The first serious study on *Miscanthus x giganteus* for biofuel in the USA was by Heaton (2000) in the Midwest.

Tolerance of warm-season grasses to low temperatures is a key trait of candidate grasses for biofuel feedstock production. Many studies reported on the ability of *M. x giganteus* to produce high biomass even when planted in cold regions, (e.g., the Midwest, which is considered a cool temperate region). Over a three-year period a mature stand of *M. x giganteus* produced two to three times more biomass than a mature stand of
switchgrass (Heaton et al., 2008). At low temperatures, the photosynthetic rate in maize was reduced by 80%, but remained unaffected in *M. x giganteus* (Naidu et al., 2003). This productivity is due in part to the ability of *M. x giganteus* to develop a canopy in a short period of time even when grown in a cold climate (Clifton-Brown et al., 2015; Dohleman and Long, 2009). Others (Lewandowski et al., 2000; Lewandowski et al., 2003c) reported yield was between 4 and 44 t DM ha\(^{-1}\). In contrast, Heaton et al. (2004a) found average yield of switchgrass in Europe was 12 t ha\(^{-1}\), which is less than half of the maximum yield for *Miscanthus*. A Midwest study of *Miscanthus* confirmed its high biomass production potential with average annual yields of 35.4, 34.7, and 22.0 t ha\(^{-1}\) at three sites in Illinois in consecutive years (Pyter et al., 2009).

The growing season of *M. x giganteus* in central Illinois begins in April and by the end of May plants reach 2 m in height and 3 m or more by the end of September. Optimal biomass production for *M. x giganteus* occurs after at least three growing seasons after plant establishment (Pyter et al., 2009). Average yield of *M. x giganteus* and switchgrass over 8–10 years in the US were 23.4 ± 1.2 and 10.0 ± 0.9 Mg ha\(^{-1}\) yr\(^{-1}\), respectively (Arundale et al., 2014b). Yield reached up to 38 t ha\(^{-1}\) in southern and mid Europe (Lewandowski et al., 2003a). In contrast, two field trials in cool temperate regions, one in the northern Great Plains (eastern South Dakota) in the US, and second study in eastern England found that the average yield within 8 – 10 years post-establishment for prairie cordgrass (*Spartina pectinata* L.) was 12 t ha\(^{-1}\)/year (Boe et al., 2009; Potter et al., 1995). A long-term study conducted in eastern South Dakota between 2000 to 2008 found a mature stand of ‘Red River’ of prairie cordgrass produced an average of 12.7 Mg ha\(^{-1}\) (Boe et al., 2009).
Regarding yield components of biomass in perennial grasses, increased tiller density is a trait that contributes to either increased or decreased biomass yield. Tiller density varies by species, plant spacing, infection by some diseases, fertilizer application and other factors. Tiller mass was more important to determine increase in biomass production than tiller density, as tiller mass had a higher response to nitrogen application than tiller density (Muir et al., 2001). Boe (2007) reported that between stem elongation and seed maturity there was strong linear correlation between yield of biomass and tiller density and yield of biomass and tiller mass.

Nitrogen fertilizer plays a major role in maximizing biomass production depending on response of species, weather conditions, and soil type. A side-by-side trial in central Illinois found biomass production of *M. x giganteus*, when no fertilizer was applied, was greater than fertilized corn by 60%. In addition, average yield across the state was 30 Mg ha\(^{-1}\) (Dohleman et al., 2009; Heaton et al., 2008).

There was inconsistency between some studies in the US and Europe about decline of biomass production of *Miscanthus x giganteus* and switchgrass due to many reasons, one being age of stand. Fourteen-year-old stands *M. giganteus* with two fertilization treatments, 0 and 120 kg ha\(^{-1}\) year\(^{-1}\), found no significant differences in biomass yield (Christian et al., 2008). Also Brummer et al. (2000) indicated that after two years of applying N at 112 kg ha\(^{-1}\) the yield of ‘Cave-In-Rock’ switchgrass was increased nearly 4.3 Mg ha\(^{-1}\) with optimum management and weed control.

A long-term study in Illinois found that peak production of *M. x giganteus* was reached at year five after establishment with little decline until year eight. The decline in yield over years 8-10 was less in *P. virgatum* (switchgrass) than *M. x giganteus*. In
contrast, long term studies in western Europe found that *M. x giganteus* had little or no yield decline. Stand age had higher effect on *Miscanthus* and yield decline in *M. x giganteus* over long-term of establishment was greater than for switchgrass (Arundale et al., 2014b). Stockpiled overwinter biomass yields had many environmental advantages but at the same time delaying harvest until end of winter can cause significant decline in biomass production due to leaf shatter and inflorescences detachment.

Furthermore, dry matter drops of *Miscanthus* from 17 to 14 t ha\(^{-1}\), equal to 35% of mean yield, happened between fall and winter harvest. A ten-year study in Europe found yield declined by 9% each month after the first frost in fall (Clifton-Brown et al., 2001a). The major loss of *Miscanthus* yield was due to detachment of upper stems and leaf when harvest was delayed to late winter. A study in Europe on 15 different genotypes of *Miscanthus*, including *M. x giganteus*, found that reduction in yield overwinter in some genotypes was due to week stems leading to detached and broken stems (Clifton-Brown et al., 2001b; Lewandowski et al., 2003a). Heaton et al. (2009) recommended harvesting *M. x giganteus* after senescence in December instead of March or April to avoid biomass loss during late winter which found the feedstock had low mineral contents and that improved the quality of feedstock for biofuel and no extra nutrient removal from soil. Furthermore, overwintering stockpiled switchgrass can provide good moisture to soil by trapping more snow during winter and enhance wildlife habitat in the northern Great Plains (Lee and Boe, 2005).

A long-term study in England found that additional N had no significant effect on yield of prairie cordgrass (*Spartina pectinata*). The average yield over 6 years was 12 t ha\(^{-1}\) with increased stand age showing no decline in yield (Potter et al., 1995).
Harvest frequency is critical for *M. x giganteus*. Multiple cutting through one growing season lead to exhaustion of the rhizomes and stand mortality. A single harvest is preferred for quality and quantity of biomass (Lewandowski et al., 1998). Over 14 years of growing *M. x giganteus* in southern United Kingdom mean yield was stable after year eight until year 14, with no evidence of decline in yield (Christian et al., 2008). In another study, stand age had effect on biomass yield in *M. x giganteus* with biomass yield declining after 8 to 10 years, compared to 5 years of stand age (Arundale et al., 2014a). A study in Italy (temperate climate region) with 100 kg ha\(^{-1}\) N applied to *M. x giganteus* over 12 growing seasons showed that average biomass yield from year 2 to 12 was 28.7 t ha\(^{-1}\) (Angelini et al., 2009).

In Illinois biomass production of *M. x giganteus* exceeded that for switchgrass by 12 Mg ha\(^{-1}\). Response to precipitation and fertilization were different in *M. x giganteus* and switchgrass. Yield of *Miscanthus* was influenced significantly more by precipitation than switchgrass. Conversely, N fertilizer rate had no significant effect on *M. x giganteus* whereas response of switchgrass was significant; however, no significant responses occurred in *M. x giganteus* for yield (Heaton et al., 2004a). Applying 50 kg N ha\(^{-1}\) with sufficient precipitation for switchgrass achieved nearly 15 Mg ha\(^{-1}\) as a sustainable yield (Parrish and Fike, 2005). A study in Texas on switchgrass found biomass production was highly increased when 112 kg N ha\(^{-1}\) or more was applied, with increases in both tiller mass and tiller density (Muir et al., 2001).

Both switchgrass and prairie have been studied extensively in the northern Great Plains whereas little is known about biomass production of *Miscanthus x giganteus* in the region. The current is the first side-by-side study of 10-year old stands of *M. x*
giganteus in South Dakota with two native species, switchgrass and prairie cordgrass, for their growth and biomass production on marginal cropland in response to nitrogen fertilizer.

MATERIALS AND METHODS

The study was conducted on the South Dakota State University Agricultural Experiment Station Felt Farm near Brookings, SD (44.3652° N, 96.7965° W). Three perennial warm-season biofuel-candidate grass species were evaluated for biomass production during 2017 and 2018 (Figs. 1 and 2). Two grasses native to the northern Great Plains, switchgrass (Panicum virgatum L.) and prairie cordgrass (Spartina pectinata Link), were compared with Miscanthus (Miscanthus x giganteus), an exotic species from Southeast Asia.

Experimental design was a split-plot for overwinter stockpiled biomass and split-split-plot for growing season biomass estimates in a randomized complete block with four replications. Species were whole plots and nitrogen fertilizer (0 kg N ha\(^{-1}\) or 112 kg N ha\(^{-1}\)) was the sub plot treatment. Individual plots of each species were established from transplanted seedlings (switchgrass and prairie cordgrass) and (Miscanthus) in June 2009. Plant spacing was on 0.6-m centers. Sub-plot size was 3 m x 8 m. N fertilizer was applied in the form of urea on 4 May 2017 when the grasses were in early vegetative development. The soil type was a McKranz (fine-silty, mixed, superactive, frigid, Calciudolls)-Badger (fine, smectitic, frigid, vertical, Argiaquolls) silty clay loam. This soil is considered marginal for conventional crop production due to slow drainage that impedes timely planting in the spring. Prior to initiation of this study, the experimental
plots were occasionally burned in the spring to remove excessive accumulation of standing dead material and litter.

**Data Collection and Analysis**

Over-winter stockpiled biomass was harvested during April 2018 and April 2019 for each of the 2017 and 2018 growing seasons, (Figs. 3 and 4). Harvest was with a sickle-bar mower at a stubble height of 10 cm. Individual plot size was 0.8 m x 8 m. Biomass was weighed in the field using a spring-loaded balance with 0.1 kg accuracy (Fig. 5). Grab samples were dried at 40°C for two weeks to determine dry matter concentration for biomass calculations (Fig. 6). Data were analyzed using the Linear Models/General Analysis of Variance procedure in Statistix 8 (Analytical Software, 2000).

For all three species, growing-season biomass and stand morphology measurements were collected on 16 June, 13 July, 7 August, and 12 September during 2017 and 23 June, 23 July, 23 August, and 27 September during 2018. Within each sub plot, 0.18 m² sub samples were harvested at ground level by hand with a rice knife for each of the above dates (Fig. 5). For 2017, one sub sample was taken per sub plot whereas, in 2018 two sub samples were taken per sub plot. Immediately after harvest, sub samples were separated into target species and non-target species components. For each sub sample those components were placed in separate paper bags and dried at room temperature for three weeks (Fig. 6). Data collected for each sub sample were: 1) dry matter biomass for the target species and non-target species (i.e., weeds), 2) number of tillers, and 3) tiller height. Dry weight of biomass for target species and non-target
species was measured to 0.1 g accuracy. Biomass data were analyzed using the split-plot and split-split-plot procedures within the Analysis of Variance menu in Statistix 8 (Analytical Software, 2003). Species, N fertilizer rate, and day of year (DOY) were considered fixed effects. Replications were considered random.

Fig. 1. Plots of Miscanthus x giganteus, switchgrass, and prairie cordgrass at South Dakota Agricultural Experiment Station (SDAES) Felt Farm, Brookings, SD early spring 2017.
Fig. 2. Plots of *Miscanthis x giganteus*, switchgrass, and prairie cordgrass at (SDAES) Felt Farm Brookings, SD during August 2018.
Fig. 3. SDAES Felt Farm during winter 2018 showing snow drifting and trapping by *Miscanthus*. Native species were completely covered by snow.
Fig. 4. Overwinter sample collection procedures at SDAES Felt Farm Brookings, SD during 2018 and 2019.
Fig. 5. Procedures of sample collection at SDAES Felt Farm Brookings, SD during growing seasons 2017 and 2018.
Fig. 6. Drying and separating samples into 3 components (target species and non-target species and weeds) for three perennial grass candidate biofuel species at Brookings, SD for growing season 2017.
RESULTS

2017 Growing Season

Analysis of variance indicated significant main effects for the three fixed independent variables (i.e., bioenergy crop species, N rate, and DOY) on two biomass production parameters and two stand morphological traits during the 2017 growing season, with the exception of N rate on weed biomass. However, eight of the twelve first-order interactions involving the three fixed main effects with biomass and stand morphology variables were also significant. In addition, the second-order interaction involving all three main effects was significant for all four variables, other than tiller m$^{-2}$ (Table 1).

The species x N rate interaction for crop biomass was quantitative, with no change in species ranks between N rates. Target biomass yield was similar for all three species in the 0 kg N ha$^{-1}$ rate (control) treatment. Biomass yield means for Miscanthus, prairie cordgrass, and switchgrass were 5.08, 5.74, and 4.49 Mg ha$^{-1}$, respectively. Mean biomass yield for the 112 kg N ha$^{-1}$ treatment was 19.43, 12.94, and 9.46 Mg ha$^{-1}$ for Miscanthus, prairie cordgrass, and switchgrass respectively. Miscanthus had a 2-fold greater yield response to 112 kg N ha$^{-1}$ than the native species, averaged across the four DOY harvest dates (Fig. 7A). The species x N rate interaction for plant height showed a similar pattern to that of crop biomass yield, with Miscanthus having a greater rate of increase than the two native species (Fig. 7B and 8).

In contrast, the species x N rate interaction for weed biomass was qualitative, with starkly different patterns among crop species. Weed biomass was lowest for prairie cordgrass and did not vary between N rates. Whereas, weed biomass in switchgrass
swards increased by 220% in fertilized compared with control sub-plots. *Miscanthus* showed an opposite pattern with a 50% decrease in weed biomass in fertilized, compared with control sub-plots (Figures 7C and 9).

Analysis of variance showed significant effects of DOY on target biomass yield, plant height, weed biomass yield, and tillers m$^{-2}$. In addition, partitioning DOY sums of squares revealed significant linear effects for all variables other than weed biomass and significant quadratic trends for weed biomass and plant height. However, the species x DOY interaction was significant for target biomass yield, tiller m$^{-2}$, and plant height, but not weed biomass (Table 1). *Miscanthus* and switchgrass biomass increased linearly from June through September. Mean biomass yields were 2.28, 9.01, 15.54, and 22.19 Mg ha$^{-1}$ during June, July, August, and September, respectively for *Miscanthus* and 1.99, 5.60, 9.61, and 10.71 Mg ha$^{-1}$, respectively for switchgrass. Biomass of switchgrass peaked (9.41 Mg ha$^{-1}$) in July and was maintained through the rest of the growing season, whereas for prairie cordgrass yield increased from 9.53 to 14.30 mg ha$^{-1}$ between August and September. Differences between *Miscanthus* and the native species for crop biomass yield increased during the growing season, from similar yields in June to a 2-fold yield advantage for *Miscanthus* in September with mean biomass yield 22.19 Mg ha$^{-1}$ (Fig. 10A).

The species x DOY interaction for tillers m$^{-2}$ was quantitative with no change in species rank across DOY. Tiller density was relatively constant for all three species. Mean tillers m$^{-2}$ were 74.9, 82.0, 77.4, and 106.2 in June, July, August, and September for *Miscanthus*, 403.6, 384.9, 439.5, and 443.4 for switchgrass, and 972.4, 851.7, 577.2, and 687.4 for prairie cordgrass. Tiller density was highest for prairie cordgrass.
throughout the growing season (maximum 1377 tillers m$^{-2}$) in June, including during an inexplicable decline of about 30% in August (Fig. 10B). The species x DOY interaction for plant height was also quantitative with no change in rank for species across DOY.

Differences between Miscanthus and the native species tended to increase as the growing season progressed. Significant linear and quadratic regression sums of squares explained the seasonal pattern in plant height (Fig. 10C). Weed biomass varied sporadically across the growing season but was lowest for prairie cordgrass from June through August with biomass means of 0.40, 0.90, 1.42, and 1.12 Mg ha$^{-1}$ in June, July, August, and September, respectively. Weed biomass for the same periods of year for Miscanthus and switchgrass were 1.24, 1.35, 2.63, and 1.02 Mg ha$^{-1}$ and 1.09, 2.55, 1.88, and 2.16 Mg ha$^{-1}$, respectively (Fig. 10D).

The N rate x DOY interactions for target biomass yield and plant height were quantitative and similar in pattern, with no change in N rate rankings across the growing season. The range in height during late growing season (mid-September) for Miscanthus, switchgrass and prairie cordgrass were 138–252, 116–170, and 119–192 cm, respectively. The easily recognizable pattern for both was sequential increase in the magnitude of difference between the control and fertilized treatments at each DOY harvest date (Fig. 11A, B).

2018 Growing Season

In general, results from 2018 were similar to those obtained in 2017. Analysis of Variance indicated the three fixed main effects were significant for all traits, with the exception of N rate effect on weed biomass. As for interactions, there were no significant second-order interactions in 2018; whereas, only the second-order interaction for tiller
density was non-significant in 2017. For first order interactions, significance of the crop species x N rate interaction differed between years for tiller density and target species x DOY interaction differed for weed biomass and tiller density between years (Table 2).

Target biomass yield was about 30% lower in 2018 than in 2017. Nevertheless, the species x N rate response pattern was similar. That was comparable yields for *Miscanthus* and natives in the control treatment with a greater response to fertilizer for *Miscanthus*. *Miscanthus* mean biomass yield was 5.08 Mg ha\(^{-1}\) with 0 kg N ha\(^{-1}\) and 19.43 Mg ha\(^{-1}\) with 112 kg N ha\(^{-1}\) compared with the natives. Switchgrass and prairie cordgrass showed significant responses to fertilizer in 2017. Mean biomass yields were 9.46 and 12.94 Mg ha\(^{-1}\), respectively; whereas the unfertilized treatment means of switchgrass and prairie cordgrass were 4.49 and 5.74 Mg ha\(^{-1}\), respectively (Fig. 7A) but only prairie cordgrass responded in 2018 (Fig. 12A). The significant species x N rate interaction for tiller density and plant height were quantitative with no change in rank between N rates (Fig. 12B, C). The significant species x N rate interaction patterns for weed biomass were very similar for the two years (Fig. 7C, Fig. 12D).

In general, the nature of the species x DOY interactions were similar in 2017 and 2018. For crop biomass yield in 2018, the pattern was for the difference between *Miscanthus* and the natives to increase as the season progressed (Fig. 13A). The same was true for plant height (Fig. 13C). Similar to what occurred in 2017, weed biomass was lowest for prairie cordgrass and remained stable throughout the growing season. In contrast, weed biomass in switchgrass and *Miscanthus* fluctuated resulting in a qualitative interaction between the two crop species (Fig. 13B). The species x DOY interaction for
tiller density was non-significant in 2018, with prairie cordgrass having the highest
density throughout the growing season (Fig. 13D).

The N rate x DOY interactions for target biomass yield and plant height were
quantitative and similar in pattern, with no change in N rate rankings across the growing
season. The easily recognizable pattern for both was sequential increase in the magnitude
of difference between the control and fertilized treatments at each DOY harvest date (Fig.
14A, B).

Linear and quadratic regression components of the DOY sums of squares were
significant for target biomass and plant height in 2018 (Table 2; Fig. 13A, C). Polynomial
contrasts applied for data of 2017 for DOY showed 98%, 47%, and 86% of variation
among the means of biomass yield, tillers m$^{-2}$ and plant height respectively of
*Miscanthus*, prairie cordgrass, and switchgrass across fertility could be explained by a
linear model but 73% of variation among means of weed biomass could be explained by
quadratic model (Table 1). In 2018, the polynomial contrast showed that 69%, 96%, and
81% of variation for biomass yield, tillers m$^{-2}$, and plant height due to DOY and across N
fertility levels could be explained by a linear model, and 42% of variation among weed
biomass means could be explained by quadratic model (Table 2).

**Overwinter biomass production of 2017 and 2018**

Analyses of variance of stockpiled overwinter crop biomass produced in growing
seasons 2017 and 2108 indicated differences among species and between N rates were
significant in both years; whereas, the species x N rate interaction was significant only in
2017 (Table 3). Significant differences were found among the three species in 2017.
Mean yields of fertilized *Miscanthus*, switchgrass, and prairie cordgrass were 21.33, 12.18, and 13.50 Mg ha\(^{-1}\), respectively; whereas, with 0 kg N ha\(^{-1}\), results showed 10.09, 6.54, and 9.89 Mg ha\(^{-1}\), respectively (data not shown). In comparison, in 2018 mean yields of *Miscanthus* and prairie cordgrass, averaged across N rates were similar, and 2.5 times the yield of switchgrass (Table 3). The response to fertilization was similar in both years, with about 65% increase in biomass yield.
Table 1. Mean squares for target biomass yield, weed biomass yield, tillers m$^{-2}$, and plant height for three perennial grass candidate biofuel species at Brookings, SD for growing season of 2017.

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Target yield (Mg ha$^{-1}$)</th>
<th>Weed (Mg ha$^{-1}$)</th>
<th>Tillers m$^{2}$</th>
<th>Height (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>REP (A)</td>
<td>3</td>
<td>94.15</td>
<td>0.3729</td>
<td>3142</td>
<td>1331.0</td>
</tr>
<tr>
<td>SPECIES (B)</td>
<td>2</td>
<td>223.75 *</td>
<td>7.5463 *</td>
<td>3777701**</td>
<td>13765.7 **</td>
</tr>
<tr>
<td>Error A x B</td>
<td>6</td>
<td>28.47</td>
<td>1.4270</td>
<td>8736</td>
<td>220.9</td>
</tr>
<tr>
<td>FERTILITY (C)</td>
<td>1</td>
<td>1876.93 **</td>
<td>0.8510</td>
<td>322596 **</td>
<td>38520.1 **</td>
</tr>
<tr>
<td>B x C</td>
<td>2</td>
<td>192.18 **</td>
<td>10.4862 **</td>
<td>61768</td>
<td>937.5 *</td>
</tr>
<tr>
<td>Error A x B x C</td>
<td>9</td>
<td>16.10</td>
<td>1.2265</td>
<td>18785</td>
<td>217.1</td>
</tr>
<tr>
<td>DOY (D)</td>
<td>3</td>
<td>721.62 **</td>
<td>4.7276 *</td>
<td>59631 *</td>
<td>32614.4 **</td>
</tr>
<tr>
<td>Linear trend</td>
<td>1</td>
<td>(2131.5) **</td>
<td>(3.5483)</td>
<td>(85343) *</td>
<td>(85083) **</td>
</tr>
<tr>
<td>Quadratic trend</td>
<td>1</td>
<td>(32.592)</td>
<td>(10.394) *</td>
<td>(67901)</td>
<td>(12190) **</td>
</tr>
<tr>
<td>B x D</td>
<td>6</td>
<td>64.36 **</td>
<td>2.0227</td>
<td>96343 **</td>
<td>450.7 **</td>
</tr>
<tr>
<td>C x D</td>
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<td>282.89 **</td>
<td>0.8282</td>
<td>33692</td>
<td>3215.2 **</td>
</tr>
<tr>
<td>B x C x D</td>
<td>6</td>
<td>70.56 **</td>
<td>5.9107 **</td>
<td>38541</td>
<td>676.8 **</td>
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<tr>
<td>Error A x B x C x D</td>
<td>54</td>
<td>14.09</td>
<td>1.5627</td>
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<td>136.5</td>
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<tr>
<td>Total</td>
<td>95</td>
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*, ** Significant at the 0.05 and at the 0.01 level of probability, respectively.
Table 2. Mean squares for target biomass yield, weed biomass yield, tillers m\(^{-2}\), and plant height for three perennial grass candidate biofuel species at Brookings, SD for growing season of 2018.

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Target yield (Mg ha(^{-1}))</th>
<th>Weed (Mg ha(^{-1}))</th>
<th>Tillers m(^{-2})</th>
<th>Height (cm)</th>
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</thead>
<tbody>
<tr>
<td>REP (A)</td>
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<td>115.415</td>
<td>13.255</td>
<td>53952</td>
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<td>SPECIES (B)</td>
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<td>358.858**</td>
<td>114.152 **</td>
<td>6625218 **</td>
<td>93778.8 **</td>
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<tr>
<td>Error A x B</td>
<td>6</td>
<td>13.059</td>
<td>5.399</td>
<td>28096</td>
<td>1081.5</td>
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<tr>
<td>FERTILITY (C)</td>
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<td>411.563**</td>
<td>1.477</td>
<td>143172*</td>
<td>26932.7 **</td>
</tr>
<tr>
<td>B x C</td>
<td>2</td>
<td>140.790**</td>
<td>35.550 **</td>
<td>223430 **</td>
<td>4572.2 **</td>
</tr>
<tr>
<td>Error A x B x C</td>
<td>9</td>
<td>5.597</td>
<td>3.362</td>
<td>22456</td>
<td>404.7</td>
</tr>
<tr>
<td>DOY (D)</td>
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<td>272.251**</td>
<td>15.564 **</td>
<td>95977</td>
<td>44689.6 **</td>
</tr>
<tr>
<td>Linear trend</td>
<td>1</td>
<td>(567.90) **</td>
<td>(2.1482)</td>
<td>(276947) *</td>
<td>(109896) **</td>
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<tr>
<td>Quadratic trend</td>
<td>1</td>
<td>(182.16) **</td>
<td>(19.622) *</td>
<td>(6153.2)</td>
<td>(23394) **</td>
</tr>
<tr>
<td>B x D</td>
<td>6</td>
<td>86.045**</td>
<td>8.223 **</td>
<td>55592</td>
<td>4240.9 **</td>
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<tr>
<td>C x D</td>
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<td>48.544**</td>
<td>2.303</td>
<td>21218</td>
<td>1362.9 **</td>
</tr>
<tr>
<td>B x C x D</td>
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<td>23.230</td>
<td>4.527</td>
<td>16531</td>
<td>383.2</td>
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<td>28931</td>
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</tr>
<tr>
<td>Error</td>
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<td>4.792</td>
<td>1.353</td>
<td>8402</td>
<td>88.5</td>
</tr>
<tr>
<td>Total</td>
<td>191</td>
<td></td>
<td></td>
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*, ** Significant at the 0.05 and at the 0.01 level of probability, respectively.
Table 3. Means yield of overwinter stockpiled biomass of three different grass species as influenced by N rates (kg ha$^{-1}$) for three perennial grass candidate biofuel species at Brookings, SD for growing seasons of 2017 and 2018.

** Differences between means significant at the 0.01 level of probability.

<table>
<thead>
<tr>
<th>Species (S)</th>
<th>2017</th>
<th>2018</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS</td>
<td>15.71 **</td>
<td>12.93 **</td>
</tr>
<tr>
<td>SW</td>
<td>9.36</td>
<td>5.52</td>
</tr>
<tr>
<td>PC</td>
<td>11.70</td>
<td>12.32</td>
</tr>
</tbody>
</table>

| LSD (0.05) | 1.93    | 3.02    |

<table>
<thead>
<tr>
<th>N rate (N)</th>
<th>2017</th>
<th>2018</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>8.84 **</td>
<td>7.90 **</td>
</tr>
<tr>
<td>112</td>
<td>15.67</td>
<td>12.62</td>
</tr>
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</table>
Fig. 7. Species x N rate interaction and its effect on (A) target biomass yield, (B) plant height, and (C) weed biomass yield for three perennial grass candidate biofuel species (MS=Miscanthus, SW=switchgrass, PC=prairie cordgrass) at Brookings, SD in growing season of 2017.
Fig. 8. (a) Response of *Miscanthus giganteus* to different N rate (0 and 112 N kg ha\(^{-1}\)) in both years; fertilized *Miscanthus* (left) and unfertilized (right). (b) Fertilized (right) and unfertilized prairie cordgrass (left) at Brookings, SD during July 2017.
Fig. 9. (a) Weed (w) competition with different N rate for fertilized and non-fertilized Miscanthus (right) during mid-August; (b) fertilized and non-fertilized switchgrass and prairie cordgrass (left) at Brookings, SD during late September of growing season 2018.
Fig. 10. DOY x species interaction effect on (A) target biomass yield, (B) tillers m$^{-2}$, (C) plant height, and (D) weed biomass yield for three perennial grass candidate biofuel species at Brookings, SD in growing season of 2017.
Fig. 11. DOY x fertility interaction and its effect on (A) target biomass yield, (B) plant height for three perennial grass candidate biofuel species at Brookings, SD in growing season of 2017. For fertility, 0=control, 112=112kg N ha$^{-1}$. 
Fig. 12. Species x N rate interaction effect on (A) target biomass yield, (B) tillers m$^{-2}$, (C) plant height, and (D) weed biomass for three perennial grass candidate species for biofuel of Brookings, SD growing season of 2018.
Fig. 13. DOY x Species interaction and its effect on (A) target biomass yield, (B) weeds biomass yield, (C) plant height and (D) tillers m$^{-2}$ for three perennial grass candidate biofuel species at Brookings, SD in growing season of 2018. For species, MS=\textit{Miscanthus}, SW=switchgrass, PC=prairie cordgrass.
Fig. 14. DOY x N rate interaction effect on (A) target biomass yield, (B) plant height for three perennial grass candidate biofuel species at Brookings, SD in growing season of 2018. For DOY, June = 174, July = 204, August = 235, September = 270.
Fig. 15. Monthly precipitation and temperature at Brookings, SD during 2016, 2017, and 2018 and the 30–years averages.
DISCUSSION

Weather conditions

Average annual precipitation for Brookings, SD is 616 mm. Average annual temperature is 6.15°C. Precipitation in 2017 was 55% of normal in April, 164% of normal in May, 34% of normal in June, 151% of normal in July, and 158% of normal in August. Precipitation in 2018 was 15% of normal in April, 44% of normal in May, normal in June, 290% of normal in July, and 122% of normal in August (Fig. 15).

Overall, 2018 received 49 mm more rainfall during April through September than 2017. Average temperature for May, June, and August in 2018 were higher than 2017 by 7.9, 3.05, and 3.65°C respectively. Total precipitations were 741.7 mm in 2016, 670.9 mm in 2017, 788.0 mm in 2018, and 617.4 mm for the 30-year average. In addition, precipitation rate during growing season from April through September, were 80.5 mm in 2016, 84.2 mm in 2017, 90.6 mm in 2018, and 77.7 mm for the 30-year average. Also, precipitations rates during June and July were 33.7 mm in 2016, 24.3 mm in 2017, 52.6 mm in 2018, and 31.0% 30-years.

2017 Growing Season

Miscanthus showed steady increasing biomass at monthly intervals June through late September, when growth was stopped by killing frost on marginal (poorly drained and saline) cropland in eastern South Dakota. At time of frost very few tillers had headed and no change in leaf color, a reliable sign of senescence and dormancy had occurred. Miscanthus stopped growing in the late September due to frost before physiological maturity with the three most apical phytomers still underdeveloped. This
agreed with a study in the Midwest at seven locations (Arundale et al., 2014b). In that study, Miscanthus stands over 10 years old produced more than two-fold the biomass of switchgrass (annual means 23.4 and 10.0 Mg ha\(^{-1}\), respectively). Also, maximum yield for Miscanthus was at the fifth growing season but declined thereafter. Furthermore, the reduction in yield in long term was greater for Miscanthus than switchgrass. Similarly, Miscanthus yielded 22 Mg ha\(^{-1}\) compared to 10 Mg ha\(^{-1}\) for switchgrass in Illinois (Heaton et al., 2004a). Miscanthus had no signs of disease infection or insect infestation compared to both natives.

Dohleman and Long (2009) attributed Miscanthus yielding twice as much as switchgrass was due to greater water and leaf nitrogen use efficiency. Most leaves of switchgrass were changed to brown prior to frost compared to M. x giganteus, for which most leaves were still green with exception of the lower leaves which were completely yellow at killing frost. I also observed that ‘Summer’ switchgrass reached physiological maturity before killing frost, indicating it was better adapted to the climate of the northern Great Plains. Because it putatively utilized more N in the growing season Miscanthus yielded 43% and 70% more than prairie cordgrass and switchgrass, respectively by the end of the growing season in 2017. Miscanthus produced more than 20 Mg ha\(^{-1}\) at 52°N, in the United Kingdom with a fully developed canopy earlier at that latitude (Beale and Long, 1995). Miscanthus can produce high biomass yield from relatively low input (nitrogen fertilizer and pesticides) resulting in reduced environment impact and sustainability for more than 15 years (Christian et al., 2008).

Precipitation during the growing season was about normal and thus adequate to support the growth of perennial warm-season grasses. Reduction in biomass yield of
switchgrass and prairie cordgrass was due, in part, to switchgrass smut (*Tilletia maclaganii*) and feeding by an insect (*Ischnodemus falicus*), respectively. In contrast, Fike et al. (2006) reported no reduction in biomass yield of switchgrass due to pests over a 10-year study.

Nitrogen is often the most critical nutrient for increasing biomass production of perennial grasses. In my study, *Miscanthus* had greater response to N fertilizer than the two native species even though *Miscanthus* was later emerging than both native species; however, its response to N was greater with significantly higher biomass yield given adequate precipitation. In contrast, Heaton et al. (2004a) reported that both *Miscanthus* and switchgrass had significant yield response to water and N application. However, *Miscanthus* produced twice (22 Mg ha\(^{-1}\)) as much biomass as switchgrass (10 Mg ha\(^{-1}\)). Conversely, to that and my study, Davis et al. (2015) reported in IL, NJ, NE, and VA, no significant effects of N rate on biomass production of *Miscanthus*, with the exception of Illinois in the fourth year of the experiment when biomass production was higher in both 60 and 120 kg N ha\(^{-1}\) than in the control treatment (0 kg N ha\(^{-1}\)). Also, Christian et al. (2008) reported no significant yield differences between fertilized and non-fertilized *Miscanthus*.

In 2017 emergence of *Miscanthus* was earlier (mid-April) compared to 2018 (early May) due to cool temperatures and snowpack until May. Prairie cordgrass emerged about two weeks earlier than switchgrass. *Miscanthus* emerged about one week later than switchgrass due to weather conditions in eastern South Dakota but rapid growth in late spring resulted in high biomass yield for *Miscanthus* in both years. A study at three sites in South Dakota found a N rate of 56 kg ha\(^{-1}\) resulted in increased biomass with
persistence of switchgrass not affected (Mulkey et al., 2006). Similarly, multiple location experiments in the Midwest found switchgrass biomass yield significantly responded to N fertilization in addition to differences in annual precipitation (Vogel et al., 2002).

In general, 10-year-old stands of three perennial biofuel grasses showed increase in biomass in response to N fertilization. However, the response of Miscanthus was 4-fold compared to 2-fold for the two natives. Without fertilization, no advantage to Miscanthus was evident.

Heaton et al. (2004a) concluded switchgrass and Miscanthus responded positively to increases in water and N. Miscanthus produced more biomass than switchgrass and responded to water to a greater degree than switchgrass, which showed high responses to N rate. In Texas, switchgrass yield was highly influenced by precipitation and N during growing season (Muir et al., 2001). In Nebraska, Vogel et al. (2002) found differences between two locations due to variation in precipitation and soil N. Tiller density (tillers m\(^{-2}\)) increased with N fertilizer treatment, with the greatest response for Miscanthus. Increasing tiller density with N fertilizer resulted in improved sward health, which in return had positive effects on biomass production, as shown by Boe and Beck (2008) for switchgrass.

Plant height of the three species increased linearly when N was applied, especially during 2017, with most rapid during the early growing season (June and July) but continuing up to mid-September before a killing frost stopped growth of Miscanthus in late September 2017. Clifton-Brown and Lewandowski (2002) found a genotype of M. sinensis which flowered early achieved less height and consequently lower biomass yield
than *M. giganteus* which had greater biomass due to later flowering and greater height, but still didn’t flower before a killing frost.

Rapid growth of N-fertilized compared to non-fertilized *Miscanthus* caused high competition and reduced weed growth. My results indicated fertilizer was a limiting factor to weed competition in a 10-year-old stand of *Miscanthus*. However, applying fertilizer to ‘Summer’ switchgrass substantially increased weed competition. In contrast, stability of prairie cordgrass for weed biomass across N treatments was due to its ability to form a dense sod composed of strong rhizomes. Weeds can be problematic during stand establishment of switchgrass and using herbicides in the first year is important to control broadleaf weeds (Lewandowski et al., 2003b). Another study reported *Miscanthus* had poor competition with weeds during establishment and the same herbicides used for maize could be used for *Miscanthus* (Lewandowski et al., 2003b). Furthermore weed competition with *Miscanthus* has been shown to decline overtime as the soil surface becomes covered with leaf litter in addition to canopy closure that limits sunlight that reaches weeds (Jones and Walsh, 2001).

Favorable growing conditions resulted in large increases in biomass yield during the growing season. *Miscanthus* had highest positive response to DOY. Switchgrass and prairie cordgrass had the similar patterns of growth but severe disease infection by smut (*Tilletia maclaganii*) in switchgrass and insect (*Ischnodemus falicus*) infestation in prairie cordgrass caused decline in production. If not, diseased ‘Summer’ switchgrass would be expected to reach maximum yield in mid-August at anthesis. In comparison *Miscanthus* growth continued until killing frost at the end of September at which time the three or so most apical phytomers were still not fully developed.
Vogel et al. (2002) reported biomass yield peaked in mid-August for switchgrass in the Midwest. In central South Dakota 90% of maximum yield variation could be explained by variation in amount of precipitation during April through May. In general, peak production was between early July to early August (stage of seed development) for ‘Dacotah’ switchgrass after which significant reduction in biomass yield resulted during senescence and seed shattering during September through October (Lee and Boe, 2005). Lee et al. (2009) reported switchgrass yielded more in response to increased rainfall during April and May.

Prairie cordgrass has potential to produce high quantities of biomass due to rapid spring – early summer growth (Boe and Lee, 2007). However, due to infestation by *I. falcipus* no yield increase occurred beyond August. Similar negative impact on biomass production of prairie cordgrass was reported in South Dakota between 2000 to 2008 (Boe et al., 2009). That study found that heavy insect feeding had profound morphological symptoms. Similar symptoms and reductions in biomass production were reported for natural stands in Kansas (Johnson and Knapp, 1996). In Iowa, switchgrass infected by smut (*T. maclaganii*) caused a decline in biomass by one-half (Gravert et al., 2000).

Tiller density was much higher for the entire growing season, for the sod-forming prairie cordgrass than the two bunchgrass grasses (i.e., switchgrass and *Miscanthus*). A study by Heaton et al. (2008) found that *Miscanthus* had lower tiller density than switchgrass, with maximums of 797 tillers m$^{-2}$ for switchgrass and 110 tiller m$^{-2}$ for *Miscanthus*. Maximum for switchgrass was in September then declined to 542 tiller m$^{-2}$ by October, whereas for *Miscanthus* the maximum was in May then declined to 64 tiller m$^{-2}$ during the rest of the growing season. Clifton-Brown and Lewandowski
(2002) found tiller density of *Miscanthus* declined from 32.2 to 9.2 stems per plant with plant age. Mitchell et al. (1998) reported similar decline in tiller density due to plant age in switchgrass.

The three species differed widely for weed biomass at monthly intervals during the growing season. Switchgrass stands weakened by smut generally had high weed biomass from July through September. For *Miscanthus*, which had much greater litter than switchgrass, weed biomass didn’t reach its maximum until August, but declined substantially thereafter likely due to shading and competition from rapid increases in height and biomass. Heaton et al. (2008) reported weed competition in *Miscanthus* was low during May through July due to canopy closure. Leaf canopy development in *Miscanthus* was earlier than in maize (Dohleman and Long, 2009).

Prairie cordgrass had much less weed biomass in early growing season through August compared to *Miscanthus* and switchgrass. Prairie cordgrass differs from switchgrass and *Miscanthus* in that it sends up new spike-like tillers during autumn. Those tillers resume growth in the spring well before tillers emerge from the underground roots of switchgrass and *Miscanthus*.

2018 Growing season

Weather conditions in 2018 were warmer and wetter May through July than 2017 due to double the precipitation rate during June and July, yet biomass yields were only about 60% of those obtained in 2017. The reason for this is unknown but due to saturated soil, it is likely that N was leached or volatilized during June and July.
A similar level of infection by smut was noticed in 2018 in switchgrass with associated reduction in yield. Similarly, for prairie cordgrass, infestation by *I. falicus* caused severe stunting to plants, similar to what was previously reported (Boe et al., 2009; Johnson and Knapp, 1996). Switchgrass had little increase in biomass during growing season, presumably due to smut infection and saturated soil conditions (Gravert et al., 2000).

Response to N fertilizer varied among species in 2018, even though no fertilizer was applied in 2018. *Miscanthus* had the highest yield response from N, fertilized *Miscanthus* yield was 40% less than 2017. Average annual yield for *Miscanthus* was 22.0 Mg ha\(^{-1}\) when N was applied compared to 11.8 Mg ha\(^{-1}\) with no N application (Lee et al., 2017). Heaton et al. (2004a) reported effect of N was greater than that of water in *Miscanthus*. My results agree since Miscanthus yielded more in 2017 than 2018 when rainfall was less in 2017 during the growing season. Yield of unfertilized *Miscanthus* in Illinois was 23.7 t ha\(^{-1}\) compared to 6.2 t ha\(^{-1}\) for unfertilized switchgrass (Pyter et al., 2007). Another study reported that a 40% *Miscanthus* yield increased due to N fertilizer (Wang et al., 2012).

Biomass was greatly increased when N rate was increased in response to increased rainfall rate during growing season for switchgrass (Muir et al., 2001). Prairie cordgrass response to N application was significant but less than in 2017, possibly due to two reasons. First infection by *I. folicus*, and second reason, no N fertilizer was applied in the spring. A recent study reported increasing N rate tended to increase biomass yield of prairie cordgrass (Hernández et al., 2018).
Tiller density varied among species due to N with little increase in *Miscanthus* and prairie cordgrass but reduction in switchgrass. Tiller density of ‘Alamo’ switchgrass increased when N was applied but tiller mass had greater response (Muir et al., 2001). Similarly, Sanderson and Reed (2000) reported that increasing N fertilization (112 kg N ha\(^{-1}\)) had no effect on tiller density but caused an increase in tiller weight in ‘Alamo’ switchgrass.

*Miscanthus* was highly competitive with weeds when N fertilizer was applied. Conversely, switchgrass which had more weed biomass in the N-fertilized treatment. Very few weeds were found in plots of prairie cordgrass with no effect on weed biomass between N treatments. Since *Miscanthus* emerged later than prairie cordgrass it had more weeds in early spring but outcompeted weeds later in the growing season, especially with the N-fertilized treatment. One study reported more than 90% loss in biomass yield due to weed competition with *Miscanthus sacchariflorus* during first two years of establishment (Song et al., 2016). When *Miscanthus* gets taller and its canopy becomes developed, it is difficult for weeds to compete (Bellamy et al., 2009; Jones and Walsh, 2001).

**DOY Effects**

*Miscanthus* and switchgrass showed little reduction in tiller density during the growing season. Infestation by *I. falcicus* likely had more effect on tiller density in prairie cordgrass. Hernández et al. (2018) reported tiller density for ‘Red River’ prairie cordgrass (old stand) in South Dakota was 536 to 934 tillers m\(^{-2}\) between years. A study in eastern South Dakota from 2000 to 2008 found tiller density declined with age of stand with a
10-year-old-stand having 683 tiller m$^{-2}$ compared to a 4-year-old stand which had 1140 tillers m$^{-2}$ (Boe et al., 2009). Decline in tiller density of prairie cordgrass could be due to several reasons. Reduction in tiller number in both years for cordgrass, especially after mid growing season may have been due to viruses or other diseases vectored by *I. falicus*. A previous study reported that prairie cordgrass was infested by *I. falicus* which found more than 20 insects tiller$^{-1}$ that contributed in biomass reduction.

Infection by smut [*Tilletia maclaganii* (Berk)] caused severe reduction in biomass yield of switchgrass due to reduction in tiller density by this fungus (Gravert et al., 2000). A study in South Dakota found positive correlation between tiller density and biomass yield in switchgrass (Boe et al., 2009).

Weeds can be major threats to perennial grasses during the growing season through competition for water, light, minerals, and space (Buhler et al., 1998). The ability of *Miscanthus* to close canopy early in spring can reduce the effect of weeds till end of growing season. Growing weeds in early spring in cold climates could be very rapid and problematic for herbaceous perennial-warm season grasses (Buhler et al., 1998). Conversely, for *Miscanthus* planted in May weed control was not necessary after planting, and in the second year the plants’ canopy reached to 3 m and closed at the third year of stand (Pyter et al., 2007).

Prairie cordgrass emerged at least three weeks earlier than switchgrass, and tillers of prairie cordgrass had at least 2 leaves which reached 25 cm in height, whereas tillers of switchgrass had not emerged (Boe et al., 2009). This confirmed the strength of prairie cordgrass to compete with weeds more than *M. x giganteus* and switchgrass. In addition, the complex root system and rhizomes of prairie cordgrass make it difficult for
weeds to compete. Correspondingly, natural spacing between Miscanthus plants allowed for more sunlight to reach growing weeds.

Overwintering dry matter biomass production for 2017 and 2018

Reduction in biomass yield occurred for overwintered biomass for all species compared to biomass achieved during the growing season when most the stems and leaves were green, and leaves were attached to stems with high water concentration. Decline in biomass during winter is inevitable due to weather condition such as strong winds and heavy snow, that caused lodging, breaking of stems, shattering of leaves and loss of panicles, especially for switchgrass. Overall Miscanthus produced more overwintered biomass due largely to less lodging than the natives.

However, delayed harvest of perennial grasses is important due to translocation of mineral and nutrients after senescence and through winter which decreases the inputs for next season (McLaughlin and Walsh, 1998).

Killing frost happened late September during 2017 when M. x giganteus and prairie cordgrass were still green. Drydown after cell rupture was evident by gray color on 3 October 2017. Killing frost in 2018 occurred later, 10 October. Reduction in dry matter yield in 2018 was likely due, in part to no nitrogen application in the spring. However, accumulation of snow during winter caused lodging and broken stems, and flooding of plots after snow melting caused delay harvest until end of May 2019. Whereas the harvest of 2017 biomass was in the end of April 2018. The previous reasons caused drop of more than 80% of leaves of M. x giganteus and lodging more than 90% of prairie cordgrass due to snow accumulation which made harvest difficult. Lewandowski
and Heinz (2003) found heavy lodging due to snow and ice rain caused lost leaves and broke stems resulting in decline in dry matter yield. Furthermore, study in northern and central Illinois on *M. x giganteus* showed that overwinter biomass of standing *Miscanthus* was prone to loss during winter more than overwinter harvestable biomass decline in Europe (Heaton et al., 2008). Boe and Lee (2007) reported heavy snow and ice at Aurora, South Dakota caused lodging for prairie cordgrass, but switchgrass was more sturdy and good for overwintering stockpiling. Conversely, delaying harvest of switchgrass after first killing frost can cause 20% decline in yield (McLaughlin and Walsh, 1998).

Another study in the northern Great Plains suggested that for long term sustainability delayed harvest of switchgrass in late of fall or late of growing season could be beneficial (Casler and Boe, 2003). Delaying harvest date until after first of September will lead to decline in biomass yield of *M. x giganteus* and switchgrass in average 0.07 and 0.01 Mg day\(^{-1}\) respectively (Heaton et al., 2004a). Delayed harvest of switchgrass until spring caused reduction in biomass production by 40% especially when snowfall was above average (Adler et al., 2006).

In this study, decline in overwintering biomass yield of prairie cordgrass due to heavy lodging and its high susceptibility to heavy snow and strong winds during winter make it less suited for stockpiling, matching the result of a study done by Boe and Lee (2007).

For *Miscanthus*, harvest date had significant effect on biomass yield which biomass yield declined 25% when plant harvest late winter compared to biomass yield in September; when senescence happened, most leaves drooped during winter and that had significant effect on mass rate of stem:leaf which was 75:25 in September to change to
85:15 in spring. In addition, harvest of Miscanthus after completed senescence and leaving the plant during winter caused decline in biomass which when harvest was delayed till February or March; on the other hand, 18% decline in dry matter yield occurred between December and February (Lewandowski and Heinz, 2003).

*Miscanthus* produced more biomass than switchgrass in both years, most markedly in response to N fertilizer. Also, we did not notice any diseases or insect damage in plots of *Miscanthus*, whereas plots of switchgrass and prairie cordgrass were pest infested which caused decline in their biomass production.

Decline in overwintering biomass due to falling leaves and broken stems in *M. x giganteus* was an issue, yet complete lodging of prairie cordgrass and decline in biomass yield for switchgrass due to lodging and leaf losses during winter with heavy snowstorm and snow accumulation were also problematic for the natives.
REFERENCES


CHAPTER 2

ABSTRACT

MORPHOLOGICAL DEVELOPMENT OF PHYTOMERS AND DISTRIBUTION OF BIOMASS OF *MISCANTHUS X GIGANTEUS* AMONG PHYTOMER COMPONENTS

EHSAN AL AINIZI

2020

*Miscanthus x giganteus* is a C₄ perennial, rhizomatous, vegetatively propagated sterile triploid hybrid grass native to East Asia. Early cultivation was in 1980 in Europe, then later, in the United States with high potential for biomass yield with low inputs. Little is known about distribution and partitioning of biomass among and within phytomers of *Miscanthus x giganteus*. The objective of this study was to describe pattern of biomass distribution among phytomer positions and their components (internode, leaf blade, and leaf sheath) for *Miscanthus x giganteus* in the northern Great Plains. A 10-year-old stand of *Miscanthus x giganteus* was sampled during June, July, August, and September of 2017 and 2018. Each month 10 tillers were randomly collected (fertilized with N or unfertilized control) and partitioned into internode, leaf blade, and leaf sheath fractions for each phytomer. Day of year (DOY) and N fertilizer had significant effects on biomass components across phytomer, with exception for sheath weight in June 2017 and internode weight in June 2018 for N. Significant phytomer x N interactions occurred for each DOY for all biomass components, with exception for June of both years and sheath weight in July 2018. Polynomial contrasts showed 70, 90, and 95% of variation among weight of phytomer components (internode, leaf blade, and leaf sheath) was explained by the quadratic regression. At the end of growing season, 50% of the biomass
was within the most basal five phytomers. Internode weight decreased acropetally with the most apical 3-4 phytomers (lightest) due to incomplete development. Internode 2 had the highest weight in both N fertilizer treatments. At the end of the growing season, the heaviest blades were phytomer 7 through 9 and heaviest sheaths in phytomer 6 through 8. The most distal 2-4 phytomers contained less than 5% of total biomass and were not fully developed due to killing frosts during both years. This research quantified the accumulation and distribution of biomass among phytomers for *M. x giganteus*, a promising cellulosic biomass crop, and identified developmental limitation to maximizing production in a short–season climate in the northern Great Plains.

**INTRODUCTION**

Understanding phytomer development can provide critical information about phenotypic plasticity, biomass distribution, and plant morphology due to effect of genetics and environment. Knowledge about phytomer growth and development is basic to explain grass growth and biomass accumulation (Boe et al., 2000). Grass tillers consist of basic units called phytomers which are composed of leaf blade, leaf sheath, internode, node, and axillary bud components (Moore and Moser, 1995). Furthermore, phytomer position in a repeated sequence and number and size can determine the architecture and shape of a grass tiller (Moore and Moser, 1995). Based on phytomer as a basic unit and culms as part of morphology of grass we can describe biomass distribution among them (Boe et al., 2000). Boe and Beck (2008) reported that number of phytomers per tiller in switchgrass is one of the yield components which is can be used as indirect criteria to enhance the biomass yield. Moore et al. (1991) described the growth of perennial grasses
in 4 stages, germination, vegetative, elongation and reproductive stage. Koo et al. (2007) reported that most of the biomass of two species of Miscanthus consisted of internode, especially the most proximal 10 phytomers, and very little from leaves and inflorescence.

Boe and Casler (2005) reported that internodes of switchgrass increased in weight basipetally with high plasticity through environment variation. Also, they mentioned acropetal increase in internode length with greatest increase between the apical and immediately subtending phytomer; sheath weight across phytomers also increased acropetally with fifth and sixth phytomers having lighter sheath weight than the more distal second and apical phytomers. In addition, effect of phytomer position on internode weight varied greatly among cultivars of switchgrass.

Strong relationships occur between morphological development and heat unit (growing degree days), with vegetative growth sensitive to temperature; whereas reproductive growth is more sensitive to photoperiod (Sanderson and Wolf, 1995). Also, two-thirds of total switchgrass yield was found within middle phytomers, with the first (most proximal) to fifth successive internodes increasing in length while length decreased from the fifth internode to the seventh (Sims et al., 1971). Similarly, sheath weight and leaf blade weight and length decreased acropetally among phytomers in big bluestem (Boe et al., 2000).

Understanding the relationship between morphological development and morphology of individual tillers and their effects on yield in the same cultivar can help to improve management and use of forage (Redfearn et al., 1997). In general biomass in grasses followed linear acropetal decreases across phytomers (Boe, 2007; Boe and Casler, 2005). Other studies found that, leaf blade length, tiller length, tiller number per
plant, and biomass yield per plant differed significantly among 11 populations of switchgrass (Das et al., 2004).

A study conducted at Brookings, South Dakota by Boe and Beck (2008) on three cultivars of switchgrass showed number of phytomers per tiller had less effect and less consistency on biomass yield compared with large effects of mass per phytomer and tiller density. Mature plants from switchgrass seedlings with multiple tillers had different morphological traits compared to single tiller per plant seedling; the rate of leaf elongation rate for switchgrass and big bluestem was higher in single tiller plant compared to multiple tillers per plant (Smart et al., 2004).

Three cultivars of switchgrass (Cave-In-Rock, Sunburst, and Nebraska 28) were evaluated for morphological traits for biomass production during early July. All three cultivars produced similar numbers of phytomer tiller\(^{-1}\) at start of stem elongation. From July through August, ‘Cave-In-Rock’ had 25% increase in phytomers tiller\(^{-1}\) compared with 20% for ‘Sunburst’ and ‘Nebraska 28’, with 50% increase in mass per phytomer (Boe and Beck, 2008). Period of late stem elongation, which is between June and July, and senescent stage (seed maturity), which is between August through November, had different patterns on morphological characteristic for cultivars. Phytomers tiller\(^{-1}\) for Cave-In-Rock had direct effect on biomass yield in elongation stage of stem in 2005 and 2006 whereas phytomers tiller\(^{-1}\) for ‘Sunburst’ had significant effect on biomass during mature seed stages and stem elongation, whereas phytomers tiller\(^{-1}\) for Nebraska 28 had an effect on biomass only in late stem elongation stage.

Nitrogen fertilizer and precipitation rate during growing season play big roles in biomass production of Miscanthus. Beale and Long (1997) reported that Miscanthus x
*Miscanthus x giganteus* can produce high biomass with less input (N, P, K) compared to maize (*Zea mays* L.). *Miscanthus x giganteus* biomass yield ranged between 15-30 Mg ha$^{-1}$ in the Midwest of United States (Heaton et al., 2004a; Heaton et al., 2008; Maughan et al., 2012). Averaged across nitrogen rates, a mature stand of *Miscanthus* produced 22 Mg ha$^{-1}$ (Heaton et al., 2004a). Mature *Miscanthus x giganteus* responded positively to nitrogen application (Arundale et al., 2014c).

Also, *Miscanthus* was highly responsive to precipitation, and precipitation had high effect on *Miscanthus* performance during April-September (Heaton et al., 2004a) and little tolerance to drought (Anderson et al., 2011). Individual tiller weight of switchgrass increased with high nitrogen application (Sanderson and Reed, 2000). Investigation study by Maughan et al. (2012) reported no important relationship between biomass yield and phytomers per tiller for *Miscanthus x giganteus*, but they reported that strong positive correlation between growing season and tiller diameter, and also strong negative correlation between phytomer number and accumulated thermal time. Another study on switchgrass and kleingrass reported that pattern of morphological development was greater during wet and cool period and that morphological development was highly related to precipitation and temperature (Sanderson, 1992). Also, the previous study reported that morphological development in summer of kleingrass was slower than spring by 40% compared to switchgrass which had the same rate of development during spring or summer.

Very few studies discussed biomass partitioning and components in perennial grasses e.g. (Das et al., 2004; Krueger et al., 1969; Smith, 1973; Thomson et al., 1973) and most were in the northern Great Plains (Boe, 2007; Boe and Beck, 2008; Boe et al., 2014c).
2000; Boe and Casler, 2005; Boe et al., 2017; Boe et al., 2009). Furthermore, to date, there was no study conducted to explain the effect of phytomer position and development and biomass distribution among phytomer components (internode, blade, and sheath) as a basic unit of Miscanthus x giganteus.

Therefore, this is the first study of biomass partitioning and distribution among phytomers of Miscanthus x giganteus in a short-season environment the northern Great Plains. Objectives were to determine the effects of time of growing season and nitrogen fertilizer on distribution of biomass among phytomers and their relative development through the growing season.

MATERIALS AND METHODS

The study was conducted on the South Dakota State University Agricultural Experiment Station Felt Farm near Brookings, SD (44.3652° N, 96.7965° W). Miscanthus x giganteus, a perennial warm-season biofuel-candidate grass species, was evaluated for distribution of biomass production among phytomer positions and components (internode, leaf blade, and leaf sheath) during 2017 and 2018.

Experimental design was a split-split-plot in a randomized complete block with four replications. Species were whole plots and nitrogen fertilizer (0 kg N ha\(^{-1}\) or 112 kg N ha\(^{-1}\)) was the sub plot treatment. Individual plots of Miscanthus were established from transplanted ramets in June 2009. Plant spacing was on 0.6-m centers. Sub-plot size was 3 m x 8 m. N fertilizer was applied in the form of urea on 4 May 2017 when the grass was in early vegetative development. The soil type was a McKranz (fine-silty, mixed, superactive, frigid, Calciudolls)-Badger (fine, smectic, frigid, vertical, Argiaquolls) silty
clay loam. This soil is considered marginal for conventional crop production due to slow drainage that impedes timely planting in the spring. Prior to initiation of this study, in 2017 the experimental plots were occasionally burned in the spring to remove excessive accumulation of standing dead and litter material.

**Data Collection and Analysis**

Miscanthus growing-season biomass and stand morphology measurements were collected on 16 June, 13 July, 7 August, and 12 September during 2017 and 23 June, 23 July, 23 August, and 27 September during 2018. Within each sub plot, 0.18 m$^2$ sub samples were harvested at ground level by hand with a rice knife for each of the above dates. For 2017, one sub sample was taken per sub plot; whereas, in 2018 two sub samples were taken per sub plot. Each sub sample was placed in a separate paper bag and dried at room temperature for three weeks. Morphological data collected for each sub sample were for 10 random tillers selected for phytomeric partitioning analysis of leaf blade, leaf sheath, internode, and inflorescence components of biomass (e.g., Boe and Casler 2005). Dry weight of tiller components was measured to 0.1 g accuracy (Fig. 1).

Partitioning of biomass among phytomers and sampling time (DOY) data were analyzed using the linear regression and polynomial contrast procedures within the analysis of variance menu in *Statistix* 8 (Analytical Software, 2003). DOY, N fertilizer rate and phytomer position were considered fixed effects. Replications were considered random.
RESULTS

Result of analyses of variance for effects of phytomer and N fertility on biomass yield components for each DOY (June, July, August, and September) during 2017 and 2018 are presented in Tables 1 and 2, Figs. 2, 2a, 3, 3a, 4, 4a. Phytomer and N rate had significant effects on internode, leaf blade, and leaf sheath weights for each DOY with the exception of sheath weight for N rate in June 2017 and internode weight for N rate in June 2018 for June (Table 1. Figs. 2, 2a, 3, 3a, 4, and 4a). In addition, the interaction between phytomer and N rate was significant for effect on biomass components in July,

Fig. 1 Partitioning of *Miscanthus x giganteus* to three components of phytomer (internode (I), leaf blade (B), and leaf sheath (S)). Fertilized (left) and unfertilized (right).
August, and September, with exception for June (not significant) for both years, and sheath weight in July 2018 (p = 0.06) (Table 1. Figs. 2, 2a, 3, 3a, 4 and 4a).

Partitioning of the phytomer sums of squares showed that quadratic models ($y = ax^2 + bx + c$) were generally the best fits for more than 75, 100, and 75% of internode, blade, and sheath weight components, respectively for each DOY in both 2017 and 2018 (Table 2).

At the end of the growing season more than 50% of total biomass was located in the five most proximal (basal) phytomers (Tables 3A and 3B). The most apical or distal phytomer internodes remained undeveloped with less biomass as shown in Figs. 4 and 4a for 2017 and 2018, respectively. In addition, the distal (apical) phytomer internodes had the lowest mean weight for each specific DOY (June, July, August, and September) (Figs. 2 and 3) for 2017 and 2018, respectively.

Furthermore phytomer 2 internode had the greatest mean weight for each DOY (June, July, August, and September) during the growing season and at the end of the growing season in both N rates (Table 3a and b). Phytomer internode mean weight decreased linearly acropetally in spite of phytomer 2 having the greater biomass throughout the growing season (June, July, August, and September) as shown in (Figs. 2, 2a, 3, 3a, 4, 4a) for 2017 and 2018, respectively.

Mean leaf blade weight increased through each DOY, and phytomers 7, 8, and 9 had the greater biomass at the end of growing season for both years (Figs. 5, 5a, 6, and 6a).

Mean leaf sheath weight had a similar trend as blades with increased weight acropetally through phytomers 6, 7, and 8 having the greatest biomass in 2018 with
subsequent decline for more distal phytomers (Figs. 7, 7a, 8, and 8a).

Phytomers 1 to 6 had similar pattern of increasing in internode weight in response to increasing DOY. In comparison phytomer 12 did not begin development until near the end of growing season. Furthermore, phytomer 2 had the largest biomass through each DOY and at the end of growing season for both years. In general, all phytomers stopped increasing in weight after September (Fig. 4).

Mean blade weight for phytomer 2 to phytomer 4 slightly increased in weight until mid-July with no increase in weight until end of growing season. In contrast, mean weight of phytomer 6 through phytomer 10 achieved largest gain in biomass from July to September in both years in contrast to phytomer 1 which had the lowest mean weight for both years (Fig. 9).

Sheath mean weight for phytomer 2 increased slightly from June to early July with no increase in weight throughout the rest of the growing season. In contrast, phytomers 4 to 8 had large increases until August, achieving greater biomass compared to more distal phytomers 10 to 12 without or although increasing in weight had lowest biomass yield for both years (Fig. 10). At the end of growing season, the most distal phytomers (i.e., 12) and above were still meristematic and composed less than 5% of the total biomass yield (Table. 3).
Table 1. Levels of significant effects of phytomer (PHY), nitrogen rate (N), and phytomer x N rate interaction (PHY x FER) on biomass components (internode (INT), blade (BLD), and sheath (SHT)) during growing seasons of 2017 and 2018.

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Table. 2. Linear and quadratic regression coefficients for phytomer on morphological components of biomass yield in tillers of *Miscanthus* during the growing seasons of 2017 and 2018.

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<td>QUD</td>
<td>RSS/TSS</td>
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<tr>
<td>June</td>
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<td>-0.06*</td>
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<tr>
<td>July</td>
<td>-1.40**</td>
<td>-0.36**</td>
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</tr>
<tr>
<td>August</td>
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<td>-0.56**</td>
<td>0.77</td>
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<tr>
<td>September</td>
<td>-2.42**</td>
<td>-0.07 n.s</td>
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</tbody>
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<th>2018</th>
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<td>August</td>
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<td>September</td>
<td>-3.18**</td>
<td>-0.36 n.s</td>
<td>0.76</td>
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</table>

† LIN. Linear coefficient contrast; QUD. Quadratic linear contrast; RSS/TSS. Sum of linear and quadratic sums of square divided by total sum of square for phytomer effect.

* Significant at the level 0.05; ** Significant at the level 0.01; ns, Non-significant
Table 3a. Mean and standard error for weight (g) of internodes (N rate 0 kg ha\(^{-1}\)) for individual phytomere (PHY) positions through growing seasons (June through September) of 2017 and 2018.

<table>
<thead>
<tr>
<th>PHY</th>
<th>June</th>
<th>July</th>
<th>Aug</th>
<th>Sep</th>
<th>June</th>
<th>July</th>
<th>Aug</th>
<th>Sep</th>
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<td>0.83±0.01</td>
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<td>0.11±0.12</td>
<td>0.60±0.43</td>
<td>1.16±0.80</td>
<td>1.30±0.89</td>
</tr>
<tr>
<td>(29)†</td>
<td>(30)</td>
<td>(27)</td>
<td>(33)</td>
<td>(28)</td>
<td>(52)</td>
<td>(51)</td>
<td>(48)</td>
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</tr>
<tr>
<td>2</td>
<td>0.23±0.04</td>
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<td>1.35±0.02</td>
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<td>0.21±0.15</td>
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<td>(27)</td>
<td>(33)</td>
<td>(28)</td>
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<td>(51)</td>
<td>(48)</td>
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<td>3</td>
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<td>1.05±0.1</td>
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<td>1.06±0.08</td>
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<td>(51)</td>
<td>(48)</td>
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<td>0.03±0.01</td>
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PHY, Phytomer; INT, internode
† (n), number of observations
‡ ---, mean value < 0.1 g, due to multiple cases where undeveloped internode was visible but weighed less than 0.1 g.
### Table 3b. Mean and standard error for weight (g) of internodes (N rate 112 kg ha\(^{-1}\)) for individual phytomer (PHY) positions through growing season (June through September) of 2017 and 2018.

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<th>Aug Mean (g)</th>
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<td>(73)</td>
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</tbody>
</table>

PHY, Phytomer; INT, internode

† (n), number of observations
‡ ‒ ‒ ‒, mean value < 0.1 g, due to multiple cases where undeveloped internode was visible but weighed less than 0.1 g.
Fig. 2. Effect of phytomer position on internode development with two nitrogen treatments (0 and 112 kg ha\(^{-1}\)) through the growing season ((A) June, (B) July, (C) August, and (D) September) of 2017.
Fig. 2a. Internode development during June (upper left), July (upper right), August (lower left), and September (lower right) for growing season 2017. Phytomer position is from left to right (basal to apical). Position numbers in Roman numerals.
Fig. 3. Effect of phytomer position on internode development with two nitrogen treatments (0 and 112 kg ha$^{-1}$) through growing season ((A) June, (B) July, (C) August, and (D) September) of 2018.
Fig. 3a. Internode development of fertilized Miscanthus during August (upper), September (lower) with the most apical phytomers not fully developed for growing season 2018 at Brookings, SD. Phytomer position is from 1(basal) to n (apical), which is the partially emerged inflorescence, i.e, lower picture.
Fig. 4. Pattern of internode development relative to phytomere (PHY) position through growing season of 2017 (A) and 2018 (B).
Fig. 4a. Effect of fertilizer on size and biomass of internode components of phytomers of Miscanthus on September 2018 at Brookings, SD. Most apical or distal phytomers (internode) remained undeveloped with less mean weight in both nitrogen treatments. Phytomer position is from 1(basal) to n (apical), which is the partially emerged inflorescence, i.e, left to right.
Fig. 5. Effect of phytomer position on blade development with two nitrogen treatments (0 and 112 kg ha⁻¹) through growing season ((A) June, (B) July, (C) August, and (D) September) of 2017.
Fig. 5a. Effect of phytomer position on blade development with nitrogen treatments (112 kg ha\(^{-1}\)) through growing season ((left) June, (second left) July, (second right) August, and (right) September) of 2017 at Brookings, SD. Phytomer position 1 (basal) to n (apical), which is the partially emerged inflorescence, i.e, left to right.
Fig. 6. Effect of phytomer position on blade development with two nitrogen treatments (0 and 112 kg ha\(^{-1}\)) through growing season ((A) June, (B) July, (C) August, and (D) September) of 2018.
Fig. 6a. Effect of phytomer position on blade development with nitrogen treatment (112 kg ha$^{-1}$) through growing season ((upper left) June, (upper right) July, (lower left) August, and (lower right) September) of 2018 at Brookings, SD. Phytomer position is from 1 (basal) ton (apical), i.e., left to right.
Fig. 7. Effect of phytomer position on sheath development with two nitrogen treatments (0 and 112 kg ha\(^{-1}\)) through growing season ((A) June, (B) July, (C) August, and (D) September) of 2017.
Fig. 7a. Effect of phytomer position on sheath development with fertilized treatment through growing season ((upper left) June, (upper right) July, (lower left) August, and (lower right) September) of 2017 at Brookings, SD. Phytomer position 1 (basal) to n (apical), which is the partially emerged inflorescence, i.e., left to right.
Fig. 8. Effect of phytomer position on sheath development with two nitrogen treatments (0 and 112 kg ha\(^{-1}\)) through growing season ((A) June, (B) July, (C) August, and (D) September) of 2018.
Fig. 8a. Effect of phytomer position on sheath development with fertilized treatments (112 N kg ha\(^{-1}\)) through growing season ((upper left) June, (upper right) July, (lower left) August, and (lower right) September) of 2018 at Brookings, SD. Phytomer position 1 (basal) to n (apical), which is the partially emerged inflorescence, i.e., left to right.
Fig. 9. Pattern of leaf blade development relative to phytomer position through growing season (A) 2017 and (B) 2018.
Fig. 10. Pattern of leaf sheath development relative to phytomer position through growing season (A) 2017 and (B) 2018.
Fig. 11 Monthly precipitation and temperature at Brookings, SD during 2016, 2017, and 2018 and the 30-year averages.
DISCUSSION

This is the first study to evaluate phytomer position, and phytomer components (internode, leaf blade, and leaf sheath), as the basic unit of biomass accumulation in a *Miscanthus* tiller. Morphological development of this perennial grass had great impact on biomass yield during two growing seasons at Brookings, SD. Increasing biomass yield could be attained through understanding some specific criteria such as morphology and architecture of the canopy (Redfearn et al., 1997).

Both years of study showed similar effects of phytomer position on biomass components (internode, leaf blade, and leaf sheath). Even though precipitation rate differed greatly between years, each DOY and phytomer position effects on internode, leaf blade, and leaf sheath were similar. Growing season precipitation (April through September) was 80.5, 84.2, 90.6 mm for 2016, 2017, and 2018, respectively. Precipitation during June and July was 33.7, 24.3, 52.6 mm in 2016, 2017, and 2018, respectively. The 30-year monthly precipitation average from April through September is 77.9 mm with 24.3 mm during June and July.

Precipitation rate during April through May had great effect on biomass production with switchgrass cultivar ‘Dacotah’ which reached peak standing crop during July, whereas cultivar ‘Cave-In-Rock’ didn’t reach peak standing crop in the same period of time (Lee and Boe, 2005). Similarly standing crop was affected by precipitation rate during early season when peak anthesis wasn’t complete until mid-August in two switchgrass cultivars (Boe, 2007). Moore et al. (1991) reported more phytomers were developed when plants received more precipitation during July and August. In Iowa, stem
diameter of *Miscanthus* x *giganteus* increased in response to precipitation (Boersma and Heaton, 2014).

This study showed internode weight decreased acropetally with the phytomer 2 internode heaviest and the most apical (distal) the lightest. Similarly, Boe (2007) reported greater mass of internode and sheath of the basal phytomer in switchgrass cultivars. He reported the apical phytomer had lightest mass compare to the basal phytomer which had 5-fold more than the apical internode in two cultivars of switchgrass.

In this study of *Miscanthus*, the internode of phytomer 1 had lower weight than phytomer 3 or 4 due to most of that internode was located below the surface of the soil. On the other hand, two-thirds of the first phytomer (internode) was belowground and for this reason the weight of phytomer one was lighter than for phytomers 2 and 3. Boe and Bortnem (2009) reported the proximal phytomer (internode) length of little bluestem was less than 1 cm and its weight was heavier than the three distal (acropetal) phytomers. The greatest mass was within the longest internodes of phytomers of little bluestem. The first three phytomers in natural populations of little bluestem composed 85% of primary axis of biomass.

Components of biomass (internode, sheath, and blade) responded significantly to increase in DOY across phytomers. In addition, quadratic regression was the best fit to explain variation among phytomer means for internode, blade, and sheath biomass. A 3-year study in the northern Great Plains to evaluate and provide morphological description and distribution of biomass production among stem components of little bluestem showed a strong relationship between blade and sheath lengths, and effect of phytomer position was explained better by a quadratic regression than linear regression (Boe and
Bortnem, 2009). Also, they reported phytomer 1 had the longest sheath, and the pattern of sheath length decreased acropetally. In contrast, the first two phytomers had the longest blades.

Boe and Beck (2008) reported DOY had positive effect on mass per phytomer. During early July, which is the time of stem elongation, three cultivars of switchgrass had the same number of phytomer per tiller, for example ‘Cave-In-Rock’ was increased by 25% and Nebraska 28 and Sunburst was increased by 25%. They concluded number of phytomers per tiller had less impact on biomass than mass per phytomer. Forty-four percent of their samples had significant effect of number of phytomers per tiller on biomass yield, whereas 90% of samples had significant effect of mass per phytomer and tiller density on biomass yield.

Sheath weight of Miscanthus increased acropetally with phytomer 5 the heaviest in July through September then declined acropetally from phytomer 6 through 9 in both N fertility treatments. Similarly sheath mass increased basipetally in the study of Boe (2007) on two switchgrass cultivars. The first two leaves of Miscanthus senesced before the end of growing season resulting in decline in leaf weight after mid-July. Also, the leaf of phytomers 3 and 4 had low weight after mid-August, likely for the same previous reason. Overall patterns of leaf blades and leaf sheaths were similar in both years, even with fluctuation in the weight of leaves during growing seasons, due to many leaves being dropped or otherwise incomplete.

Result of this study showed that internode developmental pattern across phytomers and biomass distribution among internodes were more consistent than leaf blade and leaf sheath. Development of internodes of phytomers 2 through 6 had similar
pattern in both years compared to the other phytomers, but all phytomers (internodes) had the same consistency of development between growing seasons. Similarly Boe et al. (2000) reported a pattern of consistency of internodes compared to sheaths of phytomers across warm-season grasses. These results indicated that the internode component of phytomers is more important as a stable source of biomass production compared to sheath and blade fractions.

Phytomers tiller\(^{-1}\) for *Miscanthus* was consistent between years even though there was greater precipitation during June and July in 2018. Only one extra phytomer developed in August and in September in 2018 compared to 2017. Similarly, Boe (2007) reported that switchgrass cultivars had the same number of phytomers tiller\(^{-1}\) in consecutive years with a large difference between mid-summer precipitation.

Both years showed similar patterns of development for blade and sheath weights among phytomers through the growing season. These results matched somewhat results of study by Boe (2007) for sheath weights of two cultivars of switchgrass.

Greater development and biomass occurred for all phytomers in fertilized compared to unfertilized *Miscanthus* during growing seasons of the two years. This result showed importance of N fertilizer on morphological traits of phytomers and their components, leading to positive effect on increasing biomass production of *Miscanthus x giganteus*.

Redfearn et al. (1997) predicted biomass yield in switchgrass through several primary yield components such as stem dry weight per tiller, leaf sheath, leaf blade, tiller per unit area. Also, for predicting yield of Trailblazer and Pathfinder of switchgrass they found dry weight of leaf sheath and leaf blade were significant factors. Sanderson and
Wolf (1995) reported variation between vegetative growth and reproductive growth was found which was photoperiod affected reproductive growth, whereas temperature had a greater effect on vegetative growth. In addition, they reported strong relationship between morphological development and growing degree days. Boe and Lee (2007) reported in the natural habitats of prairie cordgrass in southeastern South Dakota, internodes, leaf blades, and blade sheaths weights and lengths had similar pattern within the symmetric phytomers of reproductive tillers.

In the northern Great Plains reduced stand persistence and winter hardiness could be a result of delayed maturity from selection for increased phytomers tiller$^{-1}$ (Boe, 2007). In this study, due to killing frost, the most distal 3 phytomers (apical phytomers) were undeveloped and their weights much less than more proximal fully developed and mature phytomers (the first five basal phytomers). For the previous reason, the first proximal phytomers consisted more than 50% of the total biomass components. The upper 3 apical phytomers were not used in data analysis due to immature development halted due to killing frost. Boe (2007) reported that biomass yield had strong linear relationship with mass tiller$^{-1}$ (tiller size) within population of switchgrass. Also, he compared two cultivars of switchgrass, which showed biomass was affected by mass tiller$^{-1}$ linearly and its utility as the best indicator to predict biomass yield, better than tillers m$^{-2}$.

Results of current study on Miscanthus showed importance of N fertilizer on phytomer weight and consequently on biomass yield. In addition, phytomer number had great effect on biomass yield. Also killing frost was the major limiting factor in phytomer development in the northern Great Plains.
In addition, understanding of distribution of biomass among phytomers and phytomer development during growing season can be used to improve and enhance biomass production in perennial grasses. Even though *Miscanthus x giganteus* does not reach physiological maturity before a killing frost at Brookings, it still produces large amounts of biomass year-after-year and does not show decline in stand density, relative to switchgrass and prairie cordgrass, because of more phytomers tiller \(^1\) and phytomer mass. Also, its ( Miscanthus) resistance to lodging and thus ability to trap snow presumably enhances soil moisture for the next growing season.
REFERENCES


Smith D. (1973) Distribution of dry matter and chemical constituents among the plant parts of six temperate-origin forage grasses at early anthesis.

